THE UNIVERSITY *of* EDINBURGH
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Anticholinergic use in the UK: longitudinal trends and associations with cognitive outcomes

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Declaration

I declare that the work presented in this thesis is my own, except where work which has formed part of jointly authored publications has been included. My contributions and those of other authors to this work are indicated below. Where reference was made within this thesis to the work of others, appropriate credit was given. This thesis has not been submitted, in whole or in part, in any previous application for a degree.

Most of the data used in the analyses presented in this thesis were pre-processed and made available by UK Biobank. This includes all lifestyle- and demographic variables, data on cognitive tests, and MRI imaging. For more details on the variables used in this study and their level of pre-processing by UK Biobank, I refer the reader to chapter 6, Supplementary Table 1 (p. 214), which contains data field IDs and hyperlinks to the relevant webpages of UK Biobank. I was responsible for full preparation of the prescription data, as it had been only minimally pre-processed by UK Biobank. My work included the homogenisation of drug names, assignment of anticholinergic potency, and transformations of the dataset. The full code used to accomplish these tasks is available at https://github.com/JuM24?tab=repositories.

The work presented in chapter 4 was previously published in the British Journal of Clinical Pharmacology as "Increase in anticholinergic burden from 1990 to 2015: Age-period-cohort analysis in UK Biobank" by Jure Mur (candidate), Simon R. Cox (PhD supervisor), Riccardo E. Marioni (PhD supervisor), Graciela-Muniz-Terrera (PhD supervisor), and Tom C. Russ (primary PhD supervisor). Author contributions: study conception and design: **J.M.**, data preparation: **J.M.**, statistical analyses: **J.M.**, draft writing: **J.M.**, draft review and editing: all authors. The work was also presented as *Anticholinergic trends in the UK from 1990 to 2016* at the GSA Annual Scientific Meeting Online (poster presentation, November 4th – 7th 2020).

The work presented in chapter 5 was previously published in Alzheimer's & Dementia: Translational Research & Clinical Interventions as "Association between anticholinergic burden and dementia in UK Biobank" by Jure Mur (candidate), Tom C. Russ (primary PhD supervisor), Simon R. Cox (PhD supervisor), Riccardo E. Marioni (PhD supervisor), and Graciela Muniz-Terrera (PhD supervisor). Author contributions: study conception and design: **J.M.**,

data preparation: **J.M.**, statistical analyses: **J.M.**, draft writing: **J.M.**, draft review and editing: all authors. The work was also presented as *Anticholinergic burden and dementia in UK Biobank* at the 25th Nordic Congress of Gerontology (oral presentation, June 2nd – 5th 2021).

The work presented in chapter 6 has been published in the British Journal of Clinical Pharmacology as "Anticholinergic burden in middle and older age is associated with lower cognitive function, but not with brain atrophy" by Jure Mur (candidate), Riccardo E. Marioni (PhD supervisor), Tom C. Russ (primary PhD supervisor), Graciela Muniz-Terrera (PhD supervisor), and Simon R. Cox (PhD supervisor). Author contributions: study conception and design: **J.M.** and S.R.C., data preparation: **J.M.**, statistical analyses: **J.M.**, draft writing: **J.M.**, draft review and editing: all authors. The work was also presented as *Anticholinergic burden, cognitive ability, and structural MRI in UK Biobank* at the 26th Nordic Congress of Gerontology in Odense, Denmark (oral presentation, June 8th – 10th, 2022), and the International Population Data Linkage Network in Edinburgh, Scotland (oral presentation, September 7th – 9th, 2022).

Signed: Date: 02.03.2023

Other publications

Some of my work conducted at the University of Edinburgh during the PhD was not directly related to the content of the main PhD project as presented in this thesis. Below I have listed two publications that resulted from this other work.

An exploration of the association between blood-based methylation in the APOE gene, cognition, cholesterol, family history of Alzheimer's disease, and cardiovascular disease in the Generation Scotland cohort:

Mur, J., McCartney, D. L., Walker, R. M., Campbell, A., Bermingham, M. L., Morris, S. W., Porteous, D. J., McIntosh, A. M., Deary, I. J., Evans, K. L., & Marioni, R. E. (2020). DNA methylation in APOE: The relationship with Alzheimer's and with cardiovascular health. *Alzheimer's & Dementia: TRCI*, *6(1)*. https://doi.org/10.1002/trc2.12026

A study on the relationship between the genetic variant rs9923231 (*VKORC1*), dementia subtypes, and the use of warfarin in UK Biobank:

Mur, J., McCartney, D. L., Chasman, D. I., Visscher, P. M., Muniz-Terrera, G., Cox, S. R., Russ, T. C., & Marioni, R. E. (2021). Variation in VKORC1 is associated with vascular dementia. *Journal of Alzheimer's Disease*, *80(3)*, 1329-1337, https://doi.org/10.3233/JAD-201256

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Abstract

Observational studies have shown an association between the use of anticholinergic drugs and various negative health outcomes. However, when studying cognitive outcomes, there is great heterogeneity in previous results. The objectives of the present thesis are threefold. First, to explore the longitudinal patterns of anticholinergic prescribing in the UK. Second, to examine the association between anticholinergic burden and dementia. Third, to probe the relationship between anticholinergic burden, general cognitive ability, and brain structural MRI in relatively healthy participants.

Chapter 1 provides an overview of the role of acetylcholine as a neurotransmitter in the human body. It begins with a description of its molecular characteristics and continues with a summary of anatomical and cellular features of cholinergic pathways in the brain. The chapter concludes with a description of the relevance of cholinergic processing in cognition and Alzheimer's disease.

Chapter 2 gives a summary of anticholinergic drugs. It describes the history of anticholinergic compounds and their present use in medicine. It then appraises the tools used to gauge the anticholinergic potency of drugs. I conclude the Chapter by evaluating the available evidence on the effects of anticholinergic drugs on various important health outcomes.

Chapter 3 focuses on UK Biobank, the sample used in all analyses presented in this thesis. The chapter briefly describes the conception of the study, the timeline of assessments, and the available variables. I focus in my descriptions on the variables that were used in the present thesis, especially cognitive tests, brain imaging, and linked health data.

Chapters 4 to 6 present the empirical work conducted as part of this thesis. Chapter 4 presents an analysis of anticholinergic prescribing trends in UK primary care from the year 1990 to 2015. I first calculate an anticholinergic burden (AChB) according to 13 different anticholinergic scales and an average to derive a "Meta-scale". I then describe the prevalence

of anticholinergic prescribing and its longitudinal trend for all scales. I use different plots of age-, period- and cohort effects on the AChB according to the Meta-scale to evaluate the contributions of these effects to the linear longitudinal trend. The study finds AChB to have increased 9-fold over 25 years and that this effect was attributable to both age- and cohort/period-related changes. In other words, ageing of the sample is not sufficient to explain the increase in anticholinergic prescribing; cohort- or period-effects must have contributed to the observed changes.

Chapter 5 explores the relationship between anticholinergic prescribing and dementia. Previous studies on this topic had provided varied results. One of the goals of the present study was to probe potential factors for this heterogeneity. We find that greater AChB according to most of the studied anticholinergic scales (standardised HRs range: 1.027-1.125), as well as the slope of anticholinergic change (HR=1.094; 95% CI: 1.068-1.119), are associated with dementia. However, we find that not all drug classes are associated with dementia. Antidepressants (HR=1.11, 95% CI=1.07-1.17), antiepileptics (HR=1.07, 95% CI=1.04-1.11), and the diuretic furosemide (HR=1.06, 95% CI=1.02-1.10) exhibit the strongest effects. Interestingly, when exploring the effects of groups of anticholinergic drugs with different anticholinergic potencies, only the moderate potency group shows significant associations with dementia (HR=1.10, 95% CI=1.05-1.15).

Chapter 6 examines the association between AChB, general cognitive ability, and brain structural MRI. It aims both to explore the potential sources of heterogeneity in previous work, as well as to expand on it by studying relatively healthy community-dwelling adults. We study brain structural MRI in a much bigger sample (at least 5x bigger) and use many more outcomes than previous studies. We find weak, but significant associations between AChB and general cognitive ability, and with 7/9 individual cognitive tests (standardised betas (β) range: -0.039, -0.003). Again, AChB in only some drug classes is associated with lower general cognitive ability, especially β-lactam antibiotics (β=-0.035, p_{FDR}<0.001) and opioids (β=-0.026, p_{FDR}<0.001). We find no associations between AChB and any measure of brain structural MRI.

Finally, chapter 7 summarizes the findings presented in chapters 4 to 6. The chapter also provides a critique of the sample and of my approach when conducting the analyses presented in the present thesis. The chapter concludes by discussing suggestions for future work on this topic.

Lay summary

Anticholinergic drugs are medicines that block the actions of acetylcholine, an important signalling molecule in the human nervous system. These drugs are not prescribed for one illness but can be found among drugs prescribed for a multitude of disorders, such as antidepressants, drugs to treat pain, and cardiovascular drugs. The use of these drugs in older adults has been linked to poorer health outcomes, including higher frailty and increased likelihood of fractures. This thesis addressed the association between the use of anticholinergic drugs, the brain, thinking skills, and dementia. We did this in three parts. First, we studied how common anticholinergic prescribing was in the UK and whether its frequency had changed from 1990 to 2015. We found that anticholinergic prescribing had indeed increased in this period, but we did not determine the exact cause of this increase. Second, we studied whether individuals prescribed anticholinergic drugs were more likely to be diagnosed with dementia. We found that to be the case, but only for certain drug classes. The prescribing of anticholinergic antidepressants, antiepileptics, and antidiuretics was associated with the increased risk of dementia, but the prescribing of most other classes of drugs was not. Third, we addressed the potential effects of anticholinergic prescribing on aspects of brain structure and functioning (cognitive ability) in healthy individuals. We calculated a score of general cognitive ability and found that anticholinergic use was linked to a slightly lower cognitive score. Again, we found this only to be the case for certain drug classes: opioid pain medications and a type of antibiotics. When we explored whether brain differences existed between individuals with varying degrees of anticholinergic use, we did not find any. Overall, the results in the present thesis show that the effects of anticholinergic use on cognitive health are best studied within distinct drug classes, as not all of them increase the risk of adverse cognitive effects. The results also show that even in mostly healthy middle-aged and older adults, anticholinergic use is associated with a slightly lower cognitive ability, and that there is no link to changes in brain structure. These results are consistent with the notion that anticholinergic use causes dementia and lower cognitive ability. However, this analysis on its own is not sufficient for such a conclusion, as the observed associations could be due to other

factors (e.g., sicker people might be both more likely to take anticholinergics, as well as to experience cognitive decline).

Acknowledgements

My supervisors - Simon, Tom, Riccardo, Graciela - offered advice, support, and helpful critique. They endured my questionable email etiquette, conveyed reassurance, and helped me grow from the many, many mistakes I made throughout the course of my PhD. They served as a constant reminder that for work to be enjoyable, the people are just as important - if not more so - as the content of the work itself.

Michelle Luciano chaired my Thesis Committee. She also managed the UK Biobank project and always patiently and promptly responded to my requests for additional data.

Bruce Guthrie helped me better understand the minefield of primary care prescribing data.

The participants of UK Biobank enabled the work presented in this thesis.

The directors of the PhD programme cared about delivering a good education and it showed - the PhD experience was instructive and meaningful. The PhD coordinators Jane Haley and Marja Main offered considerable guidance through the administrative twists and turns of the graduate experience. They always knew what needed to be known and what needed to be done.

My friends and family bestowed love, respite, and endless joy. Valérie accompanied me through most of the PhD and helped me retain my sanity when dealing with tables in the text editor. She has also rendered every day more enjoyable and more profound. In the PhD thicket you can sometimes think that your work is the most important thing on Earth. My loved ones helped me to avoid that precarious thought. Without them, I might still have managed, but I wouldn't have seen the purpose of doing so.

List of abbreviations

^{3}H-QNB	^{3}H-quiniclidinyl benzilate
AAS	Anticholinergic Activity Scale
AAS-r	Revised Anticholinergic Activity Scale
ABC	Anticholinergic Burden Classification
ABS	Anticholinergic Burden Score
ACB	Anticholinergic Cognitive Burden
AcCoA	acetyl coenzyme A
ACh	acetylcholine
AChB	anticholinergic burden
AChE	acetylcholinesterase
AChE-I	acetylcholinesterase inhibitor
ACNU	active comparator new user design
ACSBC	AntiCholinergic and Sedative Burden Catalog
AD	Alzheimer's disease
ADS	Anticholinergic Drug Scale
AEC	Anticholinergic Effect on Cognition
AIS	Anticholinergic Impregnation Scale
ALS	Anticholinergic Loading Scale
ANS	autonomic nervous system
APOE	apolipoprotein E
APP	amyloid precursor protein
ARS	Anticholinergic Risk Scale
ATS	Anticholinergic Toxicity Score
Aβ	amyloid-β
BAAS	Brazilian Anticholinergic Activity Drug Scale
BBB	blood-brain barrier
CABS	Cancelli's Anticholinergic Burden Scale

CALS	CRIDECO Anticholinergic Load Scale
ChAT	choline acetyltransferase
CI/PI	Clinical Index, Pharmacological Index
CNS	central nervous system
CPRD	Clinical Practice Research Datalink
CRAs	Clinician-Rated Anticholinergic Scale
DBI	Drug Burden Index
DBI-WHO	Drug Burden Index, International Version
DDS	Drug Delirium Scale
DRN	Drug Risk Number
DRS	Delirogenic Risk Scale
DS	Durán Scale
GABS	German Anticholinergic Burden Scale
GP	general practitioner
HPA	hypothalamic-pituitary-adrenocortical axis
HR	hazard ratio
ICD	International Classification of Diseases
IDP	image-derived phenotype
KABS	Korean Anticholinergic Burden Scale
mACB	Modified Anticholinergic Burden Scale
mAChR	muscarinic acetylcholine receptor
MARANTE	Muscarinic Acetylcholinergic Receptor ANTagonist Exposure Scale
mARS	Modified Anticholinergic Risk Scale
MMSE	Mini-Mental State Examination
MRI	magnetic resonance imaging
nAChR	nicotinic acetylcholine receptor
NBM	nucleus basalis of Meynert
NFT	neurofibrillary tangle
NHS	National Health Service

OR	odds ratio
PANS	parasympathetic nervous system
PNS	peripheral nervous system
PPV	positive predictive value
RCT	randomised controlled trial
RR	risk ratio
SAA	serum anticholinergic activity
SANS	sympathetic nervous system
SNS	somatic nervous system
TNF	tumour necrosis factor
VAChT	vesicular acetylcholine transporter
WS	Whalley Scale

Table of contents

1 Cholinergic systems

Acetylcholine (ACh) is a chemical substance that in the human body acts chiefly as a transmitter of nerve signals. It is a compound with a long history in experimental research, having played a pivotal role in early neuroscience and in providing the conception of how the nervous system communicates. Over the past century, much work has been done to elucidate the function of ACh, explore neurotransmitter signalling systems, and develop drugs that manipulate those systems to therapeutic effect. To understand more about the potential for anticholinergic medication to exert putative effects on cognitive health, it is necessary to understand the role of ACh in the nervous system and the nature of cholinergic neurotransmission as it relates to cognitive function. The purpose of this chapter is to provide context for the rest of the thesis by covering the nature of ACh, its receptors, and its role in the nervous system. The chapter then proceeds to describe how ACh has been related to cognitive functioning and Alzheimer's disease (AD). While I attempt to cover the major aspects of ACh in its actions as both a central and peripheral neurotransmitter, I will emphasise its role as a centrally acting agent with implications for human cognitive functions.

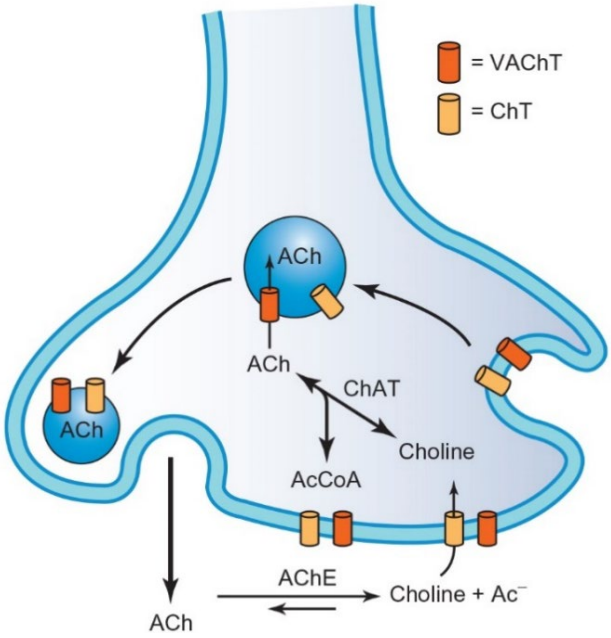
1.1 Acetylcholine: synthesis, release, and clearance

ACh was first synthesised in 1867 by Adolf von Baeyer (Fisher & Wonnacott, 2012). It then took forty years for a function of ACh in the human body to be realised: when applied to peripheral tissues, ACh stimulated parasympathetic nerves (Dale, 1914). Soon afterwards, ACh-mediated nerve-to-muscle signalling was demonstrated (Loewi, 1921), and ACh was isolated from animal tissues (Dale & Dudley, 1929). These findings paved the way for several decades of discoveries that confirmed the importance of ACh as a transmitter of signals in the nervous system.

ACh is formed from acetyl coenzyme A (AcCoA) and choline (**Figure 1**), which are derived from both extra- and intracellular sources. AcCoA is synthesised in mitochondria-rich neuronal terminals and is then translocated to the cytoplasm by a currently unknown mechanism. In

contrast, choline must be partly derived from other sources – including the diet – as there is very little *de novo* synthesis of this compound in neurons. However, it is difficult for choline to cross the blood-brain barrier (BBB), a semi-permeable barrier that regulates the movement of molecules between the blood and the interstitial fluid in the brain. Therefore, choline is mainly transported from the extracellular space as a product of the breakdown of ACh by a choline transporter (Fisher & Wonnacott, 2012; Westfall et al., 2018). This transport is the rate-limiting step in the synthesis of ACh and is in mammals realised through three types of transmembrane protein transporters (Westfall et al., 2018). The reaction between acetyl CoA and choline proceeds through the transferral of a molecule of acetate to choline (acetylation), a process catalysed by the enzyme choline acetyltransferase (ChAT). ChAT is synthesised in

Figure 1: Synthesis, release, and clearance of ACh. ACh is generated through the reaction of choline and AcCoA, catalysed by ChAT. ACh is then transported into synaptic vesicles by VAChT. Upon release and binding, ACh is broken down into products that include choline. The latter is then transported back into the cell by the choline transporter. Fisher & Wonnacott (2012). Adapted with permission.



the rough endoplasmic reticulum of the cell body and transported to the nerve terminal by axoplasmic transport (Fisher & Wonnacott, 2012; Westfall et al., 2018).

ACh is transported into synaptic vesicles by a specific vesicular ACh transporter (VAChT). Utilising an antiporter mechanism – analogous to vesicular packaging of other biogenic amine

neurotransmitters – VAChT couples ACh entry into the vesicle with the efflux of hydrogen ions. This leverages the potential energy of an ATPase-induced electrochemical gradient of hydrogen ions, to transport ACh into the vesicles against its concentration gradient (Fisher & Wonnacott, 2012; Westfall et al., 2018). The transport achieves a high concentration of ACh, with a single vesicle containing between 1,000 and 50,000 molecules, and the entire nerve terminal containing at least 300,000 vesicles (Westfall et al., 2018).

When the nerve terminal is depolarised, voltage-gated calcium channels open, allowing the entry of Ca^{2+} and promoting the action of synaptobrevins, synaptophysins, synaptotagmins, and other intrinsic vesicular proteins to fuse with the plasma membrane and release ACh into the synaptic cleft through exocytosis (Fisher & Wonnacott, 2012; Westfall et al., 2018). There seem to exist two pools of ACh within the nerve termina: (1) the readily releasable pool contains vesicles near the plasma membrane and is rapidly deployed upon depolarization, while (2) the reserve pool replenishes the readily releasable pool and may be required during periods of sustained stimulation (Westfall et al., 2018). After its release and binding, ACh is eliminated from the synapse through hydrolysis by the enzyme acetylcholinesterase (AChE), yielding acetate and choline. This breakdown of ACh by AChE is distinct from the transporter-mediated removal of many other neurotransmitters and can be performed within one millisecond upon entry into the synaptic cleft (Fisher & Wonnacott, 2012; Westfall et al., 2018). This process holds significant clinical importance, as drugs that inhibit the breakdown of ACh at the synaptic cleft (acetylcholinesterase inhibitors, AChE-Is) represent the main form of treatment for Alzheimer's disease (see section 1.4.4).

1.2 ACh in the peripheral nervous system

ACh is widely utilised in the communication between the brain and spinal cord on the one hand, and the organs of the body on the other. This section aims to describe the classification of the nervous system and highlight the widespread utilisation of ACh-mediated neurotransmission in various functions of the human body.

The peripheral nervous system (PNS) is responsible for the communication between the central nervous system (CNS; the brain and spinal cord) and the rest of the body. Preganglionic nerve fibres from the CNS project to clusters of cell bodies in the PNS called ganglia, which in turn project postganglionic fibres to different organ systems across the body. The PNS consists of the autonomic nervous system (ANS) and the somatic nervous system (Westfall et al., 2018).

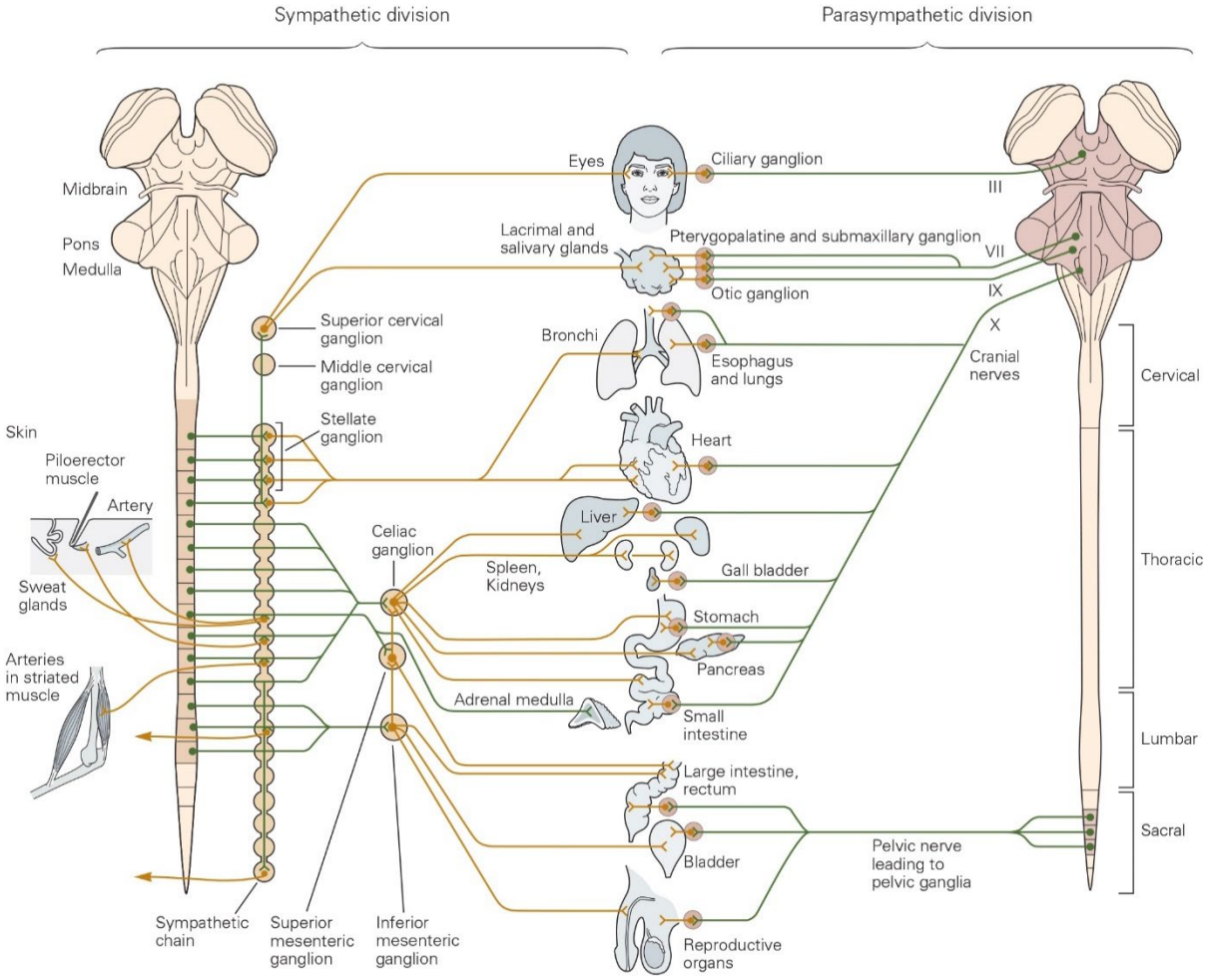
The somatic nervous system is under voluntary control and comprises nerves projecting from neurons in the brain stem and the spinal cords to skeletal muscles. The nerve fibres synapse with the muscle at an area called the end-plate. There, the motor neurons release ACh that binds to receptors on the muscle fibre and leads to the opening of sodium- and potassium channels. The channels are open for only about 1ms. However, due to the number of receptors present at the synapse, ~50,000 Na^+ and K^+ ions are admitted into the cell (Westfall et al., 2018). The resulting end-plate potential that triggers muscle contraction is consequently much larger than the postsynaptic potential at an average neuron (Kandel & Siegelbaum, 2013).

The ANS innervates smooth muscle and glands and is beyond voluntary control. Preganglionic fibres emerge from the spinal cord and first terminate in postganglionic neurons before the latter synapse on target organs. The ANS is further divided into the sympathetic (SANS) and parasympathetic (PANS) divisions (**Figure 2)**.

The differences between the two divisions are based on several criteria: the organisation of the preganglionic neurons, the locations of the ganglia that contain postganglionic neurons, the types and locations of innervated organs, the effects engendered in those organs, and the neurotransmitters used by the postganglionic neurons (Horn & Swanson, 2013). For example, while the ganglia of the SANS are distributed all along the length of the spine, the PANS projects only from the cranial and sacral parts of the spine. Additionally, while the SANS generally promotes arousal (the “fight-or-flight” response), the PANS promotes eating and mating behaviours. Thus, the two divisions of the ANS are commonly viewed as antagonistic in their effects on end-organs (Horn & Swanson, 2013; Westfall et al., 2018). In both divisions

of the ANS, preganglionic neurons synthesise and release ACh which acts on cholinergic receptors in postganglionic neurons in the ganglia (Horn & Swanson, 2013).

Figure 2: Organisation of the SANS and the PANS. Preganglionic neurons in the spinal cord and brainstem project to postganglionic neurons that in turn project to effector organs. ACh is the primary neurotransmitter for communication between the preganglionic neurons and postganglionic neurons in both divisions of the ANS and between postganglionic neurons and effector organs in the PANS. Horn & Swanson (2013). Adapted with permission.



Postganglionic neurons utilise various signalling molecules to affect the end-organs. A single neuron can simultaneously release several different neurotransmitters and a single type of neurotransmitter can act on different types of receptors, thus affecting multiple parallel mechanisms at both ends of the synapse (Horn & Swanson, 2013). However, the main neurotransmitter for parasympathetic postganglionic neurons is ACh, and noradrenaline is the main neurotransmitter for sympathetic postganglionic neurons. The release of ACh

activates receptors which – depending on the receptor type – excite or inhibit the innervated end-organs (Horn & Swanson, 2013).

The SANS innervates virtually every organ system in the body. The ganglionic neurons form synapses with blood vessels, the heart, airways, piloerector muscles (attached to hair follicles and responsible for goosebumps), salivary- and sweat glands. They also innervate the chromaffin cells of the adrenal medulla and thus modulate the release of adrenaline and noradrenaline which in the bloodstream act as hormones (Horn & Swanson, 2013). The PANS is equally widespread, although the individual ganglia usually map more precisely to single individual organs as opposed to diffusely innervating several organs. They do not innervate the skeletal muscles and the skin except in the head, where they regulate the vasculature of the jaw, lips, and tongue. The cranial outflow of the PANS controls pupillary size by innervating the ciliary muscles and the iris, production of tears by the lacrimal glands, and of saliva by the nasal, palatine, submaxillary, and sublingual glands. Additionally, it innervates the heart, lungs, liver, gall bladder, pancreas, stomach, small intestine, and more rostral sections of the gastrointestinal tract. The caudal outflow of the PANS innervates the large intestine, rectum, bladder, and reproductive organs (Horn & Swanson, 2013; Westfall et al., 2018) (**Figure 2, Table 1**).

Table 1: Responses of end-organs to muscarinic cholinergic innervation by the PANS. When several receptor subtypes are involved, the sequence represents the proportion of involvement from largest to smallest. When no subtype is given, it is yet to be identified. See section 1.3 for a discussion of receptor subtypes. Adapted from Westfall et al. (2018).

Organ	Structure/function	Effect	Receptor subtype
Eye	sphincter muscle, iris ciliary muscle lacrimal glands	contraction contraction secretion	M_3, M_2 M_3, M_2 M_3, M_2
Heart	sinoatrial node atria atrioventricular node ventricle	decrease in heart rate decreased contractility and action potential duration decrease in conduction velocity, decrease in contractility	mostly M_2 mostly M_2 mostly M_2 mostly M_2
Blood vessels	salivary glands	dilation	M_3
Endothelium		nitric oxide synthesis	M_3
Lung	tracheal and bronchial smooth muscle bronchial glands	contraction stimulation	M_2, M_3 M_2, M_3
Stomach	motility and tone sphincters secretion	increase relaxation increase	M_2, M_3 M_3, M_2 M_3, M_2
Intestine	motility and tone sphincters secretion gallbladder and ducts kidney	increase relaxation increase contraction	M_3, M_2 M_3, M_2 M_3, M_2 M
Urinary bladder	detrusor trigone and sphincter	contraction relaxation	M_3, M_2 M_3, M_2
Ureter	motility and tone	increase	M
Uterus		relaxation	M
Male sex organs		erection	M_3
Sweat glands		secretion	M_3, M_2
Pancreas	acini	secretion	M_3, M_2
Salivary glands	secretion of water and K^+	stimulation	M_3, M_2
Nasopharyngeal glands		secretion	M_3, M_2

The ANS also provides input to the enteric nervous system, a semi-autonomous division of the nervous system that regulates the propulsion and absorption of nutrients from the gastrointestinal tract from the oesophagus to the rectum, including the pancreas and the gall bladder. The enteric nervous system consists of afferent and efferent fibres and is organised into two plexuses that between them divide the functions of regulating muscle movement

and mucosal production (Horn & Swanson, 2013). ACh is crucial for the function of the enteric nervous system in two ways. First, the enteric ganglia receive cholinergic innervation from the PANS. Second, interneurons that distribute the input to the enteric nervous system, utilise ACh as their primary neurotransmitter (Westfall et al., 2018). In contrast to the skeletal muscles and neurons, some effector cells innervated by the ANS, such as the cells of the enteric nervous system and smooth cardiac cells, display intrinsic activity. This is exhibited as cycles of depolarisation much slower than those in neurons or skeletal muscle and can be regulated by parasympathetic stimulation. ACh usually leads to the opening of Na^+ and Ca^{2+} channels, causing partial depolarization, and promoting contractile activity (Westfall et al., 2018).

The ANS is also a conduit of afferent signals, as it carries information on the status of visceral organs that includes sensations on temperature and mechanical, thermal, or chemical tissue injury. These sensations are necessary for the completion of feedback loops that underlie the various peripheral reflexes, such as peristalsis and the micturition reflex (Horn & Swanson, 2013). While ACh does feature in these afferent projections, it is not the sole neurotransmitter responsible for signalling. The various signalling molecules involved have not been completely characterised, but they additionally include substance P, CGRP, SST, VIP, CCK, enkephalin, ATP, and others (Westfall et al., 2018).

While the present work focuses on the role of ACh as a neurotransmitter in the human nervous system, ACh is an evolutionary early organic molecule that other living beings, including bacteria, fungi, protozoa, and plants have evolved to employ (Wessler & Kirkpatrick, 2008). It thus should not be surprising that ACh is synthesised and released not only in human neurons but also in other cell types of the human body. Indeed, both endogenous ACh and ChAT-activity have been reported in immune cells, mesenchymal cells, epithelial cells, blood cells, the kidney, placenta, and many others. Additionally, not only neurons, but most human cell types – irrespective of the presence of cholinergic innervation – express cholinergic receptors (Wessler & Kirkpatrick, 2008). While many questions regarding non-neural functions of ACh remain, ACh seems to act as a mediator of auto- and paracrine regulatory

loops, whereby cells produce local signals to affect their functioning, and the functioning of neighbouring cells, respectively. For example, bronchial epithelial cells alter their shape, shrink, and detach from each other, thus changing their cytoskeleton, in response to ACh (Klapproth et al., 1998). In the epidermis, ACh facilitates differentiation and regulates the cell cycle, thereby directly affecting skin regeneration and wound healing (Kurzen et al., 2004; Wessler & Kirkpatrick, 2008). ACh also regulates the release of molecules from airway epithelial cells (Koyama et al., 1992) and regulates angiogenesis (formation of new blood vessels) (Heeschen et al., 2002). Furthermore, ACh can modify immune responses: T-cells, lymphocytes central to the adaptive immune response, produce ACh in response to viral infection and require it to migrate to infected tissues (Cox et al., 2020).

1.3 Cholinergic receptors

The multitude of functions supported by cholinergic neurotransmission is partly enabled through the variety in the molecular structure of receptors on which ACh acts. This section presents the consequent division of cholinergic receptors into receptor subtypes and describes their function and localisation in human organs.

In 1906, J. N. Langley observed that certain substances exerted substantial effects on muscle tissue, without acting directly on the nerves that innervated them (Glickstein, 2014, pp. 82-83; Langley, 1905). The structures upon which the substances acted are today understood as receptors - the mediators between the neurotransmitters and the effector cells. While Langley did not greatly speculate about the process of chemical transmission, the current terminology can often be traced back to him and his immediate successors. Henry Dale – a student of Langley's – observed that the drugs muscarine and nicotine partially mimicked the actions of ACh and that the relative effects of the two substances differed in strength between different types of tissues (Dale, 1914). Based on that observation, cholinergic receptors selective to either the one or the other drug, became known as *nicotinic* and *muscarinic*, respectively (Glickstein, 2014, p. 83). Both types of cholinergic receptors are widely expressed throughout the body.

Nicotinic cholinergic receptors (nAChRs) belong to the family of ligand-gated ion channels and, like all ion channels, affect the flux of ions across the plasma membrane, which is critical in regulating cellular function. Ligand-gated ion channels are activated when a specific ligand binds to a site in the channel that controls a central pore. They can be classified based on their structure, pharmacology, and ionic selectivity, with nAChRs selective specifically to ACh. When activated by the ligand, the receptor pore opens and – depending on its size and conformation – facilitates the flux of different molecules. In the case of nAChRs, the pore is relatively large (3 nm) and thus doesn't exhibit the high selectivity often seen in voltage-gated ion channels; this allows for the passage of more than one type of ion (Blumenthal, 2018). The binding of ACh to nAChRs results in a brief increase in permeability to primarily Na^+ and Ca^{2+} ions, which depolarises and excites the postsynaptic cell (Westfall et al., 2018). To date, 16 highly evolutionarily conserved genes have been identified to encode nAChRs subunits in mammals (Schaaf, 2014). A single receptor is made by the assembly of five subunits, which allows for multiple combinations. This results in several different forms of the receptor that may show different sensitivities to the same ligand. NAChRs are expressed in skeletal muscles, in the CNS, and the PNS (Westfall et al., 2018).

Muscarinic cholinergic receptors (mAChRs) are G protein-coupled receptors, a large family of transmembrane receptors. They couple to regulatory proteins called G proteins, which in turn regulate downstream effector proteins that include enzymes and ion channels. G protein-coupled receptors are integral membrane proteins, consisting of seven transmembrane segments and form a pocket that allows high-affinity binding of ACh from the extracellular space. Upon binding, conformational changes in the receptors occur, triggering internal signalling cascades that may lead either to excitation or inhibition (Bertrand & Wallace, 2020). The signalling cascades that are activated upon binding of ACh to mAChRs are slower than the permeability changes induced by nAChRs and do not necessarily involve alterations in ion permeability (Westfall et al., 2018). In addition, mAChRs can cause the endocytosis of various ion channels or the internalisation of receptors, and can regulate other pathways pertinent to cell growth, death, and physiology, including MAPK, phosphoinositide-3-kinase, RhoA, and Rac1 (Nathanson, 2008).

In mammals, mAChRs exist as five subtypes (M_1-M_5), each encoded by a separate gene (*CHRM1-CHRM5*). They are classified based on their localisation in tissue, molecular structure, and the type of intracellular signalling pathway they activate (**Table 2**) (Bertrand & Wallace, 2020). M_1, M_3, and M_5 activate the phospholipase C pathway, and promote the release of intracellular Ca^{2+}, while M_2 and M_4 inhibit adenylate cyclase, leading to a decrease in cellular cAMP and the inhibition of voltage-gated Ca^{2+} channels. Thus, while M_1, M_3, and M_5 generally excite the postsynaptic cell, M_2 and M_4 inhibit it (Bertrand & Wallace, 2020; Westfall et al., 2018).

Table 2: The mammalian mAChRs subtypes, the pathways/effects they engender, and their locations on the synapse. Summarised based on Bertrand & Wallace (2020) and Westfall et al. (2018).

Subtype	Pathway	Effect	Action type	Location
M_1	PLC-IP3/DAG-Ca^{2+}	activation of PKC and PLA2	excitatory	postsynaptic
M_2	adenylyl cyclase	decrease in cAMP	inhibitory	presynaptic autoreceptor
M_3	PLC-IP3/DAG-Ca^{2+}	activation of PKC	excitatory	postsynaptic
M_4	adenylyl cyclase	decrease in cAMP	inhibitory	presynaptic autoreceptor
M_5	PLC-IP3/DAG-Ca^{2+}	activation of PKC	excitatory	postsynaptic

The distribution of mAChR subtypes differs across tissues, with prominent roles in the CNS (see section 1.4) and PNS. M_1 is responsible for slowing the heart rate, glandular secretion, smooth muscle contraction (Eglen, 2005), and is additionally expressed in the prostate and the gastrointestinal tract (Lebois et al., 2018). M_2 is responsible for voltage-dependent changes in the contraction of the myocardium and is also expressed in the gastrointestinal tract, bladder, and submandibular gland (Harvey & Belevych, 2003; Lebois et al., 2018). Both M_3, and to a lesser extent M_2, are the primary mAChRs in the PNS. M_3 can be found in the gastrointestinal tract, glands, lung, pancreas, bladder, prostate, and testis (Lebois et al., 2018). M_4 acts to inhibit parasympathetic and sympathetic transmission (Trendelenburg et al., 2003) and is also present in the bladder and the testis (Lebois et al., 2018). Finally, M_5 is

expressed in the lung, bladder, testis, iris, oesophagus, and lymphocytes (Eglen, 2012; Lebois et al., 2018).

Human G protein-coupled receptors are targeted by almost a third of marketed drugs (Blumenthal, 2018). However, drug development for specific subtypes of mAChRs has been hampered by the highly conserved homology in the ligand-binding site, with drug candidates often producing side effects at other receptor subtypes (Bertrand & Wallace, 2020). Moreover, an individual cell can express several different receptor subtypes and the distribution of receptor subtypes will largely determine the downstream cascades activated by the binding of signalling molecules (Bertrand & Wallace, 2020; Westfall et al., 2018). The lack of specificity to receptor subtypes is pertinent to the effects of drugs known to regulate cholinergic activity: potentially non-selective binding of these compounds may cause a variety of unintended effects in different organ systems. As described in section 2.4, drugs with putatively antimuscarinic action are known to cause a variety of side effects in multiple organs, and their long-term use has been implicated in physiological changes that affect important health outcomes.

1.4 Cholinergic system in the brain

As in the PNS, cholinergic cells of the CNS release ACh to act on postsynaptic targets. However, cholinergic signalling in the CNS exhibits some distinct characteristics. The release of ACh in the CNS has classically been described as tonic and slow, with cholinergic neurons projecting diffusely throughout vast areas of the brain. It was thought that this anatomy allows for a modulatory role of the cholinergic system, where firing patterns or the nature of transmission in the postsynaptic cell is altered through cholinergic stimulation (Ballinger et al., 2016). While this notion of cholinergic innervation and the importance of slow and widespread effects of ACh in the CNS is still supported, it is now recognised that cholinergic projections and their targets can display topography in their anatomy and focal, fast release of ACh in their responding (Muñoz & Rudy, 2014). Both modes of innervation likely interact to produce the various effects that the cholinergic system exerts on cognitive functioning (Ballinger et al.,

2016). While ACh-signalling is widespread throughout vast areas of the brain, the section below focuses especially on the clusters of cholinergic neurons that have been most strongly associated with cognitive function.

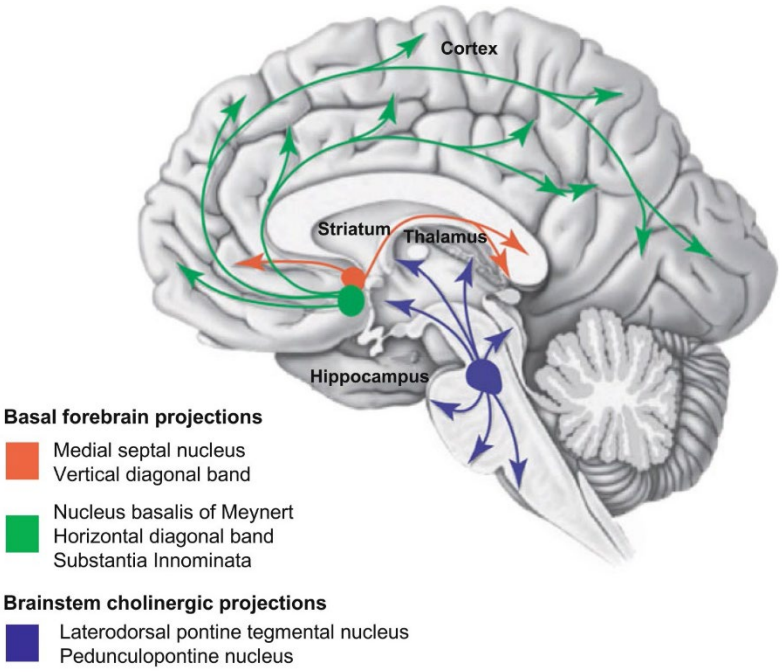
1.4.1 Central cholinergic pathways

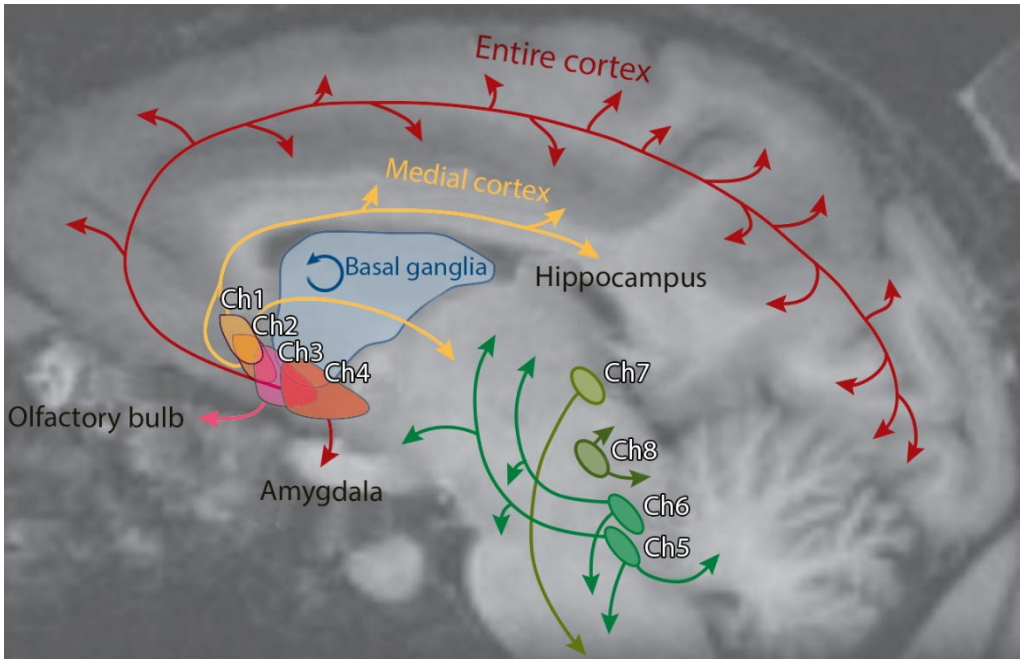
Cholinergic neurons in the CNS comprise two big groups: projection neurons that innervate other, sometimes very distant, brain areas, and interneurons that provide local connections within limited areas. Interneurons are most often associated with the modulation of dopaminergic signalling (Jett, 1998) and are located in multiple regions of the brain, including the striatum, nucleus accumbens, hypothalamus, and spinal cord (Schliebs & Arendt, 2006). The striatum is especially rich with ACh (Jett, 1998; Poppi et al., 2021): although cholinergic interneurons represent only ~1% of all striatal cells (Oorschot, 2013), their large axonal arbours release ACh across a wide area (Contant et al., 1996). Dysfunction of striatal cholinergic interneurons has been implicated in a range of developmental, psychiatric, and neurodegenerative disorders (Gonzales & Smith, 2015; Poppi et al., 2021).

Cholinergic projection neurons originate mainly from two regions: the basal forebrain and the brain stem (**Figure 3**); very few exist in the cerebral cortex, thalamus, amygdala, or hippocampus (Mesulam, 2013; Venkatesan et al., 2020). Cholinergic projections from the basal forebrain play an important role in arousal (Richerson et al., 2013). Additionally, as alluded to before, and described in further detail below (see section 1.4.3), the central cholinergic system of the basal forebrain has been implicated in a wide range of cognitive functions. Cholinergic projection neurons in the basal forebrain are located in four clusters named Ch1-4, indicating ChAT-containing neurons (Mesulam, 2013). These clusters can be found in the medial septum (Ch1), the vertical (Ch2) and horizontal (Ch3) limbs of the diagonal band of Broca, and the nucleus basalis of Meynert (NBM) (Ch4). The latter represents the largest of the areas of the basal forebrain. It also has the greatest number of neurons and has been further subdivided into several distinct sectors (Mesulam, 2013). The medial septum, the vertical, and the horizontal limbs of the diagonal band of Broca are more sparsely populated with cholinergic neurons, with Ch1, Ch2, and Ch3 neuronal populations

representing approximately 10%, 1%, and 70% of neurons in each region, respectively (Galvin et al., 2020).

Figure 3: Schematic representations of cholinergic projections in the brain. **Top**: basal forebrain- and brainstem cholinergic pathways in the human brain Bertrand & Wallace (2020). Adapted with permission. **Bottom**: cholinergic groups and their projections in the macaque brain Thiele (2013). Adapted with permission.





As alluded to previously, cholinergic projection neurons innervate most areas of the brain. Fibres from the basal forebrain terminate in most of the neocortex, the entorhinal cortices, hippocampus, amygdala, and olfactory bulb (Bertrand & Wallace, 2020; Richerson et al., 2013). Specifically, neurons of the medial septum and the vertical limb predominantly provide efferents to the hippocampus, parahippocampus, entorhinal cortex, hypothalamus, and cingulate cortex. The horizontal band (Ch3) projects to the olfactory bulbs, and the NBM (Ch4) sends projections to the amygdala, the rest of the neocortex, and may provide some innervation to the thalamus (Bertrand & Wallace, 2020; Chen & Mobley, 2019; Coyle et al., 1983; Mesulam, 2013; Richerson et al., 2013; Schliebs & Arendt, 2006). There is little overlap between axons of distinct cholinergic populations that project from the basal forebrain to the cortex (Venkatesan et al., 2020).

Two major pathways running from the basal forebrain to the cortex are the medial and lateral cholinergic pathways. The former joins the tract of the gyrus rectus and connects to the cingulum bundle to run above the corpus callosum, while the latter divides in two to separately travel within the external capsule and uncinate fasciculus on the one hand (capsular division), and within the claustrum and extreme capsule on the other (perisylvian division) (Bubb et al., 2018; Mesulam, 2013; Selden et al., 1998). The medial pathway innervates the parolfactory, cingulate, pericingulate, and retrosplenial cortices, while the two divisions of the lateral pathway innervate the frontal, parietal, and temporal neocortices (capsular division), and the frontoparietal operculum, insula, and superior temporal gyrus (perisylvian division) (Selden et al., 1998). While the overall topography of cholinergic innervation is not as differentiated as for instance in thalamocortical projections, projections from the basal forebrain to the cortex exhibit relatively high specificity. For example, individual cortical areas receive most cholinergic input from distinct sectors of Ch4 (Mesulam, 2013): from Ch4am to medial cortex, Ch4al to frontoparietal cortex and opercular regions, Ch4i to laterodorsal, frontoparietal, peristriate and midtemporal regions, and Ch4p to superior temporal and temporopolar regions (Mesulam et al., 1983). The high selectivity of cholinergic innervation contrasts with other neuromodulators, such as noradrenaline, that exhibit more divergent cortical projections (Kim et al., 2016). The sparser cholinergic neurons

in Ch1 and Ch2 project mostly within the fornix to innervate the hippocampus and within the ventral amygdalofugal pathway and stria terminalis to reach the amygdala (Selden et al., 1998).

In the pons and midbrain, cholinergic neurons are primarily found in ventrolateral and dorsomedial clusters called the pedunculopontine (Ch5) and laterodorsal tegmental (Ch6) nuclei, respectively. They provide input to the basal forebrain and most cholinergic projections to the thalamus, especially its relay- and reticular nuclei (Bertrand & Wallace, 2020; Richerson et al., 2013). Only minor cholinergic innervation is provided by the midbrain and pons directly to cortical areas (Mesulam, 2013; Schliebs & Arendt, 2006). Pontine and midbrain cholinergic neurons form part of the ascending arousal system (also known as the reticular activating system). This part of the brain is crucial for regulating arousal, and damage to it can lead to a loss of wakefulness (Liskowsky & Schliebs, 2006; Richerson et al., 2013). Finally, cholinergic neurons are also present within the medial habenula (Ch7) and the parabigeminal nucleus (Ch8), respectively (Oda & Nakanishi, 2000; Thiele, 2013). The medial habenula is a small, nAChR-rich (Sheffield et al., 2000) structure in the dorsal diencephalon that projects to the interpeduncular nucleus of the midbrain (Herkenham & Nauta, 1979). While some studies have suggested its involvement in memory, mood disorders, and addiction, more research is needed to determine its exact role (McLaughlin et al., 2017; Viswanath et al., 2013). The parabigeminal nucleus is a neuronal group in the lateral midbrain that contains up to 90% cholinergic neurons (Mufson et al., 1986). It projects to the superior colliculus and has been implicated in visual processing (Tokuoka et al., 2020).

While the regions described before represent the most prominent origins of cholinergic innervation in the brain, many of the cholinergic nuclei contain other types of nerve cells and nerve connections run in parallel with non-cholinergic nerve bundles. For example, despite being the most heavily cholinergic cluster of neurons in the basal forebrain, the NBM still contains 10% of non-cholinergic neurons; thus, the terms Ch4 (referring to cholinergic neurons) and NBM (referring to all neuronal components within the nucleus) are not synonymous (Liu et al., 2015; Mesulam, 2013). Similarly, midbrain- and pontine cholinergic

nuclei contain cholinergic and non-cholinergic neurons (Mesulam, 2013). Furthermore, within individual neurons, ACh is often co-localised with other neurotransmitters. For example, in the pedunculopontine nucleus, glutamate and GABA may be released along with ACh from the same nerve terminals. The exact distribution of different neurotransmitters within the pedunculopontine nucleus is dependent on the exact location within the nucleus, and in some areas, non-ACh neurotransmitters may even dominate (Mahaffey & Garcia-Rill, 2015).

1.4.2 Muscarinic receptor distribution

Similar to the PNS, the distribution of mAChR subtypes in the CNS (**Table 3**) differs among brain areas and is crucial for the local effects that result from cholinergic stimulation. The below summary is not intended as an exhaustive account of the distribution of different mAChR subtypes. It is intended to sensitise the reader to the variability within the cholinergic system and the complexity of processes that occur when the system is manipulated.

Table 3: Distribution of mAChR subtypes in the brain. Summarised based on Abrams et al. (2006), Bertrand & Wallace (2020), Eglen (2012), and Levey et al. (1991).

Brain region	**Receptor subtype**
Olfactory bulb	M_3, M_2
Neocortex, several areas	M_1, M_2, M_3, M_4
Hippocampus	M_1, M_2, M_3, M_4, M_5
Striatum	M_1, M_4, M_2
Amygdala	M_1, M_2, M_3
Thalamus	M_2, M_3, M_1
Hypothalamus	M_3, M_5
Ventral tegmental area	M_5
Pons	M_3
Cerebellum	M_2
Basal forebrain	M_2
Substantia nigra pars compacta	M_5, M_2

The current knowledge on the distribution of mAChRs in the brain has been achieved through various means, including the use of subtype-specific antibodies to determine mAChR protein levels, RNA profiling, radioligand binding techniques to establish receptor density, pharmacological studies that explored the effects of selective mAChR agonists, and transgenic animal models to probe behavioural effects. Most of the work on the principles of the

distribution of mAChRs has been done in rodents. However, whole-cell and field potential recordings of cortical neurons during epilepsy resection surgeries have broadly demonstrated substantial overlap of muscarinic activation between rodents and humans (Lebois et al., 2018).

M_1, M_2, and M_4 are the major mAChRs expressed in the brain, with M_3 and M_5 expressed at much lower levels (Lebois et al., 2018). Between 35% and 60% of all mAChRs in the human brain are of the M_1 type, making it the most common cortical cholinergic receptor (Flynn et al., 1995). It is expressed prominently in the frontal, temporal, parietal, and occipital cortices, the hippocampus, striatum, amygdala, and thalamus (Abrams et al., 2006; Bertrand & Wallace, 2020; Levey et al., 1991). M_1 receptors have been strongly implicated in processes of learning and memory (see section 1.4.3). M_2 is expressed as an inhibitory autoreceptor on presynaptic terminals throughout the brain, including the striatum, cerebellum, thalamus, NBM, and some limbic structures, including the amygdala and the hippocampus (Bertrand & Wallace, 2020; Fisher & Wonnacott, 2012). In the caudate-putamen, M_2 acts as a heteroreceptor, acting to regulate the effects of dopamine at the same nerve terminal. Based on genetic studies, the function of M_2 has been linked to major depression (Eglen, 2012). Additionally, while the exact nature of mAChR subtypes that mediate nociception has not been established, M_2 likely plays a predominant role in this process. Muscarinic agonists are often used as analgesics; in mice that lack both M_2 and M_4 receptors, the analgesic function of these agents has been shown to disappear (Wess et al., 2003). M_3 exhibits relatively low expression in the brain, constituting only 5-10% of all mAChRs (Levey et al., 1994). It is important in neurotransmitter regulation and is expressed in the hippocampus, cerebral cortex, striatum, and hypothalamus (Bertrand & Wallace, 2020; Eglen, 2012; Fisher & Wonnacott, 2012). While little is currently known on their exact function in the brain, M_3 receptors seem to regulate insulin secretion and may be central for promoting the release of growth hormone and longitudinal growth (Eglen, 2012; Gautam et al., 2009). Similar to M_3, M_4 is a regulatory autoreceptor, whose distribution largely overlaps with M_3. Due to its control of dopaminergic transmission in the striatum, it has been studied as a possible target for drugs to treat movement disorders such as Parkinson's disease, a neurodegenerative

illness predominantly characterised by motor symptoms (Bertrand & Wallace, 2020; Langmead et al., 2008). The increase in locomotor activity and in dopaminergic activity exhibited by transgenic mice lacking M_4 receptors (Gomeza et al., 1999) has also led to the development of selective M_4 antagonists for the treatment of the disease (Eglen, 2012). Finally, due to the antipsychotic effects of the mixed M_1/M_4 agonist xanomeline, M_4 has also been suggested as a target for schizophrenia (Eglen, 2012; Shekhar et al., 2008). M_5 is not widely expressed in the brain. While it can be found in the hippocampus, the cerebral cortex, and the striatum, it is especially prominent in dopaminergic areas, such as the ventral tegmental area and the substantia nigra. This distribution has led to suggestions that it might play a role in reward processing (Bertrand & Wallace, 2020; Fisher & Wonnacott, 2012). Indeed, M_5 knockout mice are less sensitive to the actions of addictive drugs (Fink-Jensen et al., 2003), although discrepancies have been reported in the literature (Eglen, 2012).

1.4.3 Cholinergic system in cognition

The involvement of the basal forebrain cholinergic system in cognition was first hypothesised over fifty years ago (Deutsch, 1971; Schliebs & Arendt, 2006). The proposition was soon corroborated by experiments that demonstrated impaired cognitive performance upon inhibition of cholinergic synapses in young people (Drachman & Leavitt, 1974). Since then, a plethora of experimental studies have upheld the initial findings (Schliebs & Arendt, 2006). In the past decade, novel methods of imaging, experimental stimulation of the cholinergic system, and the development of methods that selectively lesion only specific cholinergic pathways have enabled further insights into the possible role of the cholinergic system in cognition (Knox, 2016). In the following section, I describe the evidence for the role of the brain cholinergic system in memory in attention. I begin with findings from cellular- and systems-level neuroscience before moving unto behavioural work in animals. Most of the work on the cholinergic system in cognition has been done in non-human animals. Due to the importance of distinguishing between subdomains of memory and attention, I will briefly depict the predominant behavioural paradigms before describing the available evidence. This

will hopefully provide the reader with an appreciation for the breadth and complexity of cognitive processing to which the cholinergic system is thought to contribute.

1.4.3.1 Memory

Synaptic plasticity refers to the ability of neurons to change the strength of connections within and between neuronal networks in response to signalling. Synaptic plasticity is widely understood to be one of the cellular substrates of memory and hippocampal circuits are well-known for their susceptibility to this process (Ballinger et al., 2016). When applied exogenously or released endogenously, ACh profoundly alters synaptic plasticity of the hippocampus. Studies in cell cultures and hippocampal slices have demonstrated this alteration to be very time-sensitive and to depend on the coordination between cholinergic and glutamatergic inputs to the postsynaptic cell (Gu et al., 2012; Gu & Yakel, 2011). When the temporal relationship between these inputs changes, so does the type of synaptic plasticity that is induced (Gu et al., 2012). Cholinergic activity also changes the ratios of glutamatergic receptor types in postsynaptic neurons, which has implications for changes in synaptic plasticity (Mitsushima et al., 2013). Moreover, the timing of signalling also affects the types of receptors involved. For example, M_1 mAChRs have been implicated in long-term potentiation - the main form of synaptic plasticity (Gu et al., 2012; Gu & Yakel, 2011); mice genetically modified to lack M_1 mAChRs exhibit reduced long-term potentiation (Anagnostaras et al., 2003). However, this long-term potentiation is mediated by M_1 mAChRs 10ms after, but not 100ms before glutamatergic stimulation (Gu & Yakel, 2011). Finally, mAChRs might also be responsible for enhancing the excitability of axons that are responsible for transmitting the signal to other cells in the neuronal network (Martinello et al., 2015).

At the network level, the balance between oscillations of different frequencies of hippocampal activity has been implicated in synaptic plasticity and memory. Shifts in oscillations of neuronal firing have been shown to occur in situations when learning might occur (Douchamps et al., 2013), with different frequencies correlating with behavioural performance on memory tasks (Hasselmo & Stern, 2014). Cholinergic activity can induce oscillations in hippocampal neurons and modulate their strength, with inhibition of mAChRs

leading to disruptions in these oscillations (Dannenberg et al., 2015; Douchamps et al., 2013; Newman et al., 2014). Thus, at both the cellular- and network levels, evidence exists for the modulation by ACh of processes implicated in memory and learning.

When using behavioural paradigms, memory is mostly classified based on time or content (Graef et al., 2011). Time-based distinctions include sensory, short-term, and long-term memory, with some researchers equating working memory with short-term memory. However, the former is sometimes understood to additionally involve attentive and perceptive abilities that enable not only the storage but the simultaneous manipulation of stored memory (Baddeley, 1991). In animals, working memory is most often tested using the matching-to-sample test and maze tests, particularly the radial arm maze test (Graef et al., 2011). In the matching-to-sample test, the animal is presented with a "sample" stimulus and must afterwards respond when this stimulus is presented in a sequence that includes non-sample stimuli. Thus, the animal must retain the sample stimulus in short-term memory and match it to sequentially presented stimuli. In the radial arm maze test, food is stored at the end of one of several radially extending corridors of a maze. The animal must then explore the individual corridors, always returning to the centre of the maze. Thus, it must remember the corridors which it has already visited, so as not to explore them again (Graef et al., 2011).

Short-term and working memory have been shown to involve many areas of the cortex, including prefrontal, frontal, and posterior areas (Miller et al., 2018). Studies suggest that cholinergic signalling may play a profound role in this process. In experiments probing short-term memory in either non-human primates or rodents, muscarinic antagonists have been shown to reduce performance in matching-to-sample- (Granon et al., 1995; Spinelli et al., 2006), maze- (Beatty & Bierley, 1985; Kay et al., 2010; Kobayashi et al., 1995; Wirsching et al., 1984), and spatial alternation tasks (Bymaster et al., 1993) in which animals must alternate between spatial locations. The antimuscarinic effects in short-term memory seem to be relatively independent of their role in attention, as some studies on non-human primates show specific and selective deficits to tasks that require short-term memory when cholinergic innervation of the prefrontal cortex is ablated (Croxson et al., 2011). Additionally, AChE

inhibition by physostigmine – which enhances cholinergic function – has been shown to reverse the short-term memory deficits induced by muscarinic inhibition (Rupniak et al., 1991). There is also evidence for the involvement of cholinergic mechanisms in short-term memory from studies in humans: physostigmine positively affects the *encoding* of short-term spatial (Kukolja et al., 2009) memory, working memory for faces (Bentley et al., 2009; Kirrane et al., 2001), and possibly increases the efficiency of information processing (Furey et al., 1997). Moreover, systemic muscarinic inhibition of mAChRs has been associated with impairments in several tasks designed to probe working memory, including matching-to-sample task (Koller et al., 2003), n-back task (Green et al., 2005), and tests of spatial working memory (Ellis et al., 2006). Interestingly, short-term memory retrieval is associated with *reduced* cholinergic activity (Kukolja et al., 2009), suggesting a more complex role for the cholinergic system in short-term mnemonic processes. Both M_1 and M_2 cholinergic receptors seem to be linked to performance in tasks assessing short-term memory (Graef et al., 2011): although several authors have found M_1 agonism to improve, and M_1 antagonism or M_1-encoding gene knockout to disrupt performance of working memory in animal studies (Anagnostaras et al., 2003; Brandeis et al., 1995; Iwata et al., 2000; Nakahara et al., 1989; Ohno et al., 1994; Popelikova et al., 2018), research also suggest a role for M_2 receptors or a combination of both receptor subtypes in working memory performance (Ohno et al., 1994; Seeger et al., 2004).

The content-based distinction of memory applies to long-term memory, which can be differentiated into declarative and procedural memory (Cohen & Squire, 1980). While declarative memory includes semantic memory (factual knowledge) and episodic memory (memory of events and experiences), procedural memory is the result of habit learning or conditioning and is acquired through practice (Graef et al., 2011; Milner et al., 1998). In animals, the radial arm maze can also be used to study declarative memory. While the memory of already visited arms is thought to represent working memory, long-term declarative memory is reflected in the animal's ability to remember which arm contained the food in previous trials (Graef et al., 2011). Another commonly used task to study declarative memory in animals is the Morris water maze (Morris et al., 1982), where the animal is placed

in a pool of water, in which a hidden platform is positioned a few millimetres below the surface. By using visual cues placed on the walls of the maze, throughout several trials, the animal learns the spatial location of the hidden platform. Tasks probed with the Morris water maze and the radial arm maze test are understood to require spatial memory, which is dependent on an internal representation of physical space and involves spatial navigation based on that internal representation (Solari & Hangya, 2018).

Studies in non-human animals utilising these paradigms have found surprisingly little evidence for a role for the cholinergic system (Graef et al., 2011). However, in rodents, low cholinergic tone during slow-wave sleep has been shown to provide an essential environment for the consolidation of declarative memory (Gais & Born, 2004), while pharmacological blockade of cholinergic transmission during sleep stages characterised by high cholinergic tone impairs the consolidation of declarative memory (Rasch et al., 2009). This suggests that regulation of cholinergic activity during sleep is critical for the formation of declarative memories. For humans, a rich literature reports on the association between muscarinic antagonism and memory performance (Graef et al., 2011). Muscarinic blockade with hyoscine has been shown to impair paired-associate learning, episodic verbal memory (Bishop et al., 1996; Kamboj & Curran, 2006), as well as visual recognition (Sherman et al., 2003), with mixed evidence for the effects on semantic memory (Graef et al., 2011). While few studies have probed the role of distinct receptor subtypes, putative evidence suggests a role for M_1 mAChRs in these processes. M_1 mAChR knockout mice exhibit slower learning of cognitive tasks (Gould et al., 2015), while two randomised controlled trials (RCTs) found biperiden, a selective M_1 mAChR antagonist to impair episodic declarative memory in healthy human participants (Borghans et al., 2017; Wezenberg et al., 2005).

Emotional memory represents the most common subtype of procedural memory studied in animals. Two common strategies to explore emotional memory are fear conditioning and passive avoidance learning (Graef et al., 2011). In the former, an unconditioned aversive stimulus is paired with a conditioned stimulus. The latter could either be an arrangement of objects in space (contextual fear conditioning) or a cue (cued fear conditioning). In pairing this

conditioned stimulus with an aversive unconditioned stimulus, the animal learns to exhibit the same behaviour (e.g., freezing or startle reflex) to either stimulus. In passive avoidance learning, an aversive event is predictable and contingent on a certain behaviour by the animal; this could involve the active exhibition or inhibition of a behaviour to prevent the appearance of the aversive event (Graef et al., 2011).

Fear memory is a complex behaviour that relies on multiple areas of the brain, including the hippocampus, amygdala, and neocortex. Contextual fear conditioning involves processing in both the hippocampus and the amygdala, whereas cued fear conditioning mainly relies on the amygdala (Phillips & LeDoux, 1992). In animal experiments, pathways between the basal forebrain cholinergic system on the one hand, and the hippocampus, amygdala, and various cortical areas on the other, have been implicated in fear learning (Graef et al., 2011; Knox, 2016). The basal lateral amygdala receives the densest cholinergic innervation of any structure in the brain (Ballinger et al., 2016). Lesions of cholinergic neurons projecting from the NBM to the amygdala have been demonstrated to disrupt passive avoidance learning (Power & McGaugh, 2002b) and Pavlovian contextual fear conditioning (Power & McGaugh, 2002a). The disruption of passive avoidance learning is attenuated by the administration of mAChR agonists in the amygdala, which also enhances consolidation of Pavlovian fear conditioning (Power & McGaugh, 2002a). These results suggest that mAChR-mediated cholinergic transmission is crucial for the acquisition and consolidation of contextual fear memory. Furthermore, research using optogenetics – where neuronal transmission is experimentally manipulated by light – has also suggested a role for cholinergic NBM-amygdala projections in cued fear memory (Jiang et al., 2016).

As opposed to relatively delineated fibres connecting the NBM to the amygdala, NBM projections to the cortex are wide-ranging and include targets implicated in emotional learning, including the anterior cingulate and prelimbic cortices. Indeed, lesions of cholinergic projection neurons from the NBM to the cortex have been shown to attenuate both cued and contextual fear conditioning, thus demonstrating a role for cholinergic projections from the NBM to the cortex (Knox, 2016).

Hippocampal neurons receive cholinergic input almost exclusively from the medial septum and the diagonal band of Broca, and this innervation seems to play a role in Pavlovian contextual fear conditioning (Knox, 2016). This is suggested by many lines of evidence from experiments on rodents: hippocampal ACh levels increase during Pavlovian fear conditioning (Calandreau et al., 2006; Nail-Boucherie et al., 1998), while blockade of hippocampal mAChRs disrupts acquisition (Rogers & Kesner, 2004; Wallenstein & Vago, 2001) and consolidation (Izquierdo et al., 1992; Wallenstein & Vago, 2001) of Pavlovian contextual fear conditioning. Furthermore, contextual fear conditioning is enhanced by the infusion of physostigmine into the hippocampus (Rogers & Kesner, 2004) or by optogenetic stimulation of the basal forebrain (Hersman et al., 2017). Results supporting the importance of the pathway between the basal forebrain and the hippocampus in fear memory have also been demonstrated in experiments utilising avoidance learning. Disruption of avoidance learning through the inhibition of neural activity in the medial septum is reversed by enhancing the release of ACh in the hippocampus (Degroot & Parent, 2001), while mAChR inhibition in the hippocampus disturbs the acquisition (Khakpai et al., 2012; Nail-Boucherie et al., 1998) and consolidation (Zarrindast et al., 2012) of contextual avoidance memory, as well as long-term avoidance memory (Parfitt et al., 2012). Both M_1 and M_2 mAChR subtypes have been shown to play a role in passive avoidance learning (Graef et al., 2011). Considering that selective inhibition of M_1 receptors affects contextual fear conditioning, but not tone fear conditioning (Fornari et al., 2000), M_1 seems to be selectively responsible for hippocampus-dependent tasks that involve aversive motivation.

1.4.3.2 Attention

Attention can also be divided into distinct processes, e.g., divided- and sustained attention that capture the ability to process several pieces of information at a time, and the ability to focus on a task for long periods, respectively (Graef et al., 2011). Divided attention in animals can be studied using the cross-modal divided attention paradigm (Turchi & Sarter, 1997). The animals are taught to respond to stimuli in separate modalities (e.g., visual and auditory) and are then subjected to a randomised succession of trials in which either modality could be

presented. Response times in the cross-modal condition are usually longer than in the unimodal condition, where only one modality is presented. The common sustained attention test in animals is a task that consists of a random order of cued and blank trials. Responses in the former and suppression of responses in the latter are rewarded. This results in four different types of responses: hits, correct rejections, misses, and false alarms (Hasselmo & Sarter, 2011). Attention can also be divided into cue- and goal-driven attention. While the former is involuntary and is driven by stimuli from the bottom-up, the latter is driven by voluntary and modulated top-down by cortical areas. Top-down attention is the best studied of the two, as well as more pertinent to cognition, as it involves conscious attentional control (Ballinger et al., 2016).

The behaviourally different forms of attention have distinct neural correlates, as they are differentially affected by selective lesions of cholinergic neurons of the basal forebrain (Baxter & Chiba, 1999). The evidence for a role of the latter in attentional processing comes from multiple sources of animal experiments that include pharmacological, optogenetic, and lesion studies (Ballinger et al., 2016). The current evidence suggests the importance of particularly M_1 mAChRs in top-down attention (Gould et al., 2015). Attentional modulation by the cholinergic system is thought to be mediated by cholinergic projections to two broad cortical areas: the prefrontal cortex and the sensory cortex (Ballinger et al., 2016). The prefrontal cortex is composed of several regions, including the cingulate, medial prefrontal, lateral prefrontal, and orbitofrontal cortices. It represents a prominent target of cholinergic innervation from the NBM and the diagonal band of Broca, and several lines of evidence in animals indicate the importance of cholinergic signalling in this region (Ballinger et al., 2016; Chandler et al., 2013). There are several reasons why cholinergic projections from the basal forebrain to the prefrontal cortex are thought to play a role in attention. First, ACh concentrations in the prefrontal cortex have been shown to increase during behaviours that indicate the perception of a relevant cue by the animal (Howe et al., 2013; Parikh et al., 2007). In cue-detection tasks in humans, analogous behaviours were associated with activation in the prefrontal cortex (Howe et al., 2013). Moreover, convergent evidence from several human imaging studies suggests that inhibition induced by AChE facilitates stimulus-induced

increases in brain activity, especially in the prefrontal cortex (Hasselmo & Sarter, 2011). Second, optogenetic stimulation of the basal forebrain improves the detection of cues and increases false positive responses in the sustained attention task (Gritton et al., 2016). Third, in the cross-modal divided attention paradigm, blockade of ACh transmission increases the number of missed cues (Gritton et al., 2016), but does not increase the accuracy in blank trials (Hasselmo & Sarter, 2011). All this indicates that ACh release is an essential signal for cue detection.

The sensory cortex is composed of several brain regions responsible for processing various aspects of sensory information from different modalities. This region is hypothesised to support attentional processes by increasing the signal-to-noise ratio through the selective increase in the firing of neurons relevant to the task at hand, and the simultaneous "desynchronisation" of cortical noise (Ballinger et al., 2016). It has been demonstrated that this desynchronisation is mediated by cholinergic signals from the basal forebrain to various areas of the sensory cortex (Chen et al., 2015; Goard & Dan, 2009; Kalmbach & Waters, 2014; Pinto et al., 2013). Optogenetic inhibition of cholinergic neurons of the basal forebrain in rodents reduces the response reliability of sensory neurons, whereas the application of ACh improves the ability of the cortex to discriminate between stimuli (Runfeldt et al., 2014). In humans as well, inhibition of mAChRs has been associated with impairments in performance on sustained attention (Graef et al., 2011).

1.4.3.3 ACh in cognition: summary

In summary, several lines of evidence converge to suggest a role for the cholinergic basal forebrain in cognitive processing. As described before, ACh processing alters during cognitive tasks and affects cellular and network processes implicated in cognition. Also, manipulations of cholinergic activity produce parallel alterations in cognitive ability. Furthermore, pharmacological blockade of the cholinergic system consistently impairs cognitive functioning. Disruptions of cognitive processing due to inhibition of the cholinergic system can be ameliorated by the administration of cholinergic agonists. However, while the

literature on the topic strongly supports a role for muscarinic signalling in cognitive function, several caveats are worth noting.

First, as mentioned before, cholinergic innervation – especially as part of the ascending arousal system – plays an important role in arousal and shows activity patterns that correlate with the subject's state of wakefulness or sleep stage. Arousal exhibits an inverted-U function with cognitive performance under experimental conditions. Thus, substances that manipulate the activity of the cholinergic system can also affect arousal levels that may mediate the relationship between cholinergic manipulation and cognition (Graef et al., 2011). Indeed, the observation that both hypo- and hypercholinergic signalling in the human brain negatively affect cognitive performance has been reported (Bentley et al., 2011).

Second, animal lesion studies have frequently failed to produce the same impairments in cognition as does pharmacological cholinergic inhibition (Knox, 2016; Parent & Baxter, 2004). Selective – as opposed to systemic – lesions of cholinergic septohippocampal projections often do not impair a variety of cognitive tasks, including performance in the water maze, radial arm maze, and contextual fear conditioning. When impairments do occur after these lesions, they are often small in magnitude (Parent & Baxter, 2004). Several explanations have been suggested for this. First, small doses of neurotoxins – necessary to avoid off-target effects (Solari & Hangya, 2018) – cause selective lesions of cholinergic pathways where only about 70%-90% of cholinergic neurons are ablated (Parent & Baxter, 2004). However, up to 95% of those cells may need to be destroyed to produce measurable cognitive deficits (Baxter & Chiba, 1999). Second, it has been suggested that cholinergic pathways may compensate for each other and that several pathways (e.g., both hippocampal and cortical afferents) would need to be ablated to produce an impairment (Parent & Baxter, 2004). Moreover, it is possible that the basal forebrain cholinergic system is involved, but not necessary, for cognitive function (Parent & Baxter, 2004). For example, cholinergic neurons might play a modulating role in cognition that is not readily assessed with standard cognitive tasks. Third, cholinergic projections may be required for the performance in only specific tasks (Parent & Baxter, 2004). For example, while affecting the performance in tasks of fear conditioning that require

the animal to respond to a stimulus, the cholinergic projections from the NBM to the cortex do not seem to mediate Pavlovian fear conditioning that does not require an active response (Knox, 2016).

Finally, several subtypes of both mAChRs and nAChRs are distributed widely throughout the brain and studies that probe the cholinergic system often do not study a single receptor type in isolation. The lack of selective agonists and antagonists for mAChRs (Hasselmo & Sarter, 2011) renders such research highly difficult to perform. Thus, although in the previous section I write about the role of predominantly mAChRs in cognition, both types of receptors likely interact to produce or modulate cognitive function (Ballinger et al., 2016; Graef et al., 2011; Knox, 2016; Venkatesan et al., 2020).

The research on the role of the cholinergic system in cognition also reveals the high number of defined cognitive domains, which reflects the neural complexity of cognitive functioning. The role of ACh has been demonstrated for some of these domains. However, as the current section expounds, the strength of the evidence and the exact role of cholinergic processing may differ between brain regions and between cognitive domains. This has important implications for the experimental study of cognitive outcomes. The effect of an exposure may affect the performance in a cognitive task – reflecting the relative importance of that exposure on processing in a particular area of the brain – but not translate to demonstrable changes in the performance of another cognitive task. Thus, care must be taken when performing such studies in precisely specifying the cognitive domain under study. Furthermore, when available, several cognitive tests can be combined to generate a composite score of cognitive function. While more difficult to map unto distinct brain areas, measures of general cognitive ability are less susceptible to random noise and are good predictors of several health and social outcomes (Batty et al., 2007; Calvin et al., 2017; Farmer et al., 2019; Twig et al., 2018).

1.4.4 Cholinergic system in Alzheimer's disease

The importance of cholinergic processing in cognitive processes is also reflected in disorders of the brain. In the section below, I illustrate this point by focusing on AD, the most common cause of dementia. I begin by describing the features of AD, its epidemiology, and its neural correlates, and resume by linking the hallmark features of the disease to dysfunction of the cholinergic system.

AD is a progressive neurodegenerative disorder that affects wide areas of the brain. The late stage of AD is characterised by dementia (Alzheimer dementia), an impairment of cognitive function that interferes with activities of daily living (Scheltens et al., 2016). While the initial symptoms and the pace of progression vary among individuals, most people affected by Alzheimer dementia initially present with a gradual worsening of their ability to remember new information. As the disorder progresses, other cognitive domains become affected, including planning and problem solving, understanding visual images and spatial relationships, speech, and judgement of social situations (Alzheimer's Association, 2015). Due to the ageing world population and the lack of treatments that could alter the clinical course of the disease, AD represents one of the most urgent healthcare challenges today. The epidemiology of AD is notoriously difficult to study due to differences between samples and the presence of confounding variables (Masters et al., 2015). However, based on a mean incidence of 1-3% in the population over 65 years of age in western countries (Bachman et al., 1993; Evans et al., 2003; Hebert, 1995; Kawas et al., 2000), the current estimates of prevalence rates range from 10-30% (Masters et al., 2015). Moreover, the total number of people diagnosed with AD worldwide is projected to increase to 152 million by 2050 (Livingston et al., 2020).

While shared family history of the disease is common, most cases of AD are sporadic, with less than 1% of cases autosomal dominant inherited (Masters et al., 2015). The autosomal dominant inherited form results in an early onset of the disease, usually starting in the 40s. Sporadic AD usually starts late in life, with age the major risk factor. It unfolds over 8-10 years

post-diagnosis, with the symptomatic period preceded by a prodromal stage that may last several decades (Masters et al., 2015).

AD causes brain atrophy and other abnormalities of the brain tissue that can be detected by brain structural magnetic resonance imaging (MRI) (Chandra et al., 2019). Atrophy is first observed in the medial temporal lobe, including the entorhinal cortex and hippocampus, the volumes of which are reduced by over a quarter in people with AD (Du et al., 2004; Pennanen et al., 2004). As the disease progresses, limbic structures become affected, including the amygdala, the olfactory bulb, cingulate gyrus, and the thalamus (Cavedo et al., 2011; de Jong et al., 2008; Guo et al., 2010; Thomann et al., 2009). Later in the disease, atrophy spreads to the cortex, with frontal, parietal, and temporal regions exhibiting the greatest decreases in volume (de Jong et al., 2008; Du et al., 2004; Duarte et al., 2006; Kilimann et al., 2014). Additionally, atrophy affects the primary olfactory cortex, cerebellum, and brainstem (Roy et al., 2016; Tabatabaei-Jafari et al., 2017; Vasavada et al., 2015). Brain structural MRI has also revealed an increase in white matter hyperintensities that indicate the loss of axons and demyelination. In individuals with AD, white matter hyperintensities in the frontal lobes are increased (Capizzano et al., 2004) and correlate with hippocampal atrophy (de Leeuw et al., 2004).

Two hallmark neuropathological features are found in the brains of people with AD: plaques of amyloid-β (Aβ) and tau neurofibrillary tangles (NFTs) (Serrano-Pozo et al., 2011). Amyloid plaques are extracellular deposits of Aβ, a peptide that is a by-product of cellular metabolism. Aβ is derived from β-amyloid precursor protein (APP), a compound whose role in the body is unknown. This “amyloidogenic cleavage” occurs in two steps: (1) an initial cleavage by β-secretase to generate distinct secreted and membrane-bound segments of APP; and (2) a subsequent cleavage of the membrane-bound APP by γ-secretase to yield the APP intracellular domain and Aβ. Alternatively, APP can undergo non-amyloidogenic cleavage by α-secretase which precludes the production of Aβ (Jiang et al., 2014). NFTs are extra-neural aggregates of tau, a microtubule-associated protein. Tau is an essential neural component, as it facilitates axonal transport by binding to and stabilising the neuronal cytoskeleton. In AD,

tau gets translocated, hyperphosphorylated, misfolded, and aggregates to form NFTs (Serrano-Pozo et al., 2011). Both Aβ plaques and NFTs exhibit a characteristic temporal evolution and a distinct regional distribution in AD (Chen & Mobley, 2019). Aβ deposition starts in the frontal, temporal, and occipital lobes, afterwards spreads to the hippocampus, the amygdala, and the entorhinal, insular, and cingulate cortices; this is followed by the involvement of various subcortical structures. NFTs follow a different pattern of deposition in the brain, starting in the medial temporal cortex and then progressing to involve most of the neocortex. It is not entirely clear to what extent Aβ and NFTs each contribute to the cause and progress of AD or how they interact with other pathological features (Chen & Mobley, 2019).

In the late 1970s, evidence emerged of a deficiency of cholinergic markers in the brains of people with AD. This included reductions in ChAT activity in the amygdala, hippocampus, and cortex (Bowen et al., 1976; Davies & Maloney, 1976). Moreover, the cortical areas exhibiting the greatest reductions also contained the greatest density of NFTs (Davies & Maloney, 1976). It was later shown that the reduction in these markers – including ChAT and ACh itself – were positively correlated with the clinical severity of dementia (Bierer et al., 1995; Bubser et al., 2012).

The cognitive deficits in AD can be attributed primarily to abnormalities in the cortex and hippocampus (Coyle et al., 1983). Whereas cholinergic cells in the pontine reticular formation are relatively preserved in the disease (Mesulam, 2013; Schliebs & Arendt, 2006) a wealth of evidence indicates that the function of cholinergic neurons in the basal forebrain is compromised. First, in the early stages of the disease, biochemical markers for ACh are reduced in the brain, a change not consistently observed for markers for many other neurotransmitters, including noradrenaline, serotonin, and GABA (Coyle et al., 1983; Francis et al., 1999; Price et al., 1985). Neurons of people with AD exhibit decreased activity of ChAT (Bartus et al., 1982), and a reduced velocity in the uptake of choline (Rylett et al., 1983). During the disease, the release of ACh is reduced (Nilsson et al., 1986) and the NBM exhibits substantial degeneration (Whitehouse et al., 1981; Whitehouse et al., 1982) that is faster than

the rates of global brain atrophy (Grothe et al., 2013) and that likely precedes memory impairments and atrophy of neighbouring brain structures (Fernández-Cabello et al., 2020; Schmitz et al., 2016). Additionally, mAChRs exhibit alterations in AD: with the progression of the disease, the number of M_2 receptors decreases (Mash et al., 1985), while M_1 receptors may become dysfunctional (Jiang et al., 2014). Finally, the degree of cholinergic dysfunction (Perry et al., 1978) and the extent of phosphorylated tau in the NBM (Mesulam, 2004) have been found to correlate with the severity of cognitive deficits in AD. The extensive involvement of cholinergic networks in AD has led some (Bartus et al., 1982; Coyle et al., 1983) to propose that basal forebrain cholinergic neurons play a pivotal role in the disorder (the so-called *cholinergic hypothesis* of ageing and AD).

The degeneration of cholinergic neurons in the basal forebrain has been observed for several other disorders that often result in dementia or include dementia as a major symptom, including traumatic brain injury (Salmond et al., 2005), Parkinson's disease (Jellinger, 1991; Schulz et al., 2018), Creutzfeldt-Jacob disease (Arendt et al., 1984), and Korsakoff's syndrome (Arendt et al., 1983). However, for AD, there is evidence of a specific relationship between major histopathological markers of the disorder and cholinergic neurotransmission. The lines of evidence for such a relationship are as follows:

- First, cholinergic processing of the basal forebrain seems to regulate the processing of APP: cholinergic stimulation upregulates APP secretion (Müller et al., 1997), and drugs that inhibit the action of AChE (AChE-Is) increase the secretion of APP in rat brain slices (Mori et al., 1995) and cell culture (Racchi et al., 2001). Additionally, the deletion of M_1 mAChRs in transgenic mice increases the processing of the amyloidogenic form of APP (Davis et al., 2010).
- Second, cholinergic synapses are prominently characterised or affected by both hallmark features of AD: tau pathology is present in cholinergic basal forebrain neurons of people with mild cognitive impairment and with AD (Ginsberg et al., 2006), while Aβ has been shown to induce neurodegeneration at cholinergic synapses (Auld et al., 2002; Doležal & Kašparová, 2003). Moreover, Aβ plaques at autopsy are

negatively correlated with pre- and postsynaptic markers of cholinergic function, with this relationship even more pronounced in people with AD (Potter et al., 2011).

- Third, AChE co-localises with Aβ deposits in the brain of people with AD (Morán et al., 1993). Combined with its effects on APP processing (Mori et al., 1995; Racchi et al., 2001), it suggests a role for AChE in the pathogenesis of AD. Moreover, there is evidence of a direct influence of AChE on the deposition of Aβ plaques. This is supported by findings of an association of AChE activity with Aβ plaques (Apelt et al., 2002), of AChE promoting the aggregation of Aβ under some conditions (Alvarez et al., 1997; Bartolini et al., 2003), and of increased neurotoxicity of Aβ when AChE associates with it (Reyes et al., 2004). Furthermore, transgenic mice overexpressing human AChE exhibit accelerated formation of plaques that remain elevated when compared to wild-type animals (Rees et al., 2003).
- Finally, cholinergic signalling and its downstream effects have been shown to regulate aspects of AD neuropathology, as inhibition of ACh increases the levels of Aβ in animal models (Liskowsky & Schliebs, 2006). Selective activation of M_1 receptors decreases the quantity of Aβ in the cerebrospinal fluid of people with AD (Hock et al., 2003; Nitsch et al., 2000), while activation of the downstream PKC-pathway decreases cellular production of Aβ (Hung et al., 1993). Furthermore, activation of mAChRs has sometimes been shown to prevent tau phosphorylation (Hampel et al., 2018; Sadot et al., 1996).

Based on the findings of altered cholinergic neurotransmission in AD, the apparent involvement of mAChRs, and the evidence of interplay with pathological markers, pharmacological treatments have been developed that aim to stimulate cholinergic transmission. One avenue of research has involved the development AChE-Is that effectively prevent the breakdown of ACh and increase the duration of its activity. All UK-licensed therapies for AD except memantine (an NMDA receptor antagonist) – donepezil, rivastigmine, and galantamine – are AChE-Is (Masters et al., 2015).

The use of AChE-Is has been shown to improve cognitive and behavioural manifestations of AD (Hansen et al., 2008; Massoud & Gauthier, 2010), and has been associated with reductions in brain atrophy, changes in blood perfusion, and increased activation in the frontal cortex (Bentley et al., 2008; Hampel et al., 2018). However, the effects of AChE-Is on the deficits in memory and other cognitive functions in dementia have been limited (Amenta et al., 2001; Marucci et al., 2021). Most clinical studies have demonstrated AChE-Is to induce improvements mainly in mild- to moderate stages of the disease and within the first few months after starting treatment; results for severe stages of AD and those obtained years after starting treatment have been mixed (Marucci et al., 2021). Furthermore, AChE-Is can induce unwanted side effects, including nausea, sweating, salivation, and gastrointestinal problems. These side effects are thought to occur due to the enhanced activation of all mAChRs subtypes both centrally and peripherally and have often led to treatment discontinuation (Marucci et al., 2021).

A cholinergic treatment for AD that has been trialled as an alternative to AChE-Is involves mAChR agonists that directly alter mAChR signalling. However, results from clinical studies using mAChR agonist to treat AD and related disorders of cognition have been disappointing. Most drugs exhibited low efficacy and due to a lack of sufficient subtype selectivity engendered various off-target effects in the periphery (Bruno, 1986; Eglen, 2012). For example, xanomeline, a potential cognitive enhancer designed to activate M_1- and M_4 receptors, exhibited excitation of parasympathetic M_2 and M_3 subtypes, causing various cardiovascular and gastrointestinal side effects (Bodick et al., 1997). Selective regulators of M_2 mAChRs have met a similar fate. After the initial findings of decreases in cholinergic transmission and accompanying impairments in memory associated with activation of M_2 receptors, M_2 antagonists were explored as agents to improve memory and learning in disorders affecting cognition (Bertrand & Wallace, 2020; Langmead et al., 2008). However, analogous to M_1 agonists, off-target effects included both effects at other receptor subtypes in the CNS, as well as cardiovascular complications following binding in the PNS (Bertrand & Wallace, 2020; Langmead et al., 2008). The difficulty in developing highly selective mAChR agonists lies in a shared, highly conserved binding site for ACh. In response to this, some

groups started developing ligands that activate mAChRs by binding to other sites of the receptor molecule, so-called allosteric sites. Allosteric agonists activate the receptor directly without the need for ACh, while positive allosteric modulators (PAMs) bind to the allosteric site and enhance the endogenous function of Ach (Bubser et al., 2012). Several recently developed allosteric modulators were successfully tested in preclinical studies but have mostly not progressed past early clinical trials (Felder et al., 2018).

In summary, several lines of evidence suggest a consistent association between abnormalities of central cholinergic function and dementia, with cholinergic processing in especially AD possibly tightly linked to underlying neurodegenerative processes and resultant cognitive declines. Some theories (Bartus et al., 1982) have even argued that this aspect represents the hallmark feature of AD. However, as has been noted before (Hampel et al., 2018), the cholinergic hypothesis was never truly conceptualised as a proper framework of what role the cholinergic system plays in the causal chain of AD and dementia. Rather it was – and remains – a review of findings on associations between markers of neurodegeneration and cognitive decline, and cholinergic dysfunction. Questions remain about the importance, timing, and role of the cholinergic system in the disease. While some recent results suggest that neurodegeneration in AD might begin in the NBM (Fernández-Cabello et al., 2020; Schmitz et al., 2016), the history of research on cholinergic function in AD is rich with setbacks and inconclusive evidence (Arendt et al., 2015; Mesulam, 2004). More studies are needed to further illuminate the interplay between cholinergic deterioration, Aβ, and NFTs, and what role they play in the cascade that results in AD-associated cognitive decline.

2 Anticholinergic drugs

As described in the previous chapter, the stimulation of mAChRs through inhibition of AChE or directly by application of muscarinic agonists has been widely explored for therapeutic purposes. Indeed, the application of AChE-Is in medicine goes beyond the treatment of AD. They can be used prophylactically to protect against poisoning with organophosphate AChE gases, to treat urinary retention, and in the management of myasthenia gravis, an autoimmune disease of cholinergic receptors (Fisher & Wonnacott, 2012). Similarly, the opposite action of muscarinic antagonism has been widely used in medicine. Compounds that antagonise mAChRs are usually referred to as "anticholinergic", a term that is somewhat of a misnomer. While widely used in the literature, it suggests an antagonistic/inhibiting effect on all types of cholinergic receptors (Brayfield, 2014; Dowd, 2017). However, anticholinergic drugs are understood to block specifically mAChRs, while – except at extremely high doses – leaving nAChRs essentially unaffected (Dowd, 2017). Henceforth, the terms "anticholinergic" and "antimuscarinic" will – as is common in the literature – be used interchangeably to refer to compounds that block mAChRs. The purpose of this chapter is to provide a general overview of anticholinergic compounds, including their medicinal purpose, pharmacoepidemiology, and the current understanding of the potential adverse effects of their use.

2.1 The history of anticholinergic drugs

The naturally occurring anticholinergic compounds atropine and hyoscine are derived as alkaloids primarily from some plants of the family of nightshades (*Solanaceae*) that include the toxic deadly nightshade (*Atropa belladonna*), jimsonweed (*Datura stramonium*), mandrakes (genus *Mandragora*), European Scopolia (*Scopolia Carniolica*) and black henbane (*Hyoscyamus niger*) (Kersten & Wyller, 2014; Lee, 2006c). Due to its association with poison, the Swedish botanist Carl Linnaeus named the deadly nightshade after Atropos, who in Greek mythology is one of the three Fates who cuts the thread of life (Dowd, 2017). Another

anticholinergic drug, lachesine, was later given its name after the second of the three Fates – Lachesis, the one that measures the thread (Ing, 1946; Lee, 2007).

The nightshades have been known since ancient times, as evidenced by their inclusion in works of mythology and literature. For example, the Greeks believed that the souls of the dead that roamed the banks of the river Styx wore garlands of henbane (Graves & Morton, 1955; Lee, 2006b). Additionally, some authors posited that prominent works of literature feature the use of the nightshades. These include the substance that the witch Circe uses to poison the sailors aboard Odysseus' ship (Lee, 1999, 2007), and the contents of the ampoule that Claudius pours into the king's ear in Shakespeare's Hamlet (Kotsias, 2002).

The use of the nightshades as poisons was not limited to literature. Ancient Greeks, Romans, Persians, and Arabs utilised the plants' toxic effects to produce prolonged illnesses (Heller Brown et al., 2018; Kraemer, 1894; Smulyan, 2018). The Romans even used the deadly nightshade to create a paste which they used to poison the tips of their arrows (Smulyan, 2018). Military history contains several cases where the either intentional or unintentional use of the nightshades affected the outcomes of prominent historical battles. These include Mark Antony's retreat from the Parthians (Kraemer, 1894; Plutarch & Pelling, 1988), Julius Caesar's Spanish campaign (Kraemer, 1894), and a scheme by the Scottish to poison and massacre the invading Danes (Buchanan & Elzevir, 1643; Kraemer, 1894).

The popularity of the nightshades as poisons persisted well into the 17th century (Forbes, 1977; Lee, 2006b). Besides their toxic properties, however, naturally occurring anticholinergic alkaloids were purportedly also used for their cosmetic effects. Cleopatra is said to have instilled the extract of the deadly nightshade to her eyes to dilate her pupils and make her appear more attractive (Behçet, 2014; Goodman et al., 2006). Some authors have linked this cosmetic effect to the deadly nightshade's etymology (*belladonna* means "beautiful woman" in Italian) (Dowd, 2017; Kersten & Wyller, 2014), although the true origin of the name remains uncertain (Forbes, 1977; Rice, 1894).

According to some (Heller Brown et al., 2018), the recognition in modern Western medicine of the wide-ranging therapeutic utility of anticholinergic compounds can be traced to British

colonial India, where the colonialists observed the local population to burn jimsonweed and inhale the smoke to treat asthma. However, writings from the ancient Mediterranean suggest a long tradition in Western medicine of using nightshades. Among the ancient Jews of the Levant, mandrake was used to aid procreation (Lee, 2006a), while the Greeks and Romans used it as an anaesthetic, and to treat gout, pain, and insomnia (Lee, 2006a; Thompson, 1934).

In the Middle Ages, the use of the nightshades became associated with sorcery. Witches were believed to use the plants from this family to achieve several supernatural feats, including levitation, flight, and fortune-telling (Lee, 2006b, 2007). Despite such superstitions, the *Solanaceae* continued to be prized for their supposed medicinal powers (Lee, 2006a). Indeed, the possibly earliest reference to the deadly nightshade as a medicinal plant occurred in 1488 in the works of Saladin of Ascoli (Kraemer, 1894; Saladinus, 1489). In the 16th and 17th centuries, the nightshades became increasingly popular among herbalists, and the Age of Enlightenment witnessed their widespread adoption as narcotics (Lee, 2007).

The first time an anticholinergic compound was isolated was in 1824 by Dr Runges, a professor at the University of Berlin. He poisoned an animal using atropine and then boiled its stomach and intestines to concentrate the aqueous solution contained therein. When the resulting extract was applied to the eyes of a cat using a camel-hair pencil, the animal's pupils dilated ("Royal Institute of France," 1824).

Soon, more advanced methods were developed, and either in 1831 (Heller Brown et al., 2018; Mein, 1833) or 1833 (Geiger, 1833; "The history of atropine," 1902), atropine was isolated in its pure crystalline form. Within decades, it came into widespread use: it was suggested as a treatment for, among others, Parkinson's disease (Dowd, 2017), whooping cough (Corson, 1852; Garraway, 1865), asthma (Salter, 1869), myopia (Derby, 1874), constipation (Farlow, 1889; McCullough, 1901; Thom & Crief, 1879), pain (Farlow, 1889; McCullough, 1901), cancer, depression, mania (Duncan, 1803), eye inflammation (Bulley, 1842), epilepsy (Duncan, 1803; Ramskill, 1863), menstrual issues (Magoun, 1839), and as a suppressant of lactation (Trend, 1858). By the end of the century, a *belladonna* plaster was already widely employed in the United States and Europe. It was used to treat local pain, neuralgia, rheumatism, myalgia,

pleurisy, and tuberculosis, and was sold in pharmacies well into the 1950s (Kilmer, 1894; Lee, 2007).

In a monograph published at the end of the 19th century, Kilmer (1894) described what was then known of the effects and uses of atropine. The author pronounced the compound as "one of the most important drugs in medicine" (p. 2) and detailed its effects on the nervous system, voluntary muscles, gastrointestinal tract, respiratory system, urinary tract, as well as the side effects of its application. Among the indications for its use, he listed over 160 ailments. Although the precise mechanisms were unclear, atropine came to be understood as a suppressor of vagal transmission and was used to study animal organ systems (Langley, 1878; Schmiederberg & Kopper, 1869).

Hyoscine – the active anticholinergic in henbane – was isolated in the 1880s by Albert Ladenburg at the University of Kiel (Ladenburg, 1881; Lee, 2006b) and first synthesised in 1901 by the German chemist Richard Willstätter (Behçet, 2014). At the same time, Johan Scopoli, working on *Scopolia carniolica*, showed this plant to be a rich source of an alkaloid that he named scopolamine. The latter established itself as the American name for hyoscine (Lee, 2006b). Hyoscine too was tested as a therapeutic for various disorders, including angina (Bostwick, 1897), epilepsy, pain (Colman & Taylor, 1889; Duncan, 1803), eclampsia (Clowes, 1896), mania, depression (Duncan, 1803), and insomnia (MacDonald, 1887). Similar to atropine, the physiological effects and medical uses of hyoscine were well known by the end of the century (Weatherly, 1891).

Research in the first half of the 20th century provided an understanding of the chemical transmission in the nervous system. The observations that ACh exhibited a strong hypotensive action (Hunt & Taveau, 1906), that it occurred naturally in animal tissue (Dale & Dudley, 1929), and that parasympathetic nerve stimulation was conducted by chemical means (Dale & Gaddum, 1930; Loewi, 1935), provided support for the notion of ACh-mediated transmission of nerve signals in the ANS (Baumeister & Hawkins, 2005; Tansey, 2006; Valenstein, 2002). It was found that atropine blocked cholinergic transmission at sites where muscarine – but not nicotine – mimicked the effects of ACh. Based on this observation, the

sites on which ACh acted were classified as either muscarinic or nicotinic (Feldberg & Gaddum, 1934; Valenstein, 2002). In a 1934 contribution to the Physiological Society, Henry Dale introduced the term "cholinergic", thereby designating nerve fibres by the chemical that they released, as opposed to their anatomy (Tansey, 2006). The next few decades also witnessed the acceptance of the existence of receptors upon which endogenous molecules and exogenous substances might act (Maehle et al., 2002), as well as the documenting of the activity of a variety of receptor agonists and antagonists in diverse tissues (López-Muñoz & Alamo, 2009; Valenstein, 2002).

The recognition of the effects of cholinergic antagonists in affecting different organ systems (see section 2.2) led to the development of several novel agents with anticholinergic effects (Bachrach, 1958). Well into the 1960s, drugs with anticholinergic properties were still understood to be used almost exclusively for their direct effect of blocking cholinergic receptors in the body and consequently enacting changes primarily in the gastrointestinal tract, biliary structures, and the pancreas (Smith, 1964). Even though the therapeutic use of anticholinergic drugs had long been deemed relatively safe (Bachrach, 1958), the potential for anticholinergic toxicity was widely known. The literature described serious negative side effects of anticholinergic medication on heart rhythm, and the appearance of a "central anticholinergic syndrome", which included delirium, anxiety, hyperactivity, disorientation, hallucinations, and seizures (Duvoisin & Katz, 1968; Granacher & Baldessarini, 1975; Longo, 1966). In a review summarising the drugs associated with the central anticholinergic syndrome, one author (Longo, 1966) focused on atropine and hyoscine, and listed some other compounds, most of which were prescribed primarily for their anticholinergic activity, especially as antispasmodics to treat parkinsonism: quinuclidine esters, piperidyl benzilates, trihexyphenidyl, caramiphen, cycrimine, profenamine, and orphenadrine. However, treatment with drugs not developed or prescribed for their anticholinergic function – for example, the antidepressant amitriptyline (Baldessarini & Willmuth, 1968) and the antihistamine diphenhydramine (Duvoisin & Katz, 1968) – sometimes resulted in similar side effects. This, in combination with the observation that the administration of physostigmine alleviated these effects (Granacher & Baldessarini, 1975; Slovis et al., 1971), led some authors

(Granacher & Baldessarini, 1975) to hypothesise that a wide array of drugs might exhibit unknown and unwanted anticholinergic activity.

With the development of a radioreceptor assay (Tune & Coyle, 1980, 1981) to measure anticholinergic activity, researchers indeed started to detect anticholinergic effects in drugs hitherto not viewed as anticholinergic. An early study (Tune et al., 1992) found that out of 25 drugs commonly prescribed to older people, 14 exhibited detectable anticholinergic activity. This reinforced the view that a drug regimen consisting of multiple drugs might lead to unanticipated harm through the cumulative action of several mildly anticholinergic drugs.

2.2 Anticholinergic drugs in the present

2.2.1 Mechanism of action

Based on their synthesis, anticholinergic drugs can be divided into four categories: (1) naturally occurring belladonna alkaloids (atropine and hyoscine), (2) semisynthetic derivatives (e.g., homatropine) and quaternary ammonium derivatives of atropine, hyoscine, and homatropine (e.g., hyoscine methobromide, methyl bromide), (3) synthetic quaternary ammonium compounds (e.g., propantheline, ipratropium, glycopyrrolate), and (4) synthetic anticholinergic drugs that are not quaternary ammonium compounds (e.g., benztropine, trihexyphenidyl) (Dowd, 2017). Based on their chemical structure, anticholinergics can be divided into two groups. (1) tertiary amines possess a tertiary nitrogen atom. They may include naturally occurring alkaloids, semisynthetic derivatives, or synthetic compounds, whilst (2) quaternary ammonium compounds are always synthetic. In general, tertiary amines are more soluble in lipids, exhibit better absorption in the gastrointestinal tract, and more readily pass the BBB. Additionally, quaternary ammonium compounds exhibit a greater degree of antinicotinic potency, as opposed to tertiary amines that have little effect on nAChRs (Dowd, 2017). However, considering the high number of therapeutic compounds that have been reported to exhibit anticholinergic activity (see section 2.3.2), many substances listed as such will not neatly fall within one of the typical categories. While anticholinergic

compounds do not have an intrinsic effect on their muscarinic targets, they have an affinity for mAChRs. They thus block the receptors by binding to them and preventing the action of ACh, exhibiting so-called competitive antagonism (Dowd, 2017; Heller Brown et al., 2018).

When describing the effects of anticholinergics on the human body, I will mostly focus on the effects of atropine as it can be viewed as the prototypical anticholinergic drug. It has been studied for over a century and its effects have been thoroughly described. Additionally, although clinically used anticholinergic drugs seem to exert most of their effects through action on the M_1 receptor (Kay et al., 2005; Welk et al., 2021), most lack any meaningful selectivity for receptor subtypes (Heller Brown et al., 2018). Although there has been substantial progress in the development of selective mAChRs agonists, the development of selective antagonists has lagged (Lebois et al., 2018). Thus, the physiological effects of atropine can to a great extent be observed in the actions of many other anticholinergic compounds.

Generalisations about anticholinergic drugs can help develop a basic understanding of their effects, but it is important to note a few caveats. First, the effects of different anticholinergic compounds and, consequently, their therapeutic utility depend on the selectivity of the drugs to different receptors subtypes. While drugs generally lack any substantial receptor subtype selectivity, this is not true for all anticholinergic compounds. For example, pirenzepine – a drug used to reduce the secretion of gastric acid – preferentially inhibits M_1 receptors, whereas darifenacin and solifenacin – both medicines to treat an overactive bladder – selectively inhibit M_3 receptors (Dowd, 2017; Heller Brown et al., 2018). Anticholinergic compounds also differ in their pharmacokinetics. This includes differences in the propensity of absorption from the gastrointestinal tract, the skin, the conjunctiva of the eye, or by inhalation. Importantly, drugs differ in their ability to pass the BBB. For example, quaternary ammonium compounds do not readily pass the BBB and thus exert little effect on the CNS. Additionally, they more strongly block transmission between preganglionic and postganglionic neurons in the ANS (Brayfield, 2014; Dowd, 2017; Heller Brown et al., 2018). Finally, independently of the relative proportions of receptor subtypes, not all organ systems

exhibit the same susceptibility to mAChR inhibition. This is due to, among other factors, the density of receptors in those organs and the presence of various regulatory compensatory mechanisms (Dowd, 2017; Heller Brown et al., 2018).

2.2.2 Uses of anticholinergics

Anticholinergics are often utilised in therapy for their known antimuscarinic activity in effector organs. Peripherally, mAChRs are located primarily in the eyes, secretory glands, lungs, heart, gastrointestinal- and genitourinary tracts, and the skin; they are also widespread in the CNS (Brayfield, 2014; Dowd, 2017). Due to this ubiquity, anticholinergic drugs affect multiple organ systems throughout the body.

Anticholinergics alter the heart rate, which is most noticeable in young adults: on administration, transient bradycardia (slowing of the heart rate) is followed by prolonged tachycardia (increase in heart rate). Moreover, anticholinergics can abolish some types of parasympathetic reflexes of cardiac slowing. However, they have little effect on the circulation, as blood vessels exhibit only limited anticholinergic innervation. In the cardiovascular system, anticholinergics are generally used only for short-term interventions in coronary care units or surgical settings, such as in the prevention of arrhythmias associated with anaesthesia (Brayfield, 2014; Dowd, 2017; Heller Brown et al., 2018).

In the respiratory system, anticholinergics relax the smooth muscles by blocking parasympathetic innervation to the lungs an effect that is most prominent in individuals with respiratory disease. Ipratropium, tiotropium, aclidinium, and umeclidinium are used to inhibit bronchoconstriction caused by histamine and bradykinin, and – often in combination with adrenergic receptor agonists – in chronic obstructive pulmonary disease. Furthermore, anticholinergics inhibit secretions from the bronchi, pharynx, nose, and mouth and can be used to treat rhinorrhea (runny nose) (Dowd, 2017; Heller Brown et al., 2018).

In the eye, anticholinergics block the pupillary sphincter muscle of the iris and the ciliary muscle that controls the curvature of the lens. Their use dilates the pupils (mydriasis) and paralyses accommodation of the eyes (cycloplegia). These effects may occur upon systemic

administration, but they are most apparent when the agents are applied directly into the eye. Homatropine hydrobromide, tropicamide, and cyclopentolate hydrochloride are used in ophthalmological practise for examinations of the retina and the optic disc, and in the therapy of iridocyclitis (inflammation of the iris and the ciliary body) and keratitis (inflammation of the cornea) (Dowd, 2017; Heller Brown et al., 2018).

In the gastrointestinal tract, anticholinergics have wide-ranging effects. They inhibit motor activity in the stomach, duodenum, jejunum, ileum, and colon, reducing the tone and the frequency of peristalsis. However due to the relative independence of the enteric nervous system, which utilises neurotransmitter systems unaffected by cholinergic transmission, large doses of anticholinergics are required to achieve these effects. Many disorders that involve increased tone or motility of the gastrointestinal system are treated with atropine, hyoscyamine sulphate, hyoscine, or glycopyrrolate. Furthermore, anticholinergics partially block the secretion of gastric acid and strongly inhibit salivary secretion. Pirenzepine and telenzepine are used to treat peptic ulcer disease, and glycopyrrolate is prescribed for excessive salivation (Dowd, 2017; Heller Brown et al., 2018).

Anticholinergics also decrease the tone and the strength of contractions of the ureter and bladder and are often used to treat disorders of an overactive urinary bladder. By antagonising the parasympathetic innervation, anticholinergic therapy can increase bladder capacity, reduce urinary frequency, and alter bladder sensation during filling. Tolterodine, trospium, solifenacin, and darifenacin are prescribed for this purpose (Heller Brown et al., 2018).

As noted before, the ability of anticholinergics to affect the functioning of the CNS varies with their propensity to cross the BBB. For example, although toxic doses of atropine may lead to restlessness, irritability, disorientation, hallucinations, and delirium, acute effects on the CNS are minimal at therapeutic doses. Contrastingly, hyoscine prominently affects the CNS and at low therapeutic doses may cause drowsiness, amnesia, fatigue, and euphoria. Hyoscine has long been used to treat motion sickness, probably by blocking the neural pathways from the inner ear to the brainstem (Heller Brown et al., 2018). Other anticholinergics, especially

benztropine mesylate, trihexyphenidyl hydrochloride, and biperiden are prescribed for the treatment of extrapyramidal side effects of conventional antipsychotics. They may also be used for symptomatic treatment in the early stages of Parkinson's disease, although their prescribing for this purpose is rare in the UK (in comparison to Japan, for example; Yoshiyama et al., 2015). The effects of these anticholinergics are achieved primarily through the blockade of mAChRs in the basal ganglia and the selective activation or inhibition of different subpopulations of striatal neurons (Heller Brown et al., 2018).

Over the past decades, several other drugs have been ascribed anticholinergic potency. While these drugs may not be prescribed for their anticholinergic activity (i.e., their therapeutic use is not primarily tied to the blocking of transmission at mAChRs), they comprise many commonly prescribed medications and span several classes of drugs (**Table 4**). The drugs commonly thought as exhibiting anticholinergic activity are presented in chapter 4, as Supplementary Table S2 (p. 130). The classification of each drug according to the WHO Anatomical Therapeutic Chemical (ATC) classification system (https://www.whocc.no/atc_ddd_index/) (*ATC/DDD Index 2022*) is available in chapter 5, as Supplementary Table 3 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9005668/bin/TRC2-8-e12290-s001.xlsx).

Table 4: Some examples of drugs claimed to exert anticholinergic effects. For a more comprehensive presentation, please see chapter 4, Supplementary Table S2 (p. 137) and chapter 5, Supplementary Table 3 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9005668/bin/TRC2-8-e12290-s001.xlsx).

Indication	Drug group	Medication
High blood pressure	angiotensin-converting-enzyme inhibitors	captopril, trandolapril
	loop diuretic	furosemide
Blood clotting		dipyridamole, warfarin
Diabetes		metformin
Gout		colchicine
Depression	monoamine oxidase inhibitors	phenelzine, tranylcypromine
	non-selective monoamine reuptake inhibitors	trimipramine, imipramine, amitriptyline, doxepin, nortriptyline, dosulepin
	selective serotonin reuptake inhibitors	paroxetine, citalopram, escitalopram, fluoxetine, sertraline, fluvoxamine
Insomnia	benzodiazepines	temazepam, flurazepam, nitrazepam
Pain	opioids	methadone, morphine, oxycodone, codeine, tramadol, fentanyl, pethidine
Seizures		phenobarbital, clonazepam, carbamazepine, valproate, phenytoin, topiramate, midazolam
Inflammation, arthritis	non-steroidal anti-inflammatory	ketorolac, celecoxib, etoricoxib
Inflammatory bowel disease		methotrexate, azathioprine
Migraine		ergotamine, naratriptan, sumatriptan, zolmitriptan
Infection	aminoglycosides	neomycin, tobramycin, gentamicin
	beta-lactam antibiotics	cefalexin, amoxicillin, piperacillin, ampicillin, cefoxitin
Anaesthesia		ketamine

2.3 Anticholinergic scales

As alluded to before, the extent of cholinergic involvement throughout the human body, the frequent use of anticholinergic drugs, and the seemingly commonly exhibited anticholinergic activity by drugs designed for other purposes have led to concerns regarding the use of these compounds in medicine.

However, to guide medical practice and research on the potential harm of the use of anticholinergic drugs, the latter required standardisation. For this purpose, anticholinergic scales have been devised that assign to relevant compounds potency scores that are supposed to reflect the capacity of those drugs to bind mAChRs. Typically, scores range from 0 ("no anticholinergic activity") to 3 ("strong anticholinergic activity"), although some authors (Chew et al., 2008; Durán et al., 2013; Ehrt et al., 2010; Hefner et al., 2015; Xu et al., 2022) have used different ranges of values. The potency scores are then summed to yield an anticholinergic burden/load (AChB) for each person. After the generation of an anticholinergic scale, the authors usually validate it by testing its association with a pre-defined outcome measure. After publication, many scales undergo further testing, as other research groups apply them for their purposes. All such research is often referred to as "validation" in the literature (Lisibach et al., 2021). Although this is technically not always the case – many authors apply anticholinergic scales on populations and outcomes for which they were not intended – I will henceforth refer to all studies that test the association between an anticholinergic scale and an outcome measure as "validation studies". The section below aims to give an overview of anticholinergic scales, estimate their utility, and provide a critique of their use.

2.3.1 Generation of anticholinergic scores

All anticholinergic scales published to date use one of three methods – or a combination thereof – to assess the anticholinergic activity of a drug (**Table 5**): an assay to (1) determine the anticholinergic activity in serum or (2) *in vitro*, or (3) the use of lists of drugs based on expert judgement (Rudd et al., 2005).

Table 5: Characteristics of three methods used to establish anticholinergic potency. Adapted from Rudd et al. (2005).

Method	SAA	Individual mAChR affinity *in-vitro*	Drug lists and expert judgement
Assay agent	^{3}H-QNB, rat brain, serum	^{3}H-QNB, rat brain, serum	none
Commercial availability	no	no	n.a.
Individual or multiple drug assessment	multiple	individual	individual
Objective or subjective	objective	objective	subjective, objective
Considers concentrations	no	yes	no
Accounts for metabolites	yes	no	yes
Accounts for baseline physiology	yes	no	yes
Accounts for CNS penetration	no	no	yes

The first method measures anticholinergic activity in blood serum (serum anticholinergic activity, SAA). It involves the use of a receptor-ligand binding assay (Tune & Coyle, 1980, 1981): serum from a participant whose total AChB is to be measured is exposed to mAChRs in suspensions prepared from rat brain tissue before ^{3}H-quiniclidinyl benzilate (^{3}H-QNB) is added. ^{3}H-QNB is a potent antimuscarinic agent that has been tritiated to contain tritium (hydrogen-3), a radioactive isotope of hydrogen, to allow for detection with radiolabelling techniques. ^{3}H-QNB competes for binding with other anticholinergic compounds in the serum. By determining the amount of bound ^{3}H-QNB, the SAA can be quantified, usually in reference to equivalents of atropine (pmol/ml). Because SAA uses the participant's serum, it represents the total anticholinergic activity of all compounds in the blood. Individual physiology affects the metabolism of drugs. Additionally, not only initially administered anticholinergic drugs, but also their downstream metabolites and endogenously expressed compounds may exhibit antimuscarinic activity. Thus, SAA captures the *de facto* total anticholinergic activity exhibited in the body.

While SAA may represent the total peripheral anticholinergic activity for a given person, it does not enable the discernment of the contributions of individual compounds. It is therefore not useful to determine or quantify the anticholinergic effect of individual drugs present in the individual's body or to provide guidance on their potential discontinuation (Salahudeen et al., 2014). To allow for the measurement of the anticholinergic potency of individual drugs,

the assay used in SAA must be somewhat modified (Tune et al., 1993). The principles of the method remain the same, but instead of using serum, a standard concentration (usually 10^{-8} M) of the radiolabelled drug of interest is applied *in vitro*. This enables the direct comparison of the anticholinergic activity of individual drugs and even the correction for medication dosage. However, this approach disregards the unique physiology of each person and the anticholinergic effects of potential drug metabolites.

Salahudeen et al. (2016) performed a systematic review on the association between SAA and cognition, delirium, and activities of daily living in older people (n included studies = 33; n participants = 2,983). The authors found no strong link between SAA and various functional and cognitive outcome measures studied. This is especially concerning given the evidence for a good correlation between anticholinergic activity in the serum and the cerebrospinal fluid (Miller et al., 1988; Plaschke et al., 2007). However, while these results raise the question of the value of SAA as a biomarker of health outcomes, they may not signify its validity as a measure of anticholinergic activity (for a discussion on the association between anticholinergic use and health outcomes, see section 2.4). However, the authors did point out several limitations to the use of SAA to measure AChB for both research and clinical practice. First, the quantitative relationship between SAA and anticholinergic action is unclear, as associations between SAA and anticholinergic use and drug dosage have sometimes produced inconsistent results (Carnahan et al., 2006; Thienhaus et al., 1990; Vinogradov et al., 2009). Second, SAA is a peripheral measure and does not correlate with cerebral cholinergic function (Thomas et al., 2008). Thus, the worth of the assay to assess the risk of central adverse effects is questionable. Additionally, most studies have used sonicated rat cerebrum in the *in vitro* bioassay. Even though all five mAChR subtypes are represented in the rat cerebrum, the amount of each receptor subtype might differ from the human brain (Salahudeen et al., 2016). Third, it has been reported that ^{3}H-QNB binds extensively to plasma proteins, thereby prohibiting binding of the ligand to mAChRs (Cox, 2009). This raises questions about the validity of SAA to measure even peripheral anticholinergic activity. Fourth, SAA varies over time and is detectable even when no drugs are consumed (Flacker & Lipsitz, 1999; Flacker & Wei, 2001; Mangoni et al., 2013; Salahudeen & Nishtala, 2016). Fifth, SAA is expensive,

somewhat invasive, and not well standardised, as the range of measured values varies between reports and between different laboratories (Carnahan et al., 2002; Collamati et al., 2016; Kersten & Wyller, 2014). Finally, SAA is not readily accessible and difficult to interpret in clinical practice (Carnahan et al., 2002; Mangoni et al., 2013; Salahudeen et al., 2016). There have been some attempts to improve the validity of the SAA. For example, one group recently modified the procedure by using cells that stably express only the M_1 receptor subtype, which is most often associated with cognition (Chandramouleeshwaran et al., 2021; Chandramouleeshwaran et al., 2022). However, this does not circumvent the many other problems associated with SAA as described before.

The second method to assign anticholinergic activity relies on previous publications, authoritative monographs, reference databases, and the knowledge of experts involved in the construction of the anticholinergic scale to evaluate the anticholinergic potency of drugs. Anticholinergic scores for drugs in most anticholinergic scales are entirely based on this approach (Al Rihani et al., 2021; Mayer et al., 2015; Salahudeen, Duffull, et al., 2015). Authors utilising this method most often search the literature based on some criteria (e.g., reported anticholinergic side effects of drugs) and critically assess the results. They then combine the assessed accounts with personal experience and based on the rationale for the construction of the anticholinergic scale assign anticholinergic scores to the compounds of interest. The expert panel involved in the construction is usually multidisciplinary and often includes psychiatrists, geriatricians, researchers, pharmacists, and general physicians. However, the number of experts participating in the decision-making process, as well as their precise areas of expertise can differ substantially between anticholinergic scales. Due to its heavy reliance on personal expertise, the quality of implementing this method strongly depends on the knowledge and experience of the expert panel. The latter must account for a multitude of variables when assigning anticholinergic scores, including person-specific pharmacokinetics and pharmacodynamics, physiological characteristics, dosage, etc.

Even anticholinergic scales centred on either SAA or *in vitro* assays implicitly highly depend on the expertise of the authors. This is the case because – in the absence of a clear consensus

or gold standard on the measurement of anticholinergic potency – important decisions must be made at every step of the scoring process. If an anticholinergic scale is based on SAA, the choice of which drugs to include in the testing must be made. If the anticholinergic scale is based on a review on assays of muscarinic activity, contradictions in the literature must be resolved, once again drawing upon the experience of resident experts. The strong involvement of personal expertise in drug scoring necessitates a high degree of subjectivity and entails a process that is rarely transparent and scarcely reproducible (Lisibach et al., 2021).

Regardless of the choice of method to construct anticholinergic scales, AChB itself – as it is customarily conceptualised – is problematic. First, given the likely risk of publication bias in this field (Taylor-Rowan et al., 2021), the reliance on previously published literature on anticholinergic effects of drugs may lead to a self-perpetuating cycle of selection and confirmation. Drugs in the past categorised as anticholinergic are likely to be chosen for consideration for inclusion in anticholinergic scales and are by default more likely to be again judged as anticholinergic. This may lead to the exclusion of harmless anticholinergic drugs and the inclusion of potentially harmful non-anticholinergic drugs, both of which may contribute to an overestimation of the effects of anticholinergic use on health outcomes. Second, AChB assumes that anticholinergic effects are additive in a linear fashion (Lisibach et al., 2021). This may not be the case, especially when an individual combines an increasing number of different drugs (Kersten & Wyller, 2014). In fact, there is little evidence that drugs with purportedly low anticholinergic scores additively contribute towards additional risk of dementia (Fox et al., 2011; Richardson et al., 2018; Welk et al., 2021). Finally, regardless of the validity of an anticholinergic scale, the scale would need to be frequently updated to incorporate new drugs on the market; this is rarely the case (Lertxundi et al., 2013; Lisibach et al., 2021).

2.3.2 An overview of anticholinergic scales

Several reviews have described, critically appraised, or compared anticholinergic scales. (Al Rihani et al., 2021; Lertxundi et al., 2013; Lisibach et al., 2021; Lozano-Ortega et al., 2020;

Mayer et al., 2015; Naples et al., 2015; Salahudeen, Duffull, et al., 2015; Salahudeen, Hilmer, et al., 2015; Tristancho-Pérez et al., 2021; Valladales-Restrepo et al., 2020; Villalba-Moreno et al., 2016). Depending on the definition and purpose of the review, a different set of anticholinergic scales was included, and the reported numbers of published anticholinergic scales have varied: some of the recent reviews have included in their analyses 12 (Mayer et al., 2015), 10 (Villalba-Moreno et al., 2016), 11 (Al Rihani et al., 2021), 18 (Welsh et al., 2018), or 19 (Lisibach et al., 2021) anticholinergic scales. **Table 6** provides an extended list of 30 anticholinergic scales based on data included in previously published reviews and a thorough appraisal of recent literature on the topic. An anticholinergic scale was included if it provided a list of anticholinergic drugs and assigned an unambiguous potency score to each.

Table 6 (continued on pp. 55 and 56): anticholinergic scales found in the literature. The first column indicates the name and abbreviation of the scale (if available), the first author of the publication in which the scale was first presented, and the year of publication; when several years are given, the scale was updated by the authors since the first date of publication. The second column indicates the origin of the list (i.e., where the drugs were sourced from before scoring) and the size of the sample used to ascertain the drugs. The third column indicates the strategy of score assembly (i.e., which criteria were considered when assigning the scores). The final column indicates the outcome measure for which the anticholinergic score was validated.

Scale name	Origin of drug list	Score assembly	Validation/ purpose*
Drug Risk Number (DRN), Summers et al. (1978)	people undergoing cataract extraction, cardiotomy, or electroconvulsive therapy (n=84)	reported association with delirium or reported anticholinergic potency	delirium
Clinician-Rated Anticholinergic Scale (CRAs), Han et al. (2008; 2001)	people admitted to hospital (n=278)	established literature on anticholinergic potency; expert consensus	delirium
Anticholinergic Burden Score (ABS), Aizenberg et al. (2002)	people admitted to hospital (n=482)	monograph on pharmacotherapy	falls
Clinical Index, Pharmacological Index (CI/PI), Minzenberg et al. (2004)	people with schizophrenia at a medical centre (n=106)	published studies on *in-vitro* mAChR binding; expert consensus	cognitive battery
Anticholinergic Burden Classification (ABC), Ancelin et al. (2006)	patients in general practice (n=372)	published studies on SAA; expert consensus on administration mode, drug-drug interactions, and BBB-permeability	cognitive battery
Anticholinergic Drug Scale (ADS), Carnahan et al. (2006)	individuals in long-term care (n=297)	published studies on SAA and on reported drugs' anticholinergic effects	*development of a scale associated with SAA
Drug Burden Index (DBI), Hilmer et al., (2007)	Medicare recipients (n=3,075)	published studies on drugs' effects on physical and cognitive function	physical function, digit symbol substitution test
Anticholinergic Activity Scale (AAS), Chew et al. (2008)	drugs classified as frequently dispensed by a pharmacy provider, and drugs reported to produce anticholinergic side effects	*in-vitro* assay using competitive radioreceptor binding	*scale based on SAA assay

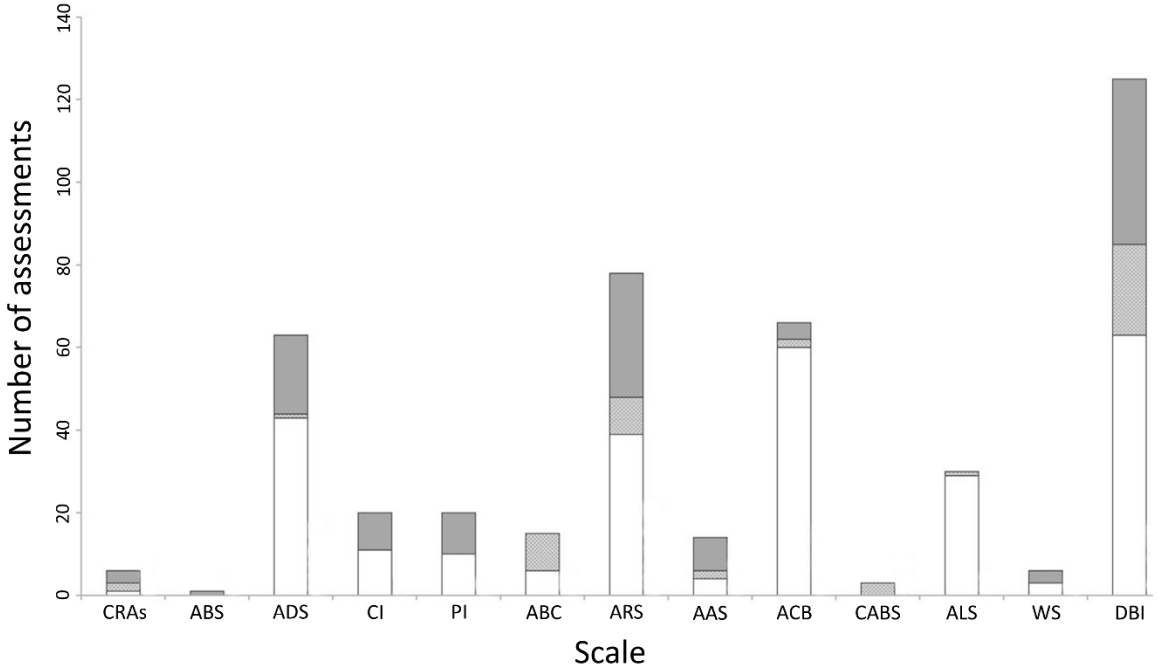
Cancelli's Anticholinergic Burden Scale (CABS), Cancelli et al. (2008)	subjects with probable AD (n=230) and a literature search for reported anticholinergic drugs	published studies on SAA and reported drugs' anticholinergic effects; expert consensus	psychosis
Anticholinergic Risk Scale (ARS), Rudolph et al. (2008)	the most prescribed drugs within a medical centre (n=132)	published studies on mAChR binding and drugs' adverse effects; expert consensus	central and peripheral side effects
Anticholinergic Cognitive Burden (ACB), Boustani et al. (2008)	database search for association between anticholinergic drugs and cognition	expert consensus	*development of a scale for central cognitive effects
Cao et al. (2008)	drugs listed as anticholinergic in a reference text		physical and cognitive function
Revised Anticholinergic Activity Scale (AAS-r), Ehrt et al. (2010)	individuals with Parkinson's disease (n=235)	AAS; expert consensus	MMSE
Anticholinergic Loading Scale (ALS), Sittironnarit et al. (2011)	participants in a study (n=1,112)	ABC, AAS, CRAs, ARS; expert consensus	cognitive battery
Whalley Scale (WS), Whalley et al. (2012)	participants in a study (n=281)	ABC, SAA (Tune & Coyle, 1980); expert consensus	cognitive battery, dementia
Durán Scale (DS), Durán et al. (2013)	database search of existing anticholinergic scales	agreement among existing anticholinergic scales; reference text	*review and harmonisation of previous anticholinergic scales
Drug Burden Index, International Version (DBI-WHO), Faure et al. (2013)	DBI		agreement with DBI
Modified Anticholinergic Risk Scale (mARS), Sumukadas et al. (2014)	ARS, ACB, ADS, the British National Formulary (BNF), and a manual addition of newer drugs	ARS, ACB, ADS; expert consensus	*update of the ARS and adaptation to the UK
Delirogenic Risk Scale (DRS), Hefner et al. (2015)	drugs listed by a previous working group, ADS, AAS, ABC	ADS, AAS, ABC; reference text	anticholinergic side effects
Muscarinic Acetylcholinergic Receptor ANTagonist Exposure Scale (MARANTE), Klamer et al. (2017)	DS	DS	*incorporation of precise dosage information

Anticholinergic Effect on Cognition (AEC), Bishara et al. (2017)	drugs commonly used in older people; database search for effects on cognitive function and reported anticholinergic activity; custom addition	database search for drugs' mAChR binding affinity, BBB penetration, and cognitive adverse effects; reference text; expert consensus	*improvement of previous anticholinergic scales
Anticholinergic Impregnation Scale (AIS), Briet et al. (2017)	French psychiatric facilities (n not reported)	published studies on SAA, scores from previous anticholinergic scales; expert consensus	*adaptation for clinical psychiatry in France
Anticholinergic Toxicity Score (ATS), Xu et al. (2017)	common drugs, representative of major drug classes, and consistently scored by the ACB and ARS	bioactivity database search for inhibition of mAChrs	*scale based on pharmacological data
German Anticholinergic Burden Scale (GABS), Kiesel et al. (2018)	database search of existing anticholinergic scales; addition of new drugs prescribed in Germany	agreement among existing anticholinergic scales; reference database; expert consensus	*adaptation for Germany
Drug Delirium Scale (DDS), Nguyen et al. (2018)	literature review; expert consensus	expert consensus	delirium
Brazilian Anticholinergic Activity Drug Scale (BAAS), Nery et al. (2019)	ATC chemical groups with reported anticholinergic effect; Beers criteria (Fick et al., 2019); previously published anticholinergic scales	agreement among previous anticholinergic scales; reference text	*adaptation for Brazil
Korean Anticholinergic Burden Scale (KABS), Jun et al. (2019)	database search of existing anticholinergic scales; addition of new drugs prescribed in Korea	agreement among previous anticholinergic scales; expert consensus	*adaptation for Korea
Modified Anticholinergic Burden Scale (mACB), Kable et al. (2019)	ACB, mARS	agreement among the ACB and mARS; expert consensus	*adaptation for Australia
AntiCholinergic and Sedative Burden Catalog (ACSBC), Al Rihani (2021)	systematic review of existing anticholinergic scales	agreement among previous anticholinergic scales; literature search	*harmonisation of previous anticholinergic scales
CRIDECO Anticholinergic Load Scale (CALS), Ramos et al. (2022)	systematic review of existing anticholinergic scales	agreement among previous anticholinergic scales; database search for medicines available in Spain	cognitive impairment

*If validation was not performed in the original publication, the reported purpose for assembling the score is given.

The published anticholinergic scales differ substantially in their popularity of use, with the DBI frequently reported as the most popular, and some newer anticholinergic scales yet unvalidated (**Figure 4**) (Lisibach et al., 2021; Lozano-Ortega et al., 2020; Mayer et al., 2015).

Figure 4: Number of validation studies for some of the commonly used anticholinergic scales by August 2015. Dark grey bars indicate a significant association between AChB and the outcome, light grey bars indicate a significant difference between anticholinergic drug users and non-users relative to an outcome, and white bars indicate no significant association between the anticholinergic exposure and the assessed outcome Mayer et al. (2015). Adapted with permission.



Anticholinergic scales also vary considerably in multiple aspects of their construction and validation. First, they are generated in different countries. One study that explored 18 different scales found nine to originate from the USA, with one each from the UK, Israel, Norway, France, Italy, Ecuador, New Zealand, and one that was international in outlook (Welsh et al., 2018). Thus, most anticholinergic scales will be more appropriate for use in the USA and less so in countries that do not provide some of the drugs available in the USA. Second, while the populations based on which scales were constructed and/or validated consist in most cases of older adults, the mean age and disease status of those individuals varies considerably (Al Rihani et al., 2021; Mayer et al., 2015). Third, the setting in which the anticholinergic scales were validated differs substantially among anticholinergic scales and includes the community, hospital, outpatients, and care homes (Welsh et al., 2018). Third, the

validation of different anticholinergic scales was undertaken with different aims and thus probing different outcome measures, including falls, fractures, cognitive ability, dementia, delirium, all-cause mortality, and hospitalisations (**Table 6**) (Lisibach et al., 2021; Mayer et al., 2015). Moreover, even when validated on the same underlying construct, different means of operationalisation are chosen – one review (Mayer et al., 2015) identified 118 different tests used to assess outcomes. Finally, the definition of anticholinergic activity and the consideration of factors accounted for when assigning potency scores vary considerably among anticholinergic scales. For example, some scales include dosage, BBB-permeability, and/or the potential to interact with other drugs, whereas others wholly exclude drugs with certain modes of administration (e.g., ophthalmic drugs, inhaled drugs, etc.) due to hypothesised differences in absorption and thus altered *de facto* anticholinergic activity (Lertxundi et al., 2013; Mayer et al., 2015).

Due to the heterogeneity in anticholinergic scale construction, the interpretations of anticholinergic scores can be subjective. Depending on the intention of the original authors it may differ between anticholinergic scales and between authors conducting subsequent validation studies. While a full analysis of relationships between anticholinergic scales is beyond the scope of this description, the case of the interpretation of the possibly first-ever published anticholinergic scale (Jun et al., 2019; Kiesel et al., 2018) may be instructive.

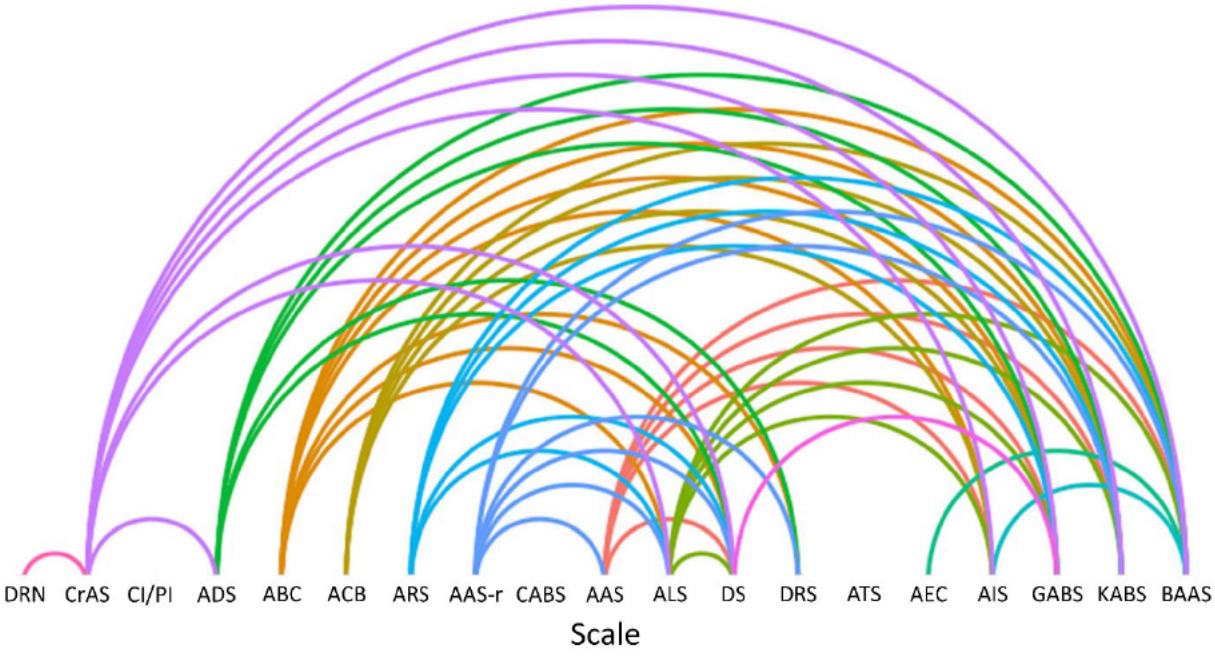
In 1978, Summers developed a method to predict the risk for a regimen of drugs to induce postoperative delirium (Summers, 1978). The author drew on previous hypotheses that explained the dysfunction as a reflection of an acute drug-induced anticholinergic syndrome. He then devised a scale (Drug Risk Number, DRN) to measure the risk of such a syndrome following pharmacological treatment. The drugs were classified into three classes, dependent on their presumed risk to cause an anticholinergic syndrome, based on the following definitions (Summers, 1978):

- "*Class I - known synergistic effect with anticholinergic agents, but not known as a direct cause of acute organic mental syndrome;*

- *Class II - known to cause delirium, but currently not documented to have CNS anticholinergic properties;*
- *Class III - known to cause delirium reversed by CNS active anticholinesterases or known to have central nervous system anticholinergic effect and to cause delirium."*

According to this definition, class I and class II drugs potentially exaggerate anticholinergic effects of other compounds and cause delirium without themselves exhibiting anticholinergic activity, respectively. However, only class III drugs were categorised based on their presumed anticholinergic effect. Moreover, the author focused on delirium specifically, and chose drugs primarily for their potential to predict the disorder, rather than on their potential to inhibit mAChRs. However, many authors (Han et al., 2001; Kersten & Wyller, 2014; Lisibach et al., 2021; Welsh et al., 2018) drawing on the DRN applied it as a measure of anticholinergic action. Although the use of the DRN in this way might be useful, it was developed for and validated on a specific population and for a specific outcome measure. Such generalisation of anticholinergic scales often constructed within a limited context, combined with great heterogeneity in the construction process, surely contribute to a lack of clarity and

Figure 5: Relationships between anticholinergic scales, sorted from earliest on the left to the latest on the right. Lines connecting scales indicate that the later scale was partially based on the earlier scale. Adapted from Lisibach et al. (2021).



reproducibility. Reviews have noted that most anticholinergic scales are strongly interdependent and rely on each other in their construction: Villalba-Moreno et al. (2016) found that 8/10 studied anticholinergic scales were directly based on previously published scales, while Lisibach et al. (2021) found that to be the case for 10/19 studied anticholinergic scales (**Figure 5**). However, despite their strong interdependence, the differences in the context in which anticholinergic scales were constructed nonetheless engenders low concordance (**Figures 6, 7**).

Figure 6: Concordance among different anticholinergic scales (n: 13 articles, 810 drugs). The heatmap depicts Spearman's rank correlation between scores for drugs common to each pair of anticholinergic scales. Coefficients that were not statistically significant ($p \geq 0.05$) were left blank in the original publication. Adapted from Al RIhani et al. (2021).

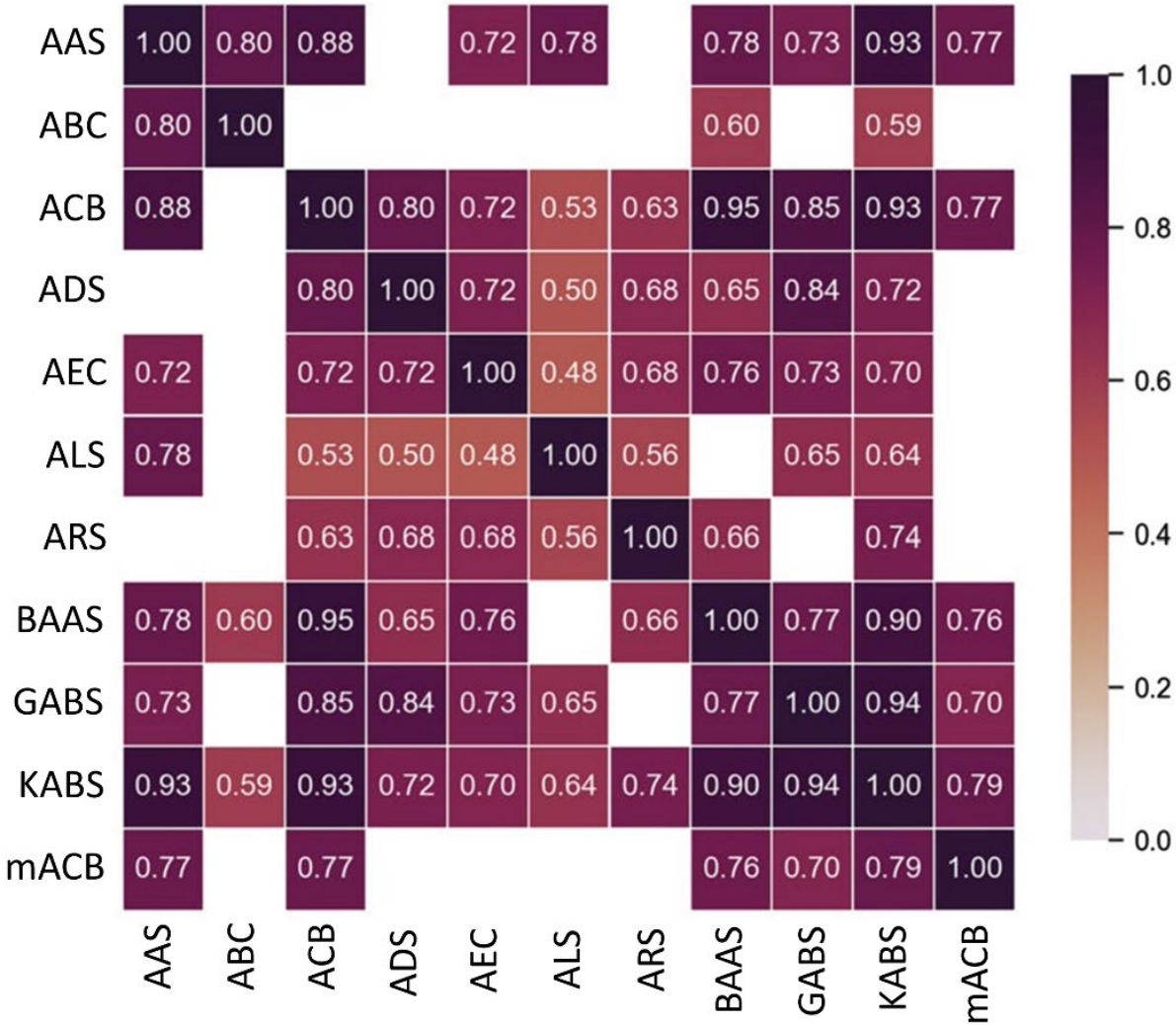
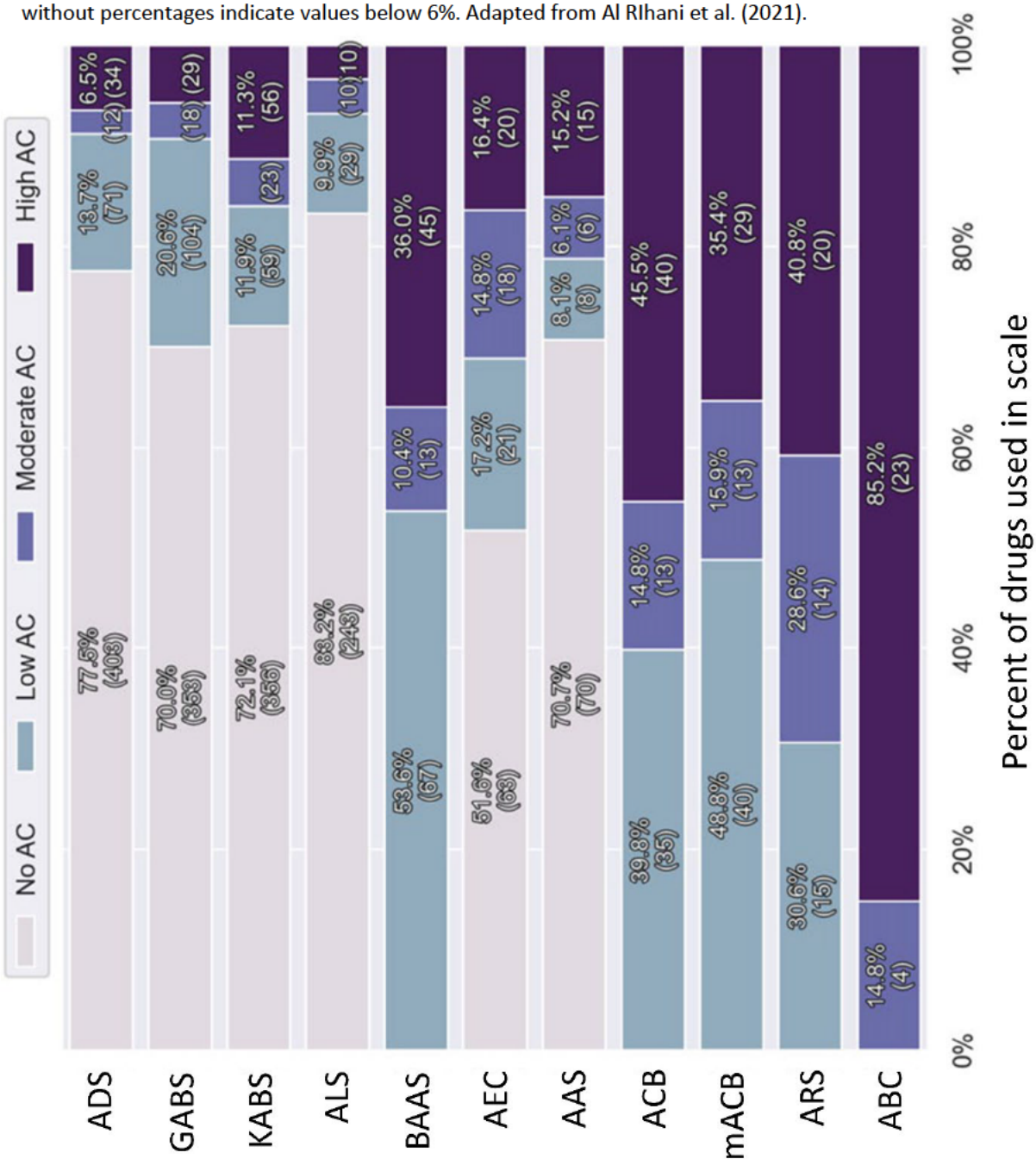


Figure 7: Percentage of drugs listed in different anticholinergic scales (n: 13 articles, 810 drugs). Differently coloured bars refer to different anticholinergic potencies, with darker hues signifying higher potencies. Bars without percentages indicate values below 6%. Adapted from Al RIhani et al. (2021).



The total numbers of drugs identified by anticholinergic scales vary widely and the lists are sometimes scarcely comparable, with the numbers of drugs identified as anticholinergic ranging from 17 to 154 (Mayer et al., 2015). Not only do the absolute numbers of included drugs differ, but so do their assigned scores. For example, when comparing 9 scales, one study

(Salahudeen, Hilmer, et al., 2015) found that out of 195 drugs, 34 (17%) were scored differently in different anticholinergic scales, while 12 (6%) were assigned a low anticholinergic potency in at least one scale and a high anticholinergic potency in another. Another review (Al Rihani et al., 2021) showed that among the 810 distinct drugs identified across 11 anticholinergic scales, 236 (29.1%) were found exclusively in one of the anticholinergic scales.

Additionally, whereas 404 drugs (49.9%) scored the same on at least two scales, 140 (17.3%) scored differently on at least one other scale. Such differences inevitably lead to estimations that strongly depend on the choice of anticholinergic scale. Thus, within a sample of 473 people with complex chronic conditions, the prevalence of anticholinergic drug use according to 10 anticholinergic scales varied between 29.6% to 79.1%, depending on the scale used (Tristancho-Pérez et al., 2021). Pairwise comparisons of anticholinergic scales paint a similar picture, with the study by Tristancho-Pérez et al. (2021) showing the scales to exhibit kappa statistics of between -0.175 and 0.708. Another study (Naples et al., 2015) of 3,055 community-dwelling older participants, showed concordance ratings for the ACB, ADS, ARS, and DBI to range from kappa=0.33 to 0.68. Similarly, in a sample of psychiatric patients, the ADS, ACB, and ADS showed poor agreement (kappa: ARS-ADS=0.20; ACB-ARS=0.25; ADS:ACB=0.21) (Lertxundi et al., 2013).

However, given their heterogeneity, the overall appraisal of anticholinergic scales has generally been remarkably positive. In a review, Al Rihani et al. (2021) evaluated the quality of studies that resulted in scale construction or validation. Using the Hawker took, comprising of nine different items to assess reporting in studies (Hawker et al., 2002), they concluded that most included studies were of good quality. Another recent systematic review (Lisibach et al., 2021) judged a total 60/104 validation studies (48.1%) to be of at least good quality. This included 1 RCT (good quality), 74 cohort studies (50 good and 24 poor quality), 9 case-control studies (6 good, 1 fair, 2 poor quality) and 20 cross-sectional studies (2 good, 18 poor quality). However, the quality differed between scales, with individual anticholinergic scales

exhibiting quality ratings from 11% to 75% according to the AGREE II tool (2017). Moreover, it is unclear which anticholinergic scale performs best under which circumstances.

Considering the large number of published anticholinergic scales, the differences that they display in the context of their generation, and in the consequent discordance in the inclusion of drugs and their scoring, great care would be expected in the choice of their use. However, a transparent account of the choice of anticholinergic scale is rarely provided by authors using these tools. This is surprising, as a consensus is lacking (Taylor-Rowan et al., 2021). As mentioned before, the DBI has been used most widely, but its popularity belies the fact that it is time-consuming to calculate and that due to copyright restrictions, it is limited in its use to healthcare practitioners registered in Australia (Welsh et al., 2018). A recent study (Lozano-Ortega et al., 2020) critically assessed and compared anticholinergic scales to determine their suitability for use in retrospective observation studies using administrative databases. Based on their analysis, the authors recommended the ACB and ADS, as they include many drugs, have been repeatedly validated, and have demonstrated the greatest inter-scale agreement (kappa=0.82). They warned against the use of the ABC, AAS, and ALS, due to their overdependence on SAA, and lack of thorough validation. For clinical use, the ACB, GABS, and AEC may be the most appropriate, as only these three anticholinergic scales provide explicit advice on their application (Lisibach et al., 2021).

In summary, although research into the effects of anticholinergic drugs hinges on their validity, anticholinergic scales are not generated according to any gold standard, and both widespread methods of assigning anticholinergic potency exhibit considerable flaws. Consequently, anticholinergic scales exhibit great heterogeneity in the context of their construction and the populations and outcomes for which they were validated. They also demonstrate scarce overlap in drugs identified as anticholinergic and in the computed AChB. However, in the absence of a clear consensus on the most appropriate way to measure anticholinergic use, many anticholinergic scales have found common use as tools to study a variety of health outcomes.

2.4 Anticholinergic drugs and health outcomes

The following section begins by describing the prescribing landscape of anticholinergics. This includes the associations between anticholinergic use and demographic characteristics, and longitudinal trends in prescribing over the past few decades. Because anticholinergic drugs are more frequently prescribed as people age, I then briefly describe the age-related changes in pharmacodynamics. The section concludes with a description the current knowledge on the long-term adverse health effects of anticholinergics. This does not include acute side effects described in section 2.4.2, but health outcomes associated with chronic use. Although this thesis focuses specifically on cognitive ability and dementia, I also devote a few sections on other important health outcomes. While cognitive ability and dementia are essential considerations for the use of these medications, they may not be the only or – depending on the context – the most important ones. The aim of this section is to enable the reader to gain an appreciation of the potential wide-ranging effects of anticholinergics and the factors that require careful consideration during prescribing and deprescribing.

2.4.1 Trends in anticholinergic prescribing

Due to the number of drugs that have been associated with anticholinergic effects and the variety of indications for which they are prescribed, their use is not limited to a homogenous, well-defined population. While most research has been conducted in older people – usually defined as aged 65 years or older – the variability in the region and time of testing and differences in the measurement of anticholinergic potency (see section 2.3), preclude a precise estimate of prevalence rates of anticholinergic use. Reports in community samples over the past three decades have ranged from 10% to 66% (Byrne et al., 2018; Fadare et al., 2021; Fox et al., 2011; Gnjidic, Bell, et al., 2012; Gnjidic, Hilmer, et al., 2012; Hilmer et al., 2009; Machado-Alba et al., 2016; Rémillard, 1996; Rhee et al., 2018; Richardson et al., 2015; Shmuel et al., 2021; Sumukadas et al., 2014), with participants in nursing homes or assisted-living facilities (Aalto et al., 2018; Blazer et al., 1983; Niznik et al., 2017), acute or long-term hospital wards (Choi et al., 2022; Kumpula et al., 2011; Lowry et al., 2011; Vickers et al., 2021;

Wawruch et al., 2012; Wilczyński et al., 2021), and those with dementia (Bhattacharya et al., 2011; Bosboom et al., 2012; Chatterjee et al., 2010; Hook et al., 2022; Kachru et al., 2015b; Kolanowski et al., 2009; Palmer et al., 2015; Sura et al., 2013) generally reported to exhibit higher prevalence rates of between 51%-65%, 14%-88%, and 27%-82%, respectively. Estimates of high-risk anticholinergic prescribing, or that which is viewed as "clinically relevant", also differ, with prevalence rates ranging from 3%-15% in community samples (Fadare et al., 2021; Fox et al., 2014; Kachru et al., 2015a; Rhee et al., 2018; Richardson et al., 2015), and 10%-37% in samples of people with dementia (Bhattacharya et al., 2011; Chatterjee et al., 2010; Kolanowski et al., 2009; Palmer et al., 2015; Sura et al., 2013).

Most AChB is attributable to prescribing within primary care, with previous estimates of anticholinergic use due to prescriptions by general practitioners found to be 40% (Reinold et al., 2021) and 53.1% (Rhee et al., 2018), with the estimate possibly higher among older participants (Reinold et al., 2021). Other important sources of anticholinergic prescribing include psychiatrists, neurologists, and urologists (Rhee et al., 2018). Accounts vary as to the contribution of individual drugs to the total AChB, with different authors attributing the greatest burden to anxiolytics, hypnotics, and antidepressants, (Byrne et al., 2018), diuretics, beta-blockers, and antipsychotics (Niznik et al., 2017), antidepressants and antihistamines (Rhee et al., 2018), and antidepressants, antispasmodics, and antipsychotics (Rémillard, 1996), among others. In a segregated analysis, Reinold et al. (2021) demonstrated the importance of age in this respect, with antihistamines, antibiotics, and glucocorticoids most common in individuals aged 19 years or younger, antidepressants in 20–49-year-olds, antidepressants, cardiovascular drugs, and antidiabetics in 50-64-year-olds, and drugs for an overactive bladder most common in older age groups.

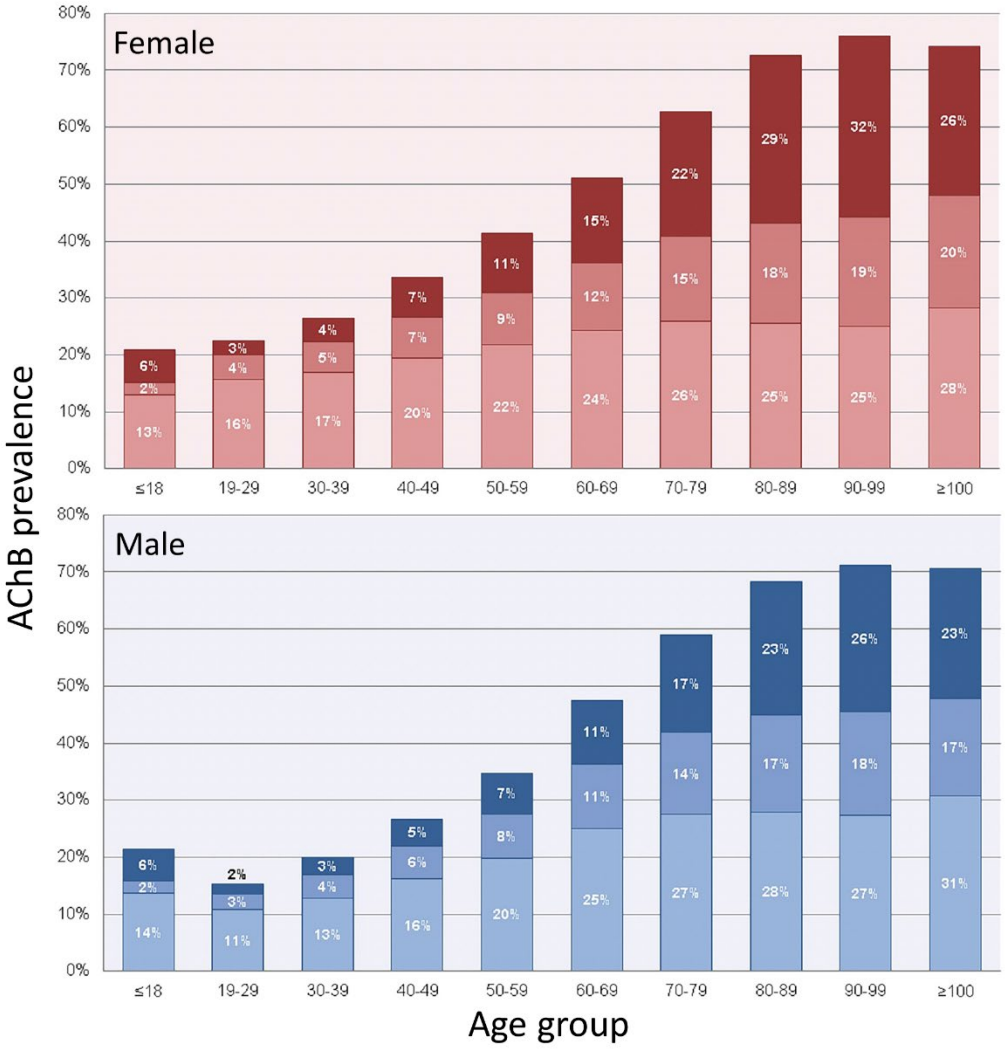
Despite the variability in precise estimates of prevalence rates, research has consistently shown certain factors to increase the odds for the use of anticholinergics. Studies that explored prescribing across a wider age range have reported age to be a major risk factor. An early study using linked healthcare data of almost 1 million participants (Rémillard, 1996) found older individuals (n=128,010) to be three times as likely to be prescribed anticholinergic

drugs compared to people younger than 65 years. Another study used the German Pharmacoepidemiological Research database that contains claims from four health insurance providers in Germany and includes 20% of the German population. They found AChB so high as to be considered clinically relevant to increase in older age (90-99 years) from 7% to 26% in men and from 10% to 32% in women when compared to a younger cohort (50-59 years) (Reinold et al., 2021). Interestingly, the association between anticholinergic use and age does not seem to be linear (**Figure 8)**. Reinold et al. (2021) classified participants into 10-year bins (19-29, 30-39, 40-49, etc.), with all individuals younger than 18 years (n=~1.4 million) and older than 100 years (n=374) in separate bins. They found AChB in the age group below 40 years (3 comparison groups) to be highest in minors. Additionally, among older people, the very old seem to be less at risk of anticholinergic prescribing (Kachru et al., 2015a; Kristensson et al., 2021; Shmuel et al., 2021). This possibly reflects the reduced health status of older individuals and the need to deprescribe to avoid side effects and harmful drug interactions.

Polypharmacy – usually defined as the concomitant prescription of either ≥5 or ≥10 drugs (Guthrie et al., 2015) – is also reported as a risk factor for high anticholinergic use (Byrne et al., 2018; Choi et al., 2022; Kersten & Wyller, 2014; Rhee et al., 2018; Shmuel et al., 2021; Sumukadas et al., 2014). One study (Byrne et al., 2018) reported anticholinergic prevalence to rise from 43% in individuals prescribed 0-4 chronic drugs to 95% in those taking 12 or more chronic drugs, with an adjusted odds ratio (OR) of 27.8 (95% CI=26.7-29.0). Unsurprisingly, another commonly reported risk factor for anticholinergic use is multimorbidity, i.e., the presence of multiple chronic health conditions (Niznik et al., 2017; Rhee et al., 2018). Some disorders are particularly associated with an increased AChB, including depression (Kumpula et al., 2011; Niznik et al., 2017), stroke (Kumpula et al., 2011), dementia (Kersten & Wyller, 2014; Wilczyński et al., 2021), Parkinson's disease (Kumpula et al., 2011), hypertension (Niznik et al., 2017), urinary incontinence (Niznik et al., 2017), psychosis (Shmuel et al., 2021), and anxiety (Kachru et al., 2015a). Additionally, frail individuals (Shmuel et al., 2021), those with intellectual disabilities (Ward et al., 2021; Ward McKernan et al., 2022), and who have been

hospitalised or have visited a medical specialist within the last year (Niznik et al., 2017) also exhibit higher anticholinergic use.

Figure 8: Prevalence of AChB according to the ACB scale in women (**top**) and men (**bottom**) from a ~20% sample of the German population. The different shades of the colours represent different categories of anticholinergic potency, with the darkest hue corresponding to the greatest potency (AChB=3) and the lightest hue corresponding to the smallest potency (AChB=1). Adapted from Reinold et al. (2021).



As alluded to previously, institutionalisation represents another major risk factor, with higher anticholinergic prescribing observed in individuals living in geriatric wards, nursing homes, and assisted living facilities (Byrne et al., 2018; Campbell et al., 2021; Haasum et al., 2012; Hook et al., 2022; Kersten & Wyller, 2014; Rémillard, 1996; Rhee et al., 2018; Shmuel et al., 2021; Sumukadas et al., 2014; Wilczyński et al., 2021). Additional demographic risk factors for

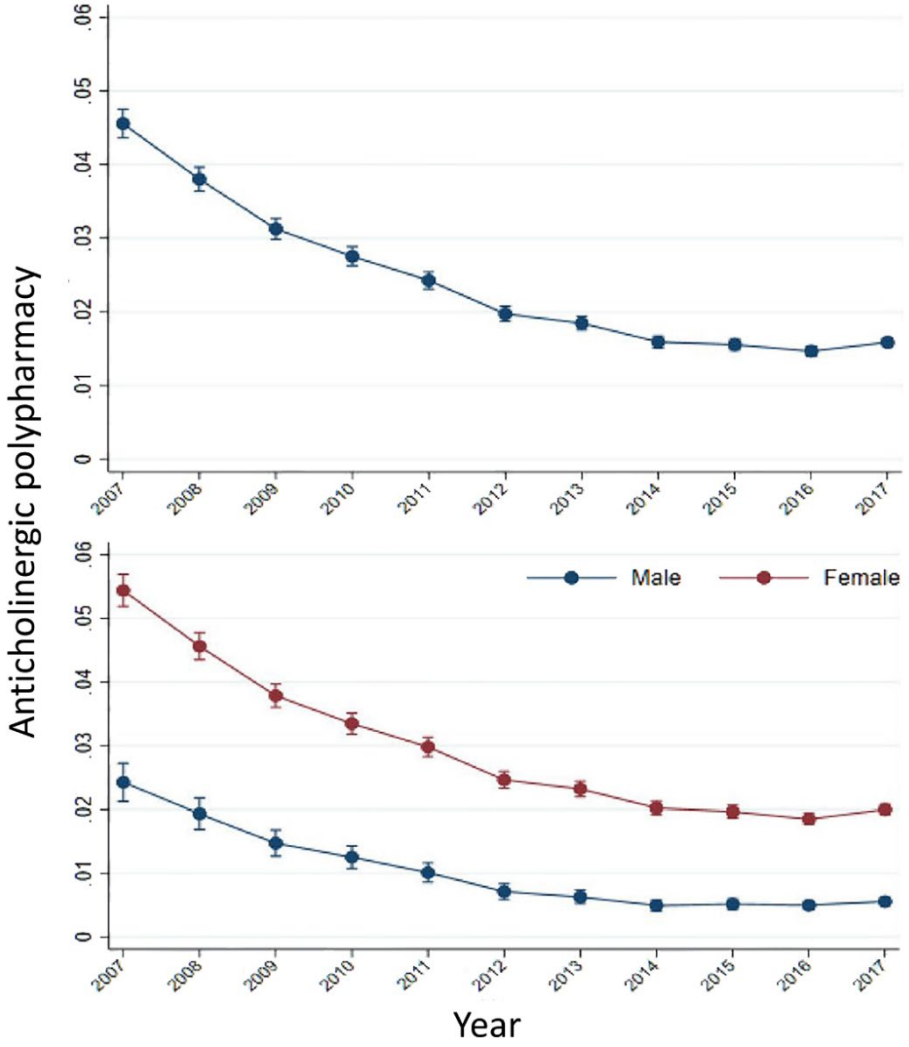
anticholinergic use are female sex (Byrne et al., 2018; Campbell et al., 2021; Kachru et al., 2015a; Reinold et al., 2021; Rémillard, 1996; Rhee et al., 2018; Shmuel et al., 2021; Sumukadas et al., 2014), socioeconomic deprivation (Sumukadas et al., 2014), and lower educational attainment (Kachru et al., 2015a).

Due to the differences in prevalence estimates, longitudinal changes in anticholinergic prescribing cannot be inferred by comparing studies that used different samples in different settings. However, analyses of different time points within the same sample are comparable. Based on data of office-based physician visits from 2006 and 2015, Rhee et al. (2018) found the rate of high-risk anticholinergic prescribing in the USA increased from 6.1% in 2006/2007 to 6.8% in 2008/2009 but decreased to 4.6% in 2014/2015. Similarly, studying claims of Medicare beneficiaries with overactive bladder between 2006 and 2017, Campbell et al. (2021) observed the share of individuals co-prescribed multiple strong anticholinergics to have decreased from 3.3% in 2006 to 1.7% in 2017 (**Figure 9**). Finally, anticholinergic prescribing has also decreased in US nursing homes. Malagaris et al. (2020) found the prevalence rates remained stable at about 34% from 2009 to 2011, but then steadily dropped to reach 24% in 2017. Thus, the trend in the USA over the past decade has been one of a steady decline in the use of anticholinergic drugs in older people. This is likely not incidental: in a cross-sectional survey of American Urogynecologic Society members (Menhaji et al., 2021), almost all were aware of the recent literature linking anticholinergics to dementia and 58.5% reported having shifted their prescribing to other agents because of that knowledge.

In Europe, the trend does not seem to be the same as in the US. In an analysis of 1.8 million older Swedes, Hovstadius et al. (2014) found that in the period between the years 2006 and 2013 six out of eight indicators of drug-prescribing quality exhibited positive developments. The frequency of anticholinergic prescribing was the only indicator to display an undesirable, increasing trend. Similar findings have been reported in the UK, where between the years 1995 and 2010, the average AChB increased for all individuals except those older than 85 years in both Scotland (Sumukadas et al., 2014) and England (Grossi et al., 2020). In this period, the prevalence of high-potency prescribing increased from 7.3% in Scotland and 5.7%

in England to 9.9% in each (Grossi et al., 2020; Sumukadas et al., 2014). However, a recent study on community-dwelling individuals older than 75 years found decreases in anticholinergic prescribing in Finland between 2009 and 2019 (Rinkinen et al., 2022). Studies in people living in nursing homes (Aalto et al., 2020) and in individuals with clinically significant cognitive impairment (Grossi et al., 2020) have also found increasing trends in anticholinergic prescribing over time. In summary, studies on longitudinal trends in anticholinergic prescribing do not paint a uniform picture, and results may differ between countries, age groups, and study populations.

Figure 9: Prevalence of anticholinergic polypharmacy (concomitant use of more than one anticholinergic drug) in all participants (**top**) and separately for each sex (**bottom**) in a sample of people with overactive bladder from the US. Adapted from Campbell et al. (2021).



2.4.2 Acute side effects of anticholinergic drugs

The side effects of anticholinergic drugs at therapeutic doses are mainly due to their inhibition of mAChRs and – at high doses – nAChRs (Brayfield, 2014). Anticholinergic compounds differ in the onset and the duration of their effects (Dowd, 2017), but do exhibit common characteristics of their actions on effector organs. Upon increasing the dose of anticholinergic medication, stepwise effects can usually be observed, with symptoms relating to antimuscarinic activity affecting in turn different processes in the body (**Table 7**) (Heller Brown et al., 2018). Their presentation is often described by the adage "hot as a hare, blind as a bat, dry as a bone, red as a beet, and mad as a hatter" (Feinberg, 1993; Peters, 1989).

Table 7: The effects of anticholinergics for the example of atropine. As the dose of the drug increases, the symptoms become more pronounced and new symptoms appear. Adapted from Heller Brown (2018).

Dose (mg)	**Effects**
0.5	slight cardiac slowing; some dryness of mouth; inhibition of sweating
1	definite dryness of mouth; thirst; acceleration of heart, sometimes preceded by slowing; mild dilation of pupils
2	rapid heart rate; palpitation; marked dryness of mouth; dilated pupils; some blurring of near vision
5	previous symptoms marked; difficulty in speaking and swallowing; restlessness and fatigue; headache; dry, hot skin; difficulty in micturition; reduced intestinal peristalsis
>= 10	previous symptoms more marked; pulse rapid and weak; iris practically obliterated; vision very blurred; skin flushed, hot, dry, and scarlet; ataxia, restlessness, and excitement; hallucinations and delirium; coma

Peripheral side effects arise due to the inhibition of receptors in the ANS, and they affect multiple organ systems (Brayfield, 2014; Dowd, 2017; Heller Brown et al., 2018). They include dry mouth (xerostomia), thirst, a burning sensation in the throat, difficulty swallowing, and flushing and drying of the skin. They also lead to transient bradycardia followed by tachycardia, disturbances in the atrial rhythm, an absence of sweating, and the dilation of cutaneous blood vessels, accompanied by a consequential increase in body temperature, and

serious airway adverse events like airway obstruction and laryngospasm (Boriosi et al., 2022). Additionally, they cause changes in the gastrointestinal- and urinary tracts, including reductions in the motility of the gastrointestinal tract and consequential constipation and urinary retention. Finally, anticholinergic drugs can cause ocular symptoms, such as blurred vision, dilation of the pupils (mydriasis), loss of accommodation (cycloplegia), and photophobia (Brayfield, 2014; Dowd, 2017; Heller Brown et al., 2018).

Overdoses – and occasionally therapeutic doses – of anticholinergics can cause central effects that include restlessness, confusion, excitement, ataxia, lack of coordination, paranoid and psychotic symptoms, hallucinations, delirium, and cognitive impairment, especially diminished short-term memory. Cases of severe overdose can result in CNS depression, followed by coma, circulatory and respiratory failure, and death (Brayfield, 2014). Although fatalities are rare, the poisoning through the ingestion of belladonna alkaloids represents a major cause of poisonings (Heller Brown et al., 2018). However, most people that exhibit symptoms caused by anticholinergic toxicity, recover within a few hours or days (Dowd, 2017), and the effects are usually reversible upon cessation of anticholinergic medication (Brayfield, 2014).

2.4.3 Side effects in older people

As mentioned before, most studies on anticholinergic prescribing have been done in older people. One of the reasons for this is that the risk of side effects from any medication generally increases with age (McLean & Le Couteur, 2004). As people age, the pharmacokinetics of drug use undergo a multitude of changes. The gastric pH is altered, gastric emptying is delayed, and the blood flow to the small intestine is reduced (Reidenberg, 1982; Swift, 1988). These changes could affect the bioavailability of drugs; although studies on passive absorption have not demonstrated great differences between age groups (Reidenberg, 1982; Swift, 1988). Next, due to reductions in muscle mass and plasma albumin, and an increase in body fat, the distribution of drugs in the body changes (Reidenberg, 1982; Swift, 1988). Additionally, a smaller liver, reduced hepatic blood-flow, diminished clearance of some oxidatively metabolised compounds, and reductions in the function of some

cytochrome P450 enzymes lead to changes in drug metabolism (Kinirons & O'Mahony, 2004) – compared to young adults, older adults seem to metabolise drugs up to 35% slower (Reidenberg, 1982). Next, due to a decline in glomerular function and tubular secretion, renal excretion is altered and any renally eliminated compounds accumulate more readily (Trenaman et al., 2021).

In older age, homeostatic mechanisms, such as postural stability, orthostatic responses, and thermoregulation are also more prone to disruption (Feinberg, 1993; Swift, 1988), and autonomic reflexes, including cardiovascular, respiratory, and gastrointestinal reflexes are weakened (Feinberg, 1993). Chronic diseases also become more common in old age, and individuals are more likely to suffer from multimorbidity, the co-occurrence of several chronic conditions, which decreases their level of physical functioning (Brockmöller & Stingl, 2017; Navickas et al., 2016; Williamson & Chopin, 1980). Additionally, polypharmacy – which is more common among the older population – may affect the drugs' respective pharmacokinetics (Guthrie et al., 2015; Hajjar et al., 2005). Finally, ageing is accompanied by a rise in the risks for a reduced income, greater deprivation, and loneliness, which can affect an individual's ability to cope with environmental insults (Williamson & Chopin, 1980).

For the specific case of anticholinergics, previous research has confirmed many of the general observations noted before. For example, the effects of hyoscine in reducing cognitive function are much more pronounced in older age (Flicker et al., 1992). Additionally, fatal heatstroke in older people has been associated with the combination of the use of anticholinergics and a hot environment (Peters, 1989). Along with a general disruption of multiple bodily processes, it has been argued that a specifically cholinergic deficit in the CNS accompanies the ageing process (Feinberg, 1993). Additionally, notable changes in drug absorption due to specifically anticholinergic action have been demonstrated for several drugs (Peters, 1989). Studies have also shown that compared to younger or middle-aged adults, older people exhibited higher plasma concentrations of amitriptyline, fluvoxamine, and clomipramine, an increased concentration to dose ratio of nortriptyline (Waade et al., 2012), a decreased elimination rate

of solifenacin (Doroshyenko & Fuhr, 2009) and hyoscine (Alvarez-Jimenez et al., 2016), and increased levels of bioavailability of oxybutynin (Hughes et al., 1992).

However, while older individuals are on average at a greater risk of side effects associated with anticholinergic drugs, several caveats are worth noting. First, it is not entirely clear to what extent the side effects of drugs in general or of specifically anticholinergics are affected by the changes associated with ageing. Second, these changes represent an average trend and individuals exhibit a great degree in variation in their responses to drugs (McLachlan et al., 2009; Swift, 1988). This is partly because side effects of drugs are not affected by ageing itself, but by a combination of multimorbidity, polypharmacy, and alterations in pharmacokinetics (McLean & Le Couteur, 2004), all of which can markedly differ from person to person. Third, while age-related changes may increase the body's sensitivity to some physiologically active substances (Feinberg, 1993; Reidenberg, 1982), it may also lead to decreases in sensitivity to other compounds (Reidenberg, 1982). Finally, although age is an important risk factor for both anticholinergic use and anticholinergic side effects, even in groups of younger adults, the prevalence of anticholinergic prescribing may be up to 7% (Reinold, 2021). As described below, chronic long-term use of anticholinergics may adversely affect several health outcomes and there is a considerable need for more research in younger populations.

2.4.4 Long-term side effects

Over the past two decades, research has been conducted into the potential negative health outcomes associated with anticholinergic use beyond the acute side effects described before. Mayer et al. (2015) found 448 evaluations of associations between AChB and outcome measures, 182 (41%) of which were positive. Lisibach et al. (2021) identified 15 studies on delirium, 54 on cognition, 20 on mortality, and 24 on falls. These reviews were limited to studies applying existing anticholinergic scales in their analyses. Because authors often utilise custom ways to measure anticholinergic exposure or categorise the latter based on information available in their sample, the total number of studies on this topic is even greater. What is common to most of them, is the heterogeneity of results and lack of clarity for

interpreting them (**Figure 10**). The below segment will review the evidence on the relationship between anticholinergic use and the most studied adverse outcomes and present the prevailing issues and contradictions.

2.4.4.1 Cognitive outcomes

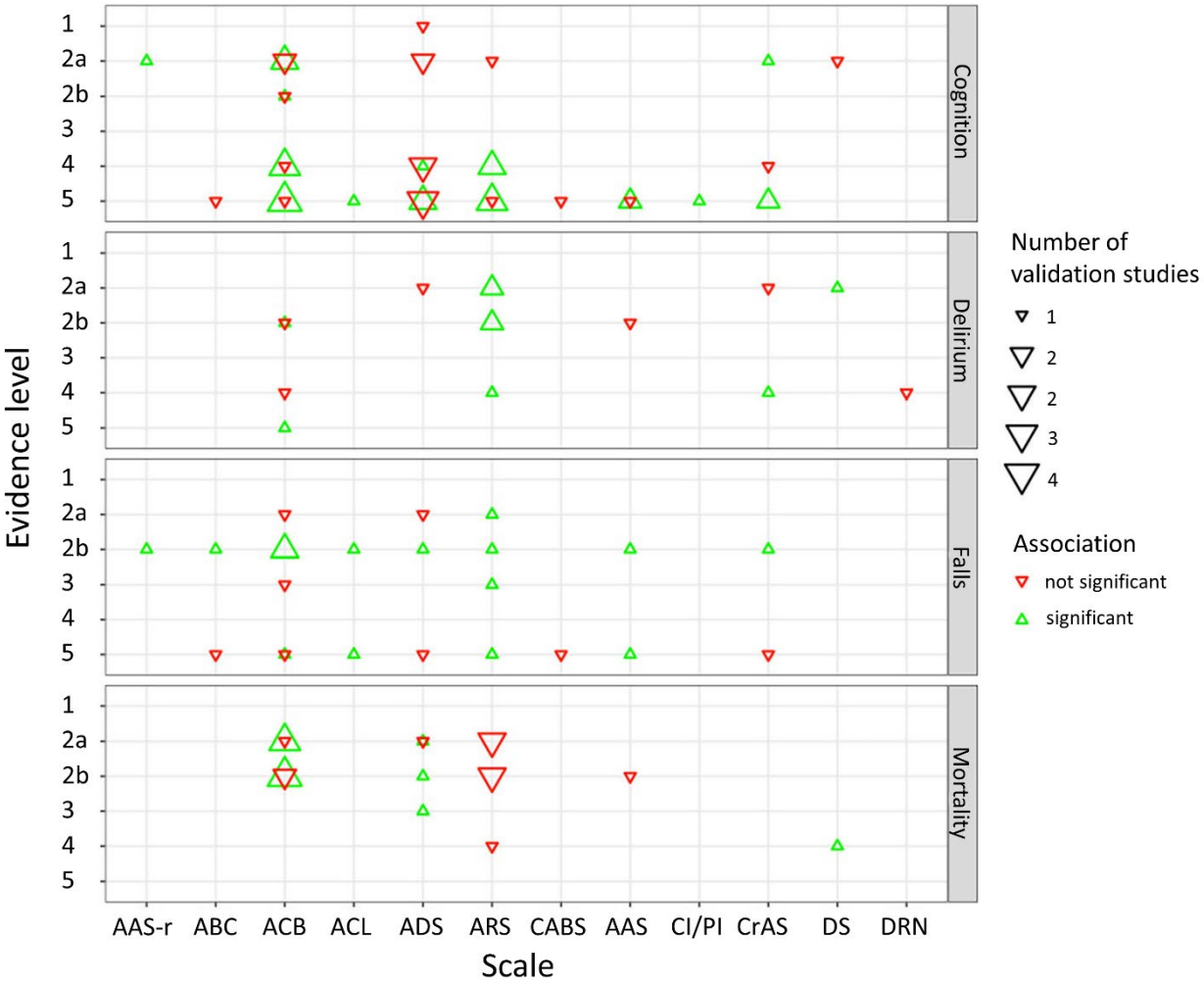
Delirium

As described before, cognitive deficits are known to occur at overdoses and occasionally at therapeutic doses of anticholinergic drugs. Delirium – an acute brain syndrome resulting in cognitive change (Inouye et al., 2014) – has been recognised as a side effect of anticholinergics for over half a decade (Duvoisin & Katz, 1968; Granacher & Baldessarini, 1975; Longo, 1966). It is thought that medications account for 12%-39% of all cases of delirium (Alagiakrishnan & Wiens, 2004). Additionally, because delirium is thought to occur due to an imbalance in the cerebral neurotransmission, the association between this condition and anticholinergic use is biologically highly plausible (Collamati et al., 2016). However, the syndrome is still poorly understood and the empirical association with anticholinergics is unclear. Reviews on the topic (Collamati et al., 2016; Egberts et al., 2021; Reisinger et al., 2022; Welsh et al., 2018) have reported discrepant findings, with the largest systematic review (n: 16 studies, 148,756 people) revealing great heterogeneity among studies and a strong dependency of the result on the anticholinergic scale used (Egberts et al., 2021). For example, when using the ARS, AChB was associated with delirium in 5/5 studies, whereas the AChB according to the ACB and ADS was associated with delirium in only 1/6 and 1/5 studies, respectively.

Until recently, most extant anticholinergic scales had not been applied to test the association between anticholinergic use and delirium. However, a recent study (Lisibach, Gallucci, Benelli, et al., 2022) that compared 19 different anticholinergic scales, found 14 or 16 (depending on the diagnostic procedure) to associate with delirium. The heterogeneity in past results possibly reflects differential effects of individual anticholinergic drugs on the risk of delirium. For example, a recent study exploring associations between drugs for overactive bladder and delirium, found substantial differences between individual compounds. They found oxybutynin, but not solifenacin to associate with delirium (Nishtala & Chyou, 2022). Finally,

some evidence suggests that even though anticholinergic use may not increase the short-term risk of delirium, the risk might increase with long-term continuous use (Welk et al., 2022), suggesting a need for more longitudinal studies.

Figure 10: Number of validation studies for different anticholinergic scales. The four brackets depict different outcome measures: cognition, delirium, falls, and mortality; the rows represent different levels of evidence quality according to the AGREE II tool: 1 RCT (good and fair quality), 2a RCT (poor quality) and prospective cohort studies (good and fair quality), 2b retrospective cohort studies (good and fair quality), 3 case-control studies (good and fair quality), 4 cohort and case-control studies (poor quality) and 5 cross-sectional studies (good, fair, and poor quality). For each outcome, the number of validation studies within each quality level is depicted, separated based on whether the results were statistically significant. Adapted from Lisibach et al. (2021).



Dementia

Over the past few years, much has been published on the association between anticholinergic use and dementia, with the literature generally suggesting a positive relationship. A recent

Cochrane systematic review and meta-analysis of longitudinal studies on the topic (Taylor-Rowan et al., 2021) found a positive association between AChB and dementia, mild cognitive impairment, or cognitive decline (OR=2.63, 95% CI=1.09-6.29, pooled from 4 studies, n=125,359). Significant risk ratios (RRs) for the association between anticholinergic use and adverse cognitive outcomes have been documented in other reviews (RR=1.46, 95% CI=1.17-1.81, pooled from 6 studies, n=645,865 (Dmochowski et al., 2021); RR=1.20, 95% CI=1.15-1.26, pooled from 14 studies, n=1,564,181 (Zheng et al., 2021)). Additionally, there have been reports of an increased risk of dementia with longer exposure to anticholinergic drugs (Dmochowski et al., 2021; Pieper et al., 2020) and evidence for cumulative effects (Taylor-Rowan et al., 2021; Zheng et al., 2021). Moreover, studies document possible dose-response (Taylor-Rowan et al., 2021; Zheng et al., 2021) and potency-response (Taylor-Rowan et al., 2021) relationships, with most studies included in the reviews reporting both higher doses and higher anticholinergic scores to correspond to an increased risk of dementia. Studies have also been conducted on vulnerable populations. Hospitalised individuals exhibited a greater risk of incident dementia when using highly potent anticholinergic drugs (Hsu et al., 2021). Additionally, people with dementia have been shown to experience greater cognitive decline when taking highly anticholinergic urinary drugs (Bishara, Perera, Harwood, Taylor, Sauer, Funnell, Stewart, et al., 2021), or anticholinergic antidepressants (Bishara, Perera, Harwood, Taylor, Sauer, Funnell, Gee, et al., 2021). However, two recent reviews on the association between anticholinergic use across different classes of drugs and cognitive outcomes in people with AD have reported mixed results (n: 18 studies, 102,684 people (Taylor-Rowan et al., 2022); n: 14 studies, 157,023 people (Wang et al., 2021)).

As is the case with other long-term outcomes associated with anticholinergic use, studies on the relationship between anticholinergics and dementia have been predominantly conducted in older individuals. In a 2009 review, the authors maintained that while there was support for mostly positive associations between anticholinergic use and cognitive dysfunction, there was no evidence for a long-term relationship (Campbell et al., 2009). However, in the last few years, at least four studies explored the associations between the use of anticholinergics in middle age and later cognitive decline or dementia. One study (Chuang et al., 2017) classified

middle-aged participants into two groups, based on whether the anticholinergic effects of the drugs that they were taking were well established ("definite") or only assumed ("possible"). Interestingly, possible users of anticholinergic drugs at a mean follow-up of 20.1 years exhibited an increased hazard ratio (HR) of incident AD (HR=1.63, 95% CI=1.02-2.61) compared to non-users, but definite users did not (HR=0.90, 95% CI=0.52-1.52). As a possible explanation for this result, the authors noted that on average, definite users exhibited both shorter duration of exposure, as well as lower frequencies of medication use. Another study (Richardson et al., 2018) found a positive association between the prescription of an anticholinergic drug in middle-age with dementia (OR=1.10, 95% CI=1.08-1.14, follow-up 4-20 years). However, not all potency groups exhibited a dose-response effect, and a higher anticholinergic potency did not always correspond to a greater effect size. The association between anticholinergic use and dementia was also dependent on the class of drugs for which AChB was computed. A third study (Coupland et al., 2019) found a dose-response effect for the association between anticholinergic use and dementia (ORs between 1.06 (95% CI=1.03-1.09) and 1.49 (95% CI=1.44-1.54), depending on dose, follow-up 1-11 years), with AChB in periods closer to the date of diagnosis exhibiting larger effect sizes. The study concluded that 10% of all dementia diagnoses were attributable to anticholinergic drug use. Finally, a recent study (Harnod et al., 2021) explored the association between bladder anticholinergics and dementia in individuals 55 years of age or older. While it is unclear what proportion of the sample consisted of middle-aged participants, the authors found a 2.46-fold increase (95% CI=2.22-2.73) in the risk of dementia in people who used bladder antimuscarinics for at least one year when compared to those who didn't use these drugs at all.

Despite the wealth of research linking anticholinergic use with dementia, several problems and unanswered questions remain. First, many of the past studies exhibited major shortcomings. A Cochrane review of longitudinal studies (Taylor-Rowan et al., 2021) judged the overall certainty of evidence to be low, indicating that the estimate is likely to change with future research. This was due to a high risk of bias in 19/25 included studies, lack of control for confounders (e.g., 12/25 included studies did not control for psychiatric conditions), lack of control for reverse causation, imprecision in effect sizes, and risk of

publication bias. A moderate-to-high risk of bias has also been reported by others (Dmochowski et al., 2021; Pieper et al., 2020). Additionally, most studies explore as the outcome the diagnosis of general dementia; data on specific subtypes or other related disorders remains limited. Recent reviews identified only four studies on mild cognitive impairment (Taylor-Rowan et al., 2021), two on vascular dementia (Zheng et al., 2021), and two on other neurodegenerative disorders (Zheng et al., 2021), with most reporting mixed or negative results (Taylor-Rowan et al., 2021; Zheng et al., 2021). Furthermore, a wide variety of anticholinergic scales has been reported in the literature, and the choice of scale can have a strong influence on the estimate (Hsu et al., 2021). There are not enough data for a thorough comparison of all different anticholinergic scales, but their relative performances are heterogeneous between studies that compared different scales (Taylor-Rowan et al., 2021). Moreover, a recent study that compared the performance of ten anticholinergic scales in predicting cognitive impairment in older complex chronic patients, found only one scale to show a significant, albeit low, discriminatory capacity (Tristancho-Perez et al., 2022). Finally, while most studies indicate a positive relationship between anticholinergic use and cognitive decline in older age, and recent research has highlighted a possible adverse role of anticholinergics even when used decades before the onset of dementia (Coupland et al., 2019; Harnod et al., 2021; Richardson et al., 2018), all anticholinergic drugs may not have this effect.

To my knowledge, five studies have formally compared drug classes in their association with dementia. Gray et al. (2015) singled out only antidepressants, as they were interested in possible prodromal symptoms affecting the hypothesised associations. They found positive associations with dementia both for antidepressants, as well as the non-antidepressants. Richardson et al. (2018) compared classes of drugs by anticholinergic potency group and by exposure period. Whereas prescriptions of anticholinergic antidepressants, antiparkinsonian drugs, and urological drugs exhibited associations with dementia, prescriptions of anticholinergic antispasmodics, antipsychotics, and antihistamines did not. The study made clear that associations between some classes of anticholinergic drugs (e.g., low-potency antidepressants and antiparkinsonian drugs) and dementia strengthen as the date of

dementia diagnosis approaches, while other classes of anticholinergics (e.g., higher-potency antidepressants and urological drugs) show consistent associations regardless of the exposure period. This indicates that some of the observed effects may be causally linked, as opposed to merely acting as markers of prodromal dementia. Coupland et al. (2019) compared classes of drugs divided into groups of different prescribed dosages. Generally, they found increases in risk associated with antidepressants, antiparkinsonian drugs, antipsychotics, urological drugs, and antiepileptic drugs. Antivertigo drugs showed mixed results and there was no effect for antihistamines, muscle relaxants, antispasmodics, antiarrhythmics, and respiratory drugs. Not all drug classes exhibited dose-response relationships. One group (Joung et al., 2019) compared antidepressants, antihistamines, and urinary drugs separately, all of which were associated with an increased risk of dementia. Finally, Hafdi et al. (2020), in addition to analysing all drugs, also analysed the sample while excluding antidepressants and antipsychotics to discount medication for possible prodromal dementia. Overall, the effect size of the association was reduced after the exclusion, suggesting that antidepressants and antipsychotics strongly contributed to the observed effect. Furthermore, studies have also shown differences between drugs within the same therapeutic class. For example, among drugs to treat overactive bladder, reviews have found positive associations with dementia for oxybutynin and tolterodine, but not for trospium, darifenacin, imidafenacin, fesoterodine, and solifenacin (Dantas et al., 2022; Duong et al., 2021; Malcher et al., 2022). Thus, as mentioned previously, not only different classes of drugs but even individual drugs within a class might exhibit distinct associations with dementia. Differences in BBB-permeability and binding specificity for receptor subtypes between these drugs may help to explain the observed disparities (Welk et al., 2021).

As the above account makes clear, not all anticholinergic drugs can be painted with the same brush. Regarding the association with dementia, there is some concordance among studies: antidepressants, antiparkinsonian drugs, and urological drugs have mostly exhibited positive associations, while cardiovascular drugs, gastrointestinal drugs, and antihistamines have not. However, even among published reviews, there is disagreement regarding the effects of antipsychotics, analgesics, and respiratory drugs (Taylor-Rowan et al., 2021; Zheng et al.,

2021). I share the view of Richardson et al. (2018), who in conclusion to their study on the relationship between AChB and dementia specifically recommended future work to further explore drug classes as opposed to examining a general anticholinergic effect.

Cognitive ability

A lot of research has been published on the association between anticholinergic use and cognitive decline in older age. However, relatively little work has been done on changes in cognition within the "normal" spectrum of cognitive functioning. In fact, as has been noted before, many studies on anticholinergic use and cognitive function in adults classify cognition in binary terms as either the absence or presence of cognitive impairment (Ghezzi et al., 2021). Alternatively, cognition in adults is assessed with questionnaires designed to detect possible dementia (e.g., the Mini-mental state examination or MMSE (Tombaugh & McIntyre, 1992)), as opposed to estimating cognitive ability within the "normal" spectrum of cognitive functioning. This line of research has generally yielded a great heterogeneity in the quality of publications (Andre et al., 2019; Wang et al., 2021), and mixed results (Andre et al., 2019; Mehdizadeh et al., 2021; Wang et al., 2021) in the association between anticholinergic use and cognition, even in samples of people with existing dementia (Wang et al., 2021), or characterised by substantial frailty (Mehdizadeh et al., 2021).

Among studies exploring the association of anticholinergic use and tests that assess distinct domains of cognitive function, the results are equally mixed. There have been reports of negative associations between anticholinergic use and tests measuring attention (Ancelin et al., 2006), visuo-spatial- and language abilities (Ancelin et al., 2006), associative learning (Low et al., 2009), executive function (Attoh-Mensah et al., 2020; Bottiggi et al., 2006; Neelamegam et al., 2020; Posis et al., 2022), and episodic memory (Ancelin et al., 2006; Moriarty et al., 2021; Papenberg et al., 2017), and mixed associations or lack thereof for verbal fluency (Carrière et al., 2009; Koyama et al., 2014; Papenberg et al., 2017; Posis et al., 2022), visual memory (Carrière et al., 2009), reaction time (Ancelin et al., 2006; Low et al., 2009), delayed recall (Koyama et al., 2014), implicit memory (Ancelin et al., 2006), semantic memory (Papenberg et al., 2017), processing speed (Papenberg et al., 2017; Posis et al., 2022),

reasoning (Ancelin et al., 2006), and executive functioning (Posis et al., 2022). While most studies have been cross-sectional, some have also reported a greater decline in cognitive functioning with anticholinergic use. Two longitudinal studies (Broder et al., 2021; Posis et al., 2022) found anticholinergic use to be associated with cognitive decline. This was true for several cognitive modalities, including executive function, episodic memory, psychomotor speed, verbal learning, and executive function. Another study (Shah et al., 2013) that aggregated multiple cognitive tests into a single global measure of cognition, found incident users (first use of anticholinergics after study entry) to exhibit a significantly more rapid decline when compared to non-users, whereas prevalent users did not. However, both user groups exhibited a negative tendency in their slopes of decline that was steeper when compared to non-users (albeit non-significantly for prevalent users). To additionally complicate the relationship between anticholinergic use and cognitive ability, there is some evidence for sex differences (Carrière et al., 2009), differences between age groups (Attoh-Mensah et al., 2020; Dos Santos et al., 2022), between individuals with different genotypes for apolipoprotein E (*APOE)* (Collin et al., 2021), and between anticholinergic scales (Kashyap et al., 2014).

In general, the research on the association between the use of anticholinergics and cognitive ability in adults has yielded studies considered to be at high risk of bias, of generally low quality, and has resulted in discordant results. This includes research in people with existing dementia, new-user designs, cross-sectional, and longitudinal studies (Andre et al., 2019). In children, however, where performance is more often measured along a continuum (Ghezzi et al., 2021), the verdict is clearer. A recent systematic review (Ghezzi et al., 2021) found no association between anticholinergic drug use and cognition in children, regardless of the specific drug, drug class, potency, duration of use, and cognitive domain. The authors hypothesised that the differences in findings, when compared to older adults, might be due to the nature of the ageing process and longer lengths of exposure, but also due to polypharmacy, study design, and other confounding factors. The possibility of confounding by indication (see section 7.2.2.1) is especially concerning – a recent study evaluating the associations between AChB and cognition found most previously significant effects to

disappear after the inclusion of various clinical variables, including smoking status and history of several common disorders (Dos Santos et al., 2022).

2.4.4.2 Evidence from neuropathology and brain imaging

In the attempt to find biological correlates of potential adverse cognitive effects of anticholinergic drugs, researchers have mainly used two approaches. The first are neuropathological studies that use tissue from brain donors to determine the distribution and severity of markers of brain damage due to neurodegenerative disease. The second are non-invasive brain-imaging techniques that attempt to detect differences or changes in either brain structure or function, principally through MRI. This section provides a brief overview of these studies and the current state of knowledge on the neural correlates of anticholinergic effects on the brain.

At least three studies have thus far attempted to relate the degeneration of brain tissue to anticholinergic use using neuropathological findings. Two of these studies were performed on tissue of people with Parkinson's disease, as anticholinergics are often prescribed to control tremor and bladder dysfunction. One study found that individuals treated with anticholinergics over a long period (2-18 years; n=18) exhibited a 2.5-fold increase (no CIs given) in plaque densities when compared to short-term users (<2 years; n=15) and non-users of anticholinergics (n=21) (Perry et al., 2003). In contrast, a study that also examined the tissue from brains of people with Parkinson's disease found no evidence for an increase in any marker of AD pathology (including plaques) when comparing moderate or heavy anticholinergic users with non-users. Moreover, heavy (RR=0.44, 95% CI=0.21-0.89) and moderate (RR=0.24, 95% CI=0.09-0.62) anticholinergic exposure was associated with a lower risk of cerebral microinfarct burden when compared to non-use (n's of heavy, moderate, and non-users were 85, 137, and 85, respectively) (Gray et al., 2018). A third study conducted in post-mortem samples of healthy volunteers (n=298) similarly found no association between anticholinergic use and various measures of macroscopic lesions, including infarcts, and focal and global atrophy (Richardson et al., 2020).

Researchers have also used fMRI to explore the effects of anticholinergics on the activation of different regions of the brain. Many studies have reported on short-term changes in brain activation that may mediate the relationship between anticholinergic use and cognitive changes. Hyoscine decreases performance in working memory and activation in the hippocampal formation, prefrontal cortex, and fusiform gyrus (Schon et al., 2005; Sperling et al., 2002). Hyoscine also leads to a decrease in verbal memory performance, which is correlated with decreased connectivity in some cortical networks when using resting-state fMRI (Chhatwal et al., 2019). However, it is unclear whether chronic anticholinergic use leads to stable changes in activation patterns. A recent study comparing anticholinergic and non-pharmacological treatment of overactive bladder found few differences in activation of brain attentional networks (Ketai et al., 2021).

Moreover, results from studies on structural brain changes following prolonged use of anticholinergics are unclear and often underpowered. To my knowledge, four studies have thus far assessed associations between anticholinergic use and measures of brain structural MRI. One cross-sectional study assessed 402 older healthy participants and divided them in users and non-users of anticholinergics based on self-reported medication use over a one-month period. In addition to reductions in verbal memory and executive function, the authors reported increased brain atrophy (difference=16.97cm^3, no CIs given) and reduced thickness in the temporal (difference=0.04mm, no CIs given) and medial cortical lobes (difference=-0.05mm, no CIs given) in users of anticholinergics when compared to non-users (Risacher et al., 2016). Another cross-sectional study evaluated 568 older participants, some of which belonged to the same cohort as in the analysis by Risacher et al. (2016). Following a similar classification of participants, the authors additionally found cognitively normal users (n=96) to have lower functional connectivity (Cohen's f=0.24, 95% CI=0.10-0.39), but comparable grey matter density of the NBM as non-users (n=97) (Meng et al., 2022). Another cross-sectional study explored associations between AChB and structural MRI volumes of the hippocampi and the basal forebrain in a sample of 3,087 adults aged 21-80 years. The authors found a negative association between AChB and the volumes of the right (β=-20.93, 95% CI=-9.84mm^3--32.02mm^3) and left hippocampi (β=-17.00mm^3 95% CI=-4.71mm^3--29.29mm^3), but

no significant effects for the basal forebrain (Kilimann et al., 2021). Finally, one study explored 723 middle-aged participants from a different cohort and followed them longitudinally for up to 20 years. They divided the sample into definite users (use of anticholinergics with clinically relevant cognitive negative effects) possible users (use of anticholinergics drugs with no clinically relevant cognitive negative effects), and non-users of anticholinergics. The authors found that compared to non-users, possible users, but not definitive users, exhibited greater rates of brain atrophy (-1.13cm^3 per year, 95% CI=-2.04cm^3--0.24cm^3) in later life (Chuang et al., 2017).

All four studies that used structural brain MRI applied the ACB scale to either categorise participants (Chuang et al., 2017; Meng et al., 2022; Risacher et al., 2016) or calculate an AChB (Kilimann et al., 2021). However, the relatively low sample sizes for this type of analyses (Marek et al., 2022) – only one study surpassed a sample size of 1,000 (Kilimann et al., 2021) – and the focus on different measures of brain structure, render the synthesis of evidence difficult. When studies of neuropathology are included into consideration, the results are conflicting and do not enable a clear interpretation for the role of anticholinergics in brain structure.

2.4.4.3 Falls or fractures

The relationship between anticholinergic use in older people and the risk of falls or fractures has been well replicated and several systematic reviews (n=610,862 (Reinold et al., 2020); n=274,647 (Stewart, Taylor-Rowan, et al., 2021); n=540,479 (Welsh et al., 2018); n=124,286 (Ruxton et al., 2015)) have found most original studies to have reported a positive relationship. This has also been shown in meta-analyses: a recent study by Ogawa et al. (2021) (n: 10 studies, 487,247 people) found a linear relationship between AChB and fractures, with each additional point according to the ACB scale corresponding to a 28% increase (95% CI=1.18-1.39) in the risk of fractures. Recent observational studies (Campbell et al., 2021) have confirmed these associations, with anticholinergic use (as opposed to no anticholinergic use) possibly doubling (95% CI=1.45-3.03) the risk of recurrent falls (Rosso et al., 2021).

Additionally, research has shown that the association between AChB and the risk of falls/fractures in older people is observed when the exposure to anticholinergics is not ongoing but occurred in the past (Suehs et al., 2019). Furthermore, some studies have demonstrated the association between anticholinergic use and falls/fractures even in middle-aged individuals (Ablett et al., 2018; Tan et al., 2020; Xu et al., 2022), with one study (Szabo et al., 2019) suggesting even more pronounced effects in this population when compared to older people.

However, despite the consistency of the literature, many confounders, including cognitive impairment, behavioural disorders, incontinence, polypharmacy, or poor physical function may explain the purported association (Collamati et al., 2016). Furthermore, the studies differ both in how falls are measured and how AChB is calculated (Reinold et al., 2020; Stewart, Taylor-Rowan, et al., 2021). Comparative studies between different anticholinergic scales are lacking, but the existing evidence indicates that the risk of falls or fractures depends on the scale used (Akgün et al., 2022; Ogawa et al., 2021). Additionally, despite the consistency of the relationship between anticholinergic use and falls/fractures, most studies exhibit a high risk of bias (Stewart, Taylor-Rowan, et al., 2021), and do not explain the underlying mechanisms, which could include changes to alertness and attention, movement coordination, or balance (Shmuel et al., 2021). Finally, there might exist a need for more granular hypotheses: extant studies have suggested differences between anticholinergic drugs (Ruxton et al., 2015; Welk et al., 2022), between classes of anticholinergic potency (Neal et al., 2020), and between individuals exhibiting varying degrees of frailty (Naharci & Tasci, 2020).

2.4.4.4 All-cause mortality and all-cause hospitalisations

Research on the association between anticholinergic use and all-cause mortality is highly heterogeneous, but systematic reviews on the topic (Ali et al., 2020; Fox et al., 2014; Graves-Morris et al., 2020; Ruxton et al., 2015; Welsh et al., 2018) reveal that most studies have demonstrated a positive relationship. Moreover, one of the largest studies conducted on the topic that included 537,387 participants (Nishtala et al., 2014) suggested a 29% increase in

mortality risk (95% CI=1.25-1.33) in people that use anticholinergics. Moreover, a recent study (Lisibach, Gallucci, Beeler, et al., 2022) compared 19 different anticholinergic scales and concluded that AChB according to each scale was associated with an increased risk of mortality, ranging from an increase of between 1.32- and 3.03-fold. Some analyses have focused on more vulnerable populations: positive associations of anticholinergic use with all-cause mortality have been shown for older hospitalised people (Herrero-Zazo et al., 2021; Lisibach, Gallucci, Beeler, et al., 2022; Sorensen et al., 2021) and for people with pre-existing dementia (Taylor-Rowan et al., 2022; Wang et al., 2021). However, there have also been reports of negative results for each of these population (Bishara, Perera, Harwood, Taylor, Sauer, Funnell, Gee, et al., 2021; Rigor et al., 2020). Moreover, when using the GRADE approach (Guyatt et al., 2008) to evaluate the reported evidence in multiple areas, the quality of studies for the association between anticholinergic use and mortality in people with dementia has been reported as low or very low (Taylor-Rowan et al., 2022). Finally, most of the research on the topic has been done in older individuals (Welsh et al., 2018), but there is some evidence for an increased risk of all-cause mortality in individuals younger than 65 years (Myint et al., 2015).

Although there has been plenty of research on the association between anticholinergic use and all-cause mortality, there is great variation in study design, the types of anticholinergic scales used, and the sizes and characteristics of the samples (Ali et al., 2020). Moreover, there is a lack of studies comparing different scales (Graves-Morris et al., 2020) and the variability attributable to that difference is thus difficult to evaluate. Additionally, many studies have involved small numbers of participants, short follow-up periods, and have been assessed as being of low quality or to exhibit a moderate to high risk of bias (Ali et al., 2020; Fox et al., 2014; Graves-Morris et al., 2020). Furthermore, some research indicated possible differences between individual anticholinergic drugs in their associations with the risk of mortality (McMichael et al., 2021; Ruxton et al., 2015; Welk, 2022) or failed to find a consistent dose-response (Graves-Morris et al., 2020) or potency-response (McMichael et al., 2021) relationship.

The relationship between anticholinergic use and all-cause hospitalisations exhibits similar trends to the association between anticholinergic use and all-cause mortality. Studies have so far been conducted only or mostly in older people (Welsh et al., 2018), but have predominantly demonstrated positive associations (Bishara et al., 2020; Hsu et al., 2021; Liang et al., 2022; Wang et al., 2021; Welsh et al., 2018), although lack of associations between AChB and risk of hospitalisation (Bishara, Perera, Harwood, Taylor, Sauer, Funnell, Stewart, et al., 2021; Cardwell et al., 2020), re-hospitalisation (Castier et al., 2021), and the duration of stay in hospital (Bishara et al., 2020; Lisibach, Gallucci, Beeler, et al., 2022) have been reported. Furthermore, some papers have noted a lack of a clear relationship between the potency of anticholinergic drugs and hospitalisations, and in differences in the predictive capacity for risk of hospitalisation when using different anticholinergic scales (Hsu et al., 2021).

2.4.4.5 Other outcomes

Although studies relating anticholinergic use with other disorders are sparser, thus precluding the formation of a clear narrative, AChB has been associated with multiple negative health outcomes. These include constipation (Rodríguez-Ramallo et al., 2021), cardiovascular disease (Lockery et al., 2021; Myint et al., 2015), frailty (Ruiz et al., 2021), vertigo (Phillips et al., 2019), reduced quality of life (Stewart, Yrjana, et al., 2021; Sura et al., 2015; Yrjana et al., 2021), reduced physical function (Fox et al., 2014; Mehdizadeh et al., 2021; Stewart, Yrjana, et al., 2021; Welsh et al., 2018), macular degeneration (Aldebert et al., 2018), undernutrition (Ates Bulut et al., 2022; Naharci et al., 2022), sarcopenia (Bag Soytas et al., 2022), dental health (Kakkar et al., 2021), post-stroke pneumonia (Gradek-Kwinta et al., 2022), psychosis in individuals with suspected AD (Cancelli et al., 2008), and swallowing dysfunction in older people undergoing stroke rehabilitation (Kose et al., 2022). For the outcome measures for which systematic reviews are available, the latter have pointed out several shortcomings in previous research. First, application of tools to assess study quality, including the Joanna Briggs Institute Critical Appraisal Tool (Aromataris & Munn, 2020; Rodríguez-Ramallo et al., 2021) and the GRADE system (Guyatt et al., 2008; Stewart, Yrjana, et al., 2021) has often

resulted in an assessment of low quality of studies. This includes studies exploring constipation (Rodríguez-Ramallo et al., 2021), quality of life (Stewart, Yrjana, et al., 2021), and physical function (Mehdizadeh et al., 2021; Stewart, Yrjana, et al., 2021). Second, the exact measures used to quantify physical function affect the direction of the association (Mehdizadeh et al., 2021). Finally, questions remain regarding the most appropriate anticholinergic scale to use (Fox et al., 2014; Rodríguez-Ramallo et al., 2021; Stewart, Yrjana, et al., 2021).

In conclusion, the literature suggests a positive association between anticholinergic use and various adverse health outcomes. However, due to the high number of possible moderating variables and the relatively low average quality of past studies, the data are often inconsistent and confusing. Despite this uncertainty, the findings of potential harm, especially in older people, have prompted several calls for reduced anticholinergic prescribing in this population. This includes several influential bodies and manuals for healthcare practice, including the National Institute for Health and Care Excellence guidelines (NICE, 2018), the American Geriatrics Society 2019 AGS Beers Criteria for Potentially Inappropriate Medication Use in Older Adults (Fick et al., 2019), STOP/START criteria for potentially inappropriate prescribing in older people (O'Mahony et al., 2015), and the 5th Canadian Consensus Conference on the diagnosis and treatment of dementia (Ismail et al., 2020). The analyses conducted as part of this thesis focus only on the associations between anticholinergic drugs and cognitive outcomes, and do not tackle other relevant health outcomes also described in the previous sections. However, it is important to acknowledge that the prescribing of medicines must take all these potential outcomes, in addition to the ability of the drugs to manage symptoms of the underlying disease, into account.

3 UK Biobank

3.1 Introduction

3.1.1 Background

UK Biobank is a large prospective study that acts as a resource for the investigation of a variety of variables on diseases of middle- and old age. It was formally conceived at a meeting hosted by the Wellcome Trust and the Medical Research Council in 1999 (Ollier et al., 2005). The idea of a longitudinal cohort study was based on scientific advances that were occurring at the time: the genomes of several animal species, including humans, were being sequenced, and genetic diversity was being studied and mapped across populations. The aim of UK Biobank was to utilise the new knowledge and methodology to study how inherited genetic variation and environmental factors interact in their effects on health outcomes. Utilising UK Biobank, researchers would be able to specify associations between subgroups of the population and certain diseases, and determine the risks based on genetic and environmental factors. By the year 2006, the recruitment and testing strategies were in place and both funding and ethical approval had been granted (Ollier et al., 2005). To access the data, an online application process exists. Applications are approved if the proposed research is judged to be in the public interest and the data are already available or will be made available in the future. While both praise and criticism has been levied against UK Biobank since its inception (Senior, 2006), it has developed into a valuable resource for researchers, with over 3,200 publications using UK Biobank data published as of December 2022 (https://www.ukbiobank.ac.uk/published-papers). In the following sections, I will depict the main characteristics of the UK Biobank sample. I will focus on variables of major interest in my analyses and describe their ascertainment, utility, and quality.

3.1.2 Study population

Eligible volunteers for UK Biobank included all residents of the UK that were registered with the National Health Service (NHS) and lived up to approximately 25 miles from one of the 22 assessment centres (Allen et al., 2012). The exact recruitment protocol has been published before (UK Biobank, 2006) and all details are available at https://www.ukbiobank.ac.uk/. The number of participants (~0.5 million) – was chosen due to the prevalence of the most common diseases and based on the sufficient statistical power of nested case-control studies to model those diseases in the next 5-10 years. To detect effect sizes with ORs 1.3-1.5 and 2.0, respectively 5,000-10,000 and 20,000 cases would be required. For the case of AD, it was estimated that the number of incident cases would increase from 800 by the year 2012, to 3,000 by the year 2017, and to 9,000 and 30,000 by the years 2022 and 2027, respectively (Ollier et al., 2005; Sudlow et al., 2015). As of January 2022, the number of diagnoses of AD in UK Biobank was only about 3,300, possibly reflecting the modest representativeness of the sample for the UK population (see section 7.2.1). The age range of the participants – 40-69 years – was the result of a compromise between recruiting participants that were healthy during baseline, but with sufficient probability of developing illness within a reasonable time frame. This would not only allow the development of relevant health outcomes, but also the assessment of exposure without the "interference" by disease or its treatment. Thus, recall bias and reverse causation bias could be avoided or minimised (Allen et al., 2012; Sudlow et al., 2015).

3.1.3 Ethics

Ethical approval for the UK Biobank was obtained from the Research Ethics Committee (11/NW/0382). All participants gave consent to the use of their anonymised data, samples for any health-related research, to be re-contacted for further analyses, and for access to their health records. This consent is valid throughout the duration of the study (Sudlow et al., 2015).

3.1.4 Baseline measurements

The baseline assessment for UK Biobank occurred between the years 2006 and 2010 in 22 assessment centres throughout the UK. It comprised electronic signed consent, touch-screen questionnaires that queried several sociodemographic factors (ethnicity, education, income, etc.), habits and lifestyle aspects (smoking, drinking, sleep, physical activity, diet, etc.), as well as cognitive performance. Additionally, it involved computer-assisted interviews, physical and functional measures, and the collection of blood, urine, and saliva for various laboratory analyses (**Table 8**) (Allen et al., 2012; Sudlow et al., 2015). The description of all measures available in UK Biobank is beyond the scope of this chapter. However, some variables were repeatedly used in most of my analyses and are described below:

- *Education* is captured by two variables: qualifications and age at which full-time education was completed. I primarily used the former, as its meaning is clearer. It consists of a list of completed qualifications that are not necessarily mutually exclusive. This list was transformed into a binary variable that indicated whether a college or university degree had been obtained.
- *Socioeconomic deprivation* was based on the Townsend deprivation index (Townsend, 1987). This was calculated immediately before the participants joined UK Biobank and used information from the preceding national census. It includes several socioeconomic indicators that are scored and summed up. The component scores are then standardised using the z-score technique. Each participant was assigned a score corresponding to the area in which their postcode was located.
- *Smoking* was ascertained by UK Biobank in several ways and captured in multiple variables, including the age when the participants smoked or started to smoke, type of tobacco smoked, number of cigarettes smoked per day, and a calculation of pack years. I used a categorical variable that indicated whether at time of ascertainment, the participant was a current smoker, past smoker, or if they had never smoked. This was chosen for easier comparisons with previous studies.

- *Alcohol consumption* was also ascertained in different ways, including frequency for different types of alcoholic beverages. I used the frequency of alcohol consumption, represented as a categorical variable (1: daily or almost daily; 2: three or four times a week; 3: once or twice a week; 4: once to three times a week; 5: only on special occasions; 6: never). This also was chosen for ease of comparison with previous studies.
- *Physical activity* was assessed by querying the duration and frequency of various types of physical activities. I used a list of five types of physical exercise that the participants may have exhibited within the past four weeks. A participant could have partaken in several activities within those five types. Therefore, the variable was transformed into three intensity categories and the participant was assigned the highest category of intensity that they displayed within those four weeks (Hanlon et al., 2020).
- *APOE genotype* was ascertained as part of the genotyping performed on 488,000 participants of UK Biobank as described in detail by Bycroft et al. (2018). *APOE* alleles are major genetic risk factors for the development of AD.

By the year 2010, recruitment and baseline assessment of UK Biobank participants was complete. Since then, UK Biobank has continued to expand, with additional measurements undertaken on subsets of participants. These have included web questionnaires on diet, mental health, occupational history, and cognitive testing. Additionally, they involved genome-wide genotyping, whole-exome sequencing, whole-genome sequencing, measurements of telomere lengths, plasma metabolites, and various other biochemical measurements (**Table 9**). Most crucially for our purposes, since the initial baseline measurements, some participants have undergone multimodal imaging – including brain structural MRI – and have had their data linked to their respective hospital and primary care records (Caleyachetty et al., 2021; Sudlow et al., 2015). The UK Biobank's Data Showcase (https://biobank.ndph.ox.ac.uk/showcase/) is publicly available and contains a list of all variables measured. This includes information on the methodology used to measure the variables, the univariate distribution of variables, and reports and manuals to describe and

explain each resource. I provide an overview of the variables used in my analyses, including corresponding data fields and hyperlinks to the UK Biobank data showcase in chapter 6, Supplementary Table 1 (p. 214).

Table 8: The measures ascertained during the baseline assessment in UK Biobank (years 2006-2010). The first column shows the types or classes of measures, the second column indicates the variable measured. Adapted from Sudlow et al. (2015) and Caleyachetty et al. (2021).

Measure type	Details
Touchscreen questionnaire and computer-assisted verbal interview	
Sociodemographic	social class; ethnicity; employment status; marital status; education; income; car ownership
Lifestyle	smoking; alcohol consumption; physical activity; diet; sleep
Family history, early life exposures	family history of major diseases; birth weight; breast feeding; maternal smoking; childhood body size; residence at birth
Psychosocial factors	neurosis; depression (including bi-polar spectrum disorder); social support
Environmental factors	current address; current (or last) occupation; domestic heating and cooking fuel; housing; means of travel; shift work; mobile phone use; sun exposure
Health status	medical history; medications; disability; hearing; sight; sexual and reproductive history
Hearing threshold	speech reception threshold
Physical measures	
Cognitive function	pairs matching; reaction time; prospective memory; fluid intelligence; numeric memory
Blood pressure, heart rate	two automated measures, one minute apart
Grip strength	left- and right-hand grip strength
Anthropometrics	standing and sitting height; weight and bio-impedance; hip and waist circumference
Spirometry	forced vital capacity, forced expiratory volume, peak expiratory flow
Bone density	calcaneal ultrasound
Arterial stiffness	pulse wave velocity
Eye examination	refractive index, intraocular pressure; acuity; retinal photograph; optical coherence tomography
Fitness test	cycle ergometry with electrocardiogram (ECG) heart rate monitoring
Sample collection	
Blood	45 ml divided into 6 tubes, includes whole blood, serum, plasma, red cells, buffy coat
Urine	9 ml in 1 tube
Saliva	25 ml in 1 tube

Table 9 (continued on next page): Follow-up assessments – completed, planned, and ongoing. Note that not all analyses were performed in the full sample. Adapted from Sudlow et al. (2015) and Caleyachetty et al.

Measure	Details	Date of data acquisition
Genetic		
Genotype	genome-wide genotyping performed on all UK Biobank participants, with direct measurement of approximately 850,000 variants and imputation of >90 million variants	2013-2015
Whole-exome sequencing		2017-2021
Whole-genome sequencing		2020-
Biomarkers		
Telomere length	leucocyte telomere length	2015-2020
Biochemical measures	34 biomarkers assayed in the plasma, serum, red blood cells, and urine samples	2006-2013
Plasma metabolites	NMR-metabolomics assay	2020-
Plasma proteins	measurement of 1,500 plasma proteins	2021-
Web-based questionnaires		
24-h dietary recall	information on consumption of over 200 food and drink items over the last 24 hours	2011
Cognitive function	*cognitive tests, of which 4 were repeated from the baseline assessment in addition to 2 further tests	2014
Occupational history	lifetime employment history, occupational exposures, and related medical information	2015
Mental health	information on lifetime mental health events	2017
Gastrointestinal health	information on gastrointestinal symptoms	2017
Food preferences	information on various food (and other) preferences	2019
Pain	included information on the causes of pain, severity, and duration of chronic pain	2019
24-h dietary recall	information on consumption of over 200 food and drink items over the last 24 hours	2011

Health record linkage		
Death registrations	cause-specific mortality	2006-
Cancer registrations	cancer diagnoses	England 1971– Scotland 1957– Wales 1971–
Hospital inpatient episodes	diagnoses	England 1997– Scotland 1977– Wales 1999–
Primary care	Read-coded information including diagnoses, measurements, referrals, prescriptions	England 1938– Scotland 1939– Wales 1948–
Repeat of baseline assessment		2012-2013
Multimodal imaging	†MRI of brain, heart and body, carotid ultrasound and whole-body DXA scan of bones and joints	2014-
Cardiac monitor	wearing of a cardiac monitor for 2 weeks	Ongoing
Accelerometry	wearing of a wrist accelerometer for 7 days	2013-2016

*Not to be confused with cognitive tests during the imaging visit as used in my analyses.
†Includes cognitive testing as used in my analyses.

3.2 Cognitive testing

As part of the baseline assessments, five cognitive tests were administered, all via a computerised touchscreen interface. Almost all participants completed the Reaction Time and Pairs Memory tests, with tests of Numeric Memory, Fluid Intelligence, and Prospective Memory completed by only a subset of participants (**Table 10**) (Fawns-Ritchie & Deary, 2020; Lyall et al., 2016).

- The Reaction Time test is a test of processing speed. It required the participants to press a button as soon as two identical cards were presented on the touchscreen. The response time in milliseconds across trials that contained matching pairs was measured.
- The Pairs Memory test is a test of visual memory. Participants were required to memorise the positions of six card pairs on a grid and to later match them from memory. The outcome was the number of errors the participants made, with higher scores reflecting poorer performance.

- The Numeric Memory test probes working memory. Participants were shown a two-digit number, which they had to recall and after a brief pause enter in reverse. The number of to-be-remembered digits was increased until the participant made an error in two successive trials or the maximum of twelve digits was reached. The outcome was the maximum number of correctly remembered digits.
- The Fluid Intelligence test is an assessment of verbal and numerical reasoning. It consisted of thirteen multiple-question tests within a two-minute time limit and awarded a point for each correct response.
- The Prospective Memory test required the participants to engage in a specific behaviour later in the assessment, after the completion of all other cognitive tests. They were then scored as correctly recalling the instruction on the first attempt, second attempt, or not at all.

A subset of participants (n=20,339) performed the tests of Fluid Intelligence, Reaction Time, Pairs Memory, and Prospective Memory again after the baseline assessment. The interval between the two assessments varied among participants (mean=4.33 years). Analysing the concordance between the baseline and the follow-up results of the cognitive tests, Lyall et al. (2016) concluded that the significant intercorrelation of the data allowed for the generation of a *g*-factor of general cognitive ability. Furthermore, they showed that the cognitive scores for reasoning and reaction time in participants with follow-up data exhibited acceptable test-retest reliability. While the short-term stability of Pairs Matching was low, the test-retest interval in that study was much longer than the usual 2-4 weeks.

Another subset of participants completed assessments of multimodal imaging (**Table 9**; see also section 3.3) that involved repetition of all tests, along with additional cognitive testing. The additional tests included the Trail Making test, Tower Rearranging test, Matrix Pattern Completion, Paired Associate Learning test, Symbol Digit Substitution test, and Picture Vocabulary:

- The Trail Making test measures executive function. The participants were presented with randomly arranged letters and numbers and were instructed to switch between

touching the letters and the numbers in alphabetical order, working as fast and as accurately as possible. The outcome was the time to complete the test.

- In the Tower Rearranging test, the participants were shown two displays, each with three differently coloured pegs and three differently coloured hoops on the pegs. The hoops were arranged in different locations on the two displays and the participants were required to plan the moves that would make one display look like the other. The outcome was the number of items answered correctly in 180 seconds.
- In Matrix Pattern Completion, non-verbal reasoning is assessed. The participants were presented with a matrix that was missing an item in the lower right-hand corner and were then required to choose the correct missing piece among a list of alternatives.
- The Paired Associate Learning test is a test of verbal declarative memory. The participants were first required to memorise twelve pairs of words. After completing a different cognitive test, they were presented with the first word of a pair and then asked to select the correct pairing from four options. The outcome was the number of correctly answered questions.
- The Symbol Digit Substitution test measures processing speed. The participants were shown a key that paired symbols to numbers and were then required to recall the correct pairings. The outcome was the number of correct matches made in 60 seconds.
- Picture Vocabulary assesses crystallised cognitive ability. The participants were shown four pictures and a word written underneath and were required to recognise the picture that was most closely related to the word. The outcome was the number of correct responses.

Table 10: Cognitive tests used in UK Biobank, with sample sizes for each each test.

UK Biobank test	Cognitive domain	Baseline (2006-2010)	Repeat (2012-2013)	Web-based (2014-2015)	Imaging (2014-)
Pairs Matching	visual declarative memory	n = 497,791	n = 20,334	n = 118,512	n = 48,202
Reaction Time	processing speed	n = 496,590	n = 20,254		n = 47,878
Numeric Memory	working memory	n = 51,799		n = 111,048	n = 36,535
Prospective Memory	prospective memory	n = 171,517	n = 20,329		n = 48,178
Fluid Intelligence	verbal and numerical reasoning	n = 165,430	n = 20,110	n = 123,597	n = 47,291
Trail Making	executive function			n = 42,766	n = 35,663
Tower Rearranging	executive function				n = 34,933
Paired Associative Learning	verbal declarative memory				n = 35,663
Matrix Pattern Completion	non-verbal reasoning				n = 35,243
Picture Vocabulary	vocabulary				n = 35,175
Symbol Digit Substitution	processing speed			n = 118,770	n = 35,339

Sample sizes as of April 2022. The numbers are correct as at the release date for project 10279 and may change with the progression of the study.

The cognitive assessment at UK Biobank was brief (approximately 5 minutes for all tests) and unsupervised, thus differing substantially from the procedure of typical well-validated cognitive batteries that are administered by trained testers. Building on the work by Lyall et al. (2016), Fawns-Ritchie & Deary (2020) aimed to assess the concurrent validity, test-retest reliability, and the presence of a *g* component in the cognitive tests. They recruited an independent sample of participants and in addition to well-validated reference tests, administered all tests used in UK Biobank on two separate occasions. They found the tests to correlate moderately to strongly with well-validated tests designed to assess the same cognitive domains. This was true for all UK Biobank tests except the Prospective memory test

(r=0.22), possibly due to ceiling effects. Furthermore, it was possible to derive a measure of general intelligence from either all tests administered at baseline, or all tests administered during the imaging assessment. Finally, using the typical 4-week interval to probe test-retest reliability, they found the latter to be moderate-to-high in all cognitive tests. Another subset of UK Biobank participants additionally completed the cognitive tests on a web-based version at home. However, due to the context of administration, these tests were even less standardised and may not exhibit comparable psychometric quality to that observed for tests completed during assessment centre visits.

3.3 Brain imaging

UK Biobank has been conducting multimodal imaging, including brain MRI, since the year 2014. During the imaging visit, each participant completed a set of cognitive tests (see section 3.2) and a questionnaire on lifestyle and demographic variables that had already been queried during the baseline assessment, including alcohol consumption, smoking, physical activity, and education.

The brain imaging document released by UK Biobank (Alfaro-Almagro et al., 2018; Miller et al., 2016; Smith et al., 2020) details the brain imaging done in the study, including data acquisition, image processing, and derivation of measures of brain structure and function.

The brain imaging data include six modalities that cover structural-, diffusion-, and functional imaging:

- T1-weighted structural images depict brain anatomy with high resolution, with strong contract between grey and white matter.
- Resting-state functional MRI reflects changes in blood oxygenation that is associated with intrinsic brain activity.
- Task functional MRI uses the same measurement technique as resting-state functional MRI but does so while the subjects are performing a particular task.

- T2-weighted FLAIR structural data is based on signal decay from interaction between water molecules. The intensity on the images primarily relates to pathology.
- Diffusion MRI reflects the ability of water molecules to move within tissue and is used to estimate structural connectivity between brain regions.
- Susceptibility weighted imaging is sensitive to magnetised tissue constituents, allowing the depiction of the venous vasculature or of microbleeds.

UK Biobank has released the raw imaging data, as well as processed versions of the data that resulted from an image processing pipeline. The latter utilised available image processing tools, primarily FSL (Jenkinson et al., 2012) and FreeSurfer (Dale et al., 1999). Both tools are free and open-source and provide a set of algorithms that quantify the properties of the human brain and automatically segment the different structures.

T1 specifically has been processed to remove non-brain parts from the image and to segment the image into different types of tissue. The pipeline has also been used to generate image-derived phenotypes (IDPs) that are intended to represent quantifications of various aspects of brain structure and function. IDPs are summary measures that can be used to relate the imaging data to non-imaging variables in UK Biobank. They have only been generated for complete and high-quality datasets. IDPs aim to describe objective and meaningful quantities in brain imaging data and can range from simple global IDPs (e.g., total brain volume) to very specific focal IDPs (e.g., mean fractional anisotropy in anterior corona radiata). From T1 images, several global IDPs were reported, including total brain matter volume, total white matter volume, total grey matter volume, volume of ventricular cerebrospinal fluid, and peripheral cortical grey matter volume. Total regional grey matter volume was estimated in 139 different regions-of-interest, defined from atlases in standard space. Additionally, the volumes of several subcortical structures were reported separately for left and right hemispheres. IDPs related to the diffusion MRI data were aligned to the white matter tract skeleton and 48 distinct regions of interest were defined using the John Hopkins University tract atlas. Probabilistic tractography yielded 27 maps for distinct tracts for which

microstructural measures were computed as a mean of all voxels within each tract for each participant.

The data also include variables that can be used as control variables in analyses. These include the location of the scanning centre (Cheadle, Reading, or Newcastle, all UK), subject head size, the X-, Y-, and Z- positions of the brain mask in MRI scanner coordinates, and the Z-position of the table/coil in scanner coordinates (Alfaro-Almagro et al., 2021).

3.4 Linked health data

To relate the recorded exposures to relevant health outcomes in participants of UK Biobank, it is necessary to provide detailed follow-up. This is achieved through linkage with data routinely collected by the NHS and in UK Biobank includes hospitalisations, primary care, and death certificates. The documents detailing the properties of these data have been published before (UK Biobank, 2019b, 2020a, 2020b). The following section describes the linked health-data available in UK Biobank and focuses on clinical diagnoses and drug prescriptions.

3.4.1 Format and availability

The first source of linked healthcare data are death certificates (UK Biobank, 2020b). These are received from NHS Digital for participants in England and Wales and from the NHS Central Register for participants in Scotland. It includes the date of death and the primary and contributory causes of death using the World Health Organisation's ICD (International Classification of Disease and Related Health Problems) system version 10.

The second source of data is from hospital stays. The information on patients treated at hospitals but whose treatment did not require an overnight stay and who did not occupy a hospital bed (outpatients) in the UK is limited and thus not appropriate for use in research (Wright et al., 2012). Hence, UK Biobank hospitalisation data only include information on inpatients – patients admitted to hospital that occupied a bed. This can include day cases and admissions where an overnight stay in the hospital had been planned. The inpatient

information includes dates of admission, discharge, and diagnostic codes. The latter are coded according to the ICD versions 9 and 10.

The data on hospital inpatient admissions in England, Wales, and Scotland are provided to UK Biobank by different providers for each nation (**Table 11**) and are managed differently by each provider. Therefore, the overlap between the three datasets for the three nations is incomplete. For example, maternity, and mental health hospital admissions in Scotland have not been made available to UK Biobank. Additionally, the records date back to the year 1981 for Scotland, but only to the years 1997 and 1998 for England and Wales, respectively. Thus, the Scottish data are not entirely comparable to Welsh and English data.

The final source of health outcomes in UK Biobank is primary care. In the UK, individuals seeking medical treatment usually first meet with a family physician (a.k.a. general practitioner or GP) that can refer them for more specialised treatment. Primary care in this context refers to work performed in general practice. There exists no nationwide system for the collection or sharing of primary care data. However, by liaising with data suppliers and other intermediaries, UK Biobank has obtained the primary care data (**Table 12**). As of September 2022, the data for ~230,000 participants have been released. These include coded clinical events (e.g., diagnoses, history, and symptoms), prescriptions, and various administrative codes (e.g., referrals to specialist clinics).

GP data is not coded using ICD codes as is the case for inpatient diagnoses. Instead, it uses Read codes, BNF codes, and dm+d codes. Read codes are a thesaurus of clinical terms that have been used in primary care since 1985. Both existing versions of Read codes (Read v2 and Read v3) can be accessed via the NHS Digital Technology Reference Data Update Distribution (TRUD) but are now deprecated. A new thesaurus was introduced in 2018 that is now being incorporated across the NHS. BNF codes are provided by the British National Formulary that issues prescribing guidance on medicines and exists as a physical and online reference guide. The codes used are annually updated and managed by the NHS Business Services Authority (NHSBSA). Finally, dm+d codes are provided by the Dictionary of Medicines and Devices and are also used widely across the NHS. UK Biobank has compiled a list of clinical codes (UK

Biobank Resource 592) allowing researchers to interpret the codes and convert between them. The linked prescriptions in UK Biobank are part of the GP electronically prescribed data. They include all prescribed – but not necessarily dispensed – medications that were written on the computer by the GP.

Table 11: Providers of inpatient data for UK Biobank. Adapted from UK Biobank (2020a).

Nation	Data provider	Dataset name	Date range
England	Data Access Request Service (DARS)	Hospital Episode Statistics (HES)[a]	1997 -
Scotland	Electronic Data Research and Innovation Service (eDRIS)	General Acute Inpatient and Day Case – Scottish Morbidity Record (SMR01)[b]	1981 -
Wales	Secure Anonymised Information Linkage (SAIL) Databank	Patient Episode Database for Wales (PEDW)[c]	1998 -

a. https://digital.nhs.uk/data-and-information/data-tools-and-services/data-services/hospital-episode-statistics/hospital-episode-statistics-data-dictionary
b. https://www.ndc.scot.nhs.uk/Data-Dictionary/SMR-Datasets/SMR01-General-Acute-Inpatient-and-Day-Case/
c. https://www.datadictionary.wales.nhs.uk/#!WordDocuments/nhswalesdatadictionary.htm

Table 12: Providers of primary care data for UK Biobank. Adapted from UK Biobank (2019).

Nation	Data provider	~N participants	Clinical coding classification	Prescription coding classification
England	TPP[a]	165,000	Read[d] v3	British National Formulary (BNF)[e]
	Vision[b]	18,000	Read v2	Read v2; Dictionary of Medicines and Devices (dm+d)[f]
Scotland	EMIS[c] / Vision	27,000	Read v2	Read v2; BNF
Wales	EMIS / Vision	21,000	Read v2	Read v2

a. TPP (https://www.tpp-uk.com/) provides the SystmOne practice management system to the NHS.
b. Vision Health (https://www.visionhealth.co.uk/) provides the Vision practice management system to the NHS.
c. EMIS Health (https://www.emishealth.com/) provides the EMIS Web practice management system to the NHS.
d. Read codes were updated biannually and distributed under Open Government License via the UKTC Terminology Reference data Update Distribution (TRUD) service (https://isd.digital.nhs.uk/).
e. British National Formulary (BNF) provides prescribing and pharmacology guidance on medicines used within the NHS (https://www.bnf.org/).
f. dm+d provides a dictionary of descriptions and codes for medicines and devices used across the NHS.

In the primary-care data, the start date of coverage and the completeness of coverage until the extract date are unknown for many participants. Another issue relates to deceased participants: for approximately 3% of participants in data extracts from the data provider TPP and in data extracts from Wales are known to have died before the data was extracted. For all data from data provider Vision and all Scottish prescription data, this figure is much lower, indicating that the information on some of those deceased individuals has been lost (UK Biobank, 2019b). Thus, whenever death is an important outcome, care is needed to prevent bias when using these data.

3.4.2 Quality

Routine data was originally collected for administrative purposes (e.g., reimbursement for medical services) and healthcare needs. Whereas their cost-effectiveness and relative imperviousness to attrition in longitudinal studies make it an attractive source of information for research, they were not intended for such use. Due to national and local policy changes over time in how the data are collected, coded, and stored, primary- and secondary care systems are subject to a range of potential biases and shortcomings.

One of the biggest potential issues with electronically derived diagnoses are incomplete or inaccurate data. These may occur for several reasons, such as failures by the clinician to spot symptoms, link the exhibited symptoms to the appropriate diagnosis, an inconsistency in coding the diagnoses, an incorrect mapping between code and illness, etc. (Chubak et al., 2012; Davis et al., 2016).

There are several measures that can be used to present the accuracy of electronic healthcare data, including sensitivity, specificity, positive predictive value (PPV), and negative predictive value (Chubak et al., 2012). When choosing an algorithm that assigns diagnoses from electronic healthcare data, researchers may prioritise different measures of accuracy, depending on the goal of the study. For example, sensitivity – the proportion of individuals with the condition that are identified as having the condition – is prioritised when the goal is to maximise the number of identified cases for a disorder. On the other hand, PPV – the

proportion of individuals identified as having the condition that truly have it – is more important when we want to ensure that only participants that truly have a condition are identified as such. Considering that PPV is directly related to the prevalence of the disorder, it is especially pertinent for conditions that are relatively infrequent in the population of interest.

Validation studies of linked routinely collected data that seek to ascertain these measures of accuracy are difficult to identify when using conventional research literature. This is because many authors report the results of these analyses in the methods, but do not include them in the title or keywords. Furthermore, the providers of administrative records often do not keep records of studies that use their data (Wright et al., 2012).

Burns et al. (2012) reviewed studies exploring the accuracy of hospital episode data. Among the 18 studies, 8 used English data sets, 8 Scottish, and two used Welsh datasets. They found no differences between the different nations, with the median diagnostic accuracy at 80.3%. Furthermore, they showed that since 2004 – the time of introduction of Payment by Results, an initiative that funds healthcare based on coding data – the accuracy of primary diagnoses increased from 73.8% to 96%. Increases in data accuracy are also reflected in results from independent audits of HES data between 2007/2008 and 2009/2010 (Audit Commission, 2010).

Additionally, analyses have been performed that explore data accuracy within individual groups of disorders. In a study of linked cardiovascular diagnoses, Wright et al. (2012) compared HES records of ~1,000 women to the information provided by their general practitioners. For those diagnosed with vascular disease, they found an agreement of 93% between the two sources, with even higher accuracy (97%) for those that had not been diagnosed with vascular disease. Other authors (Boekholdt et al., 2004; Kirkman et al., 2009) that performed validation studies of English hospital records on vascular outcomes have found a similarly high concordance, demonstrating that routinely collected information on vascular disease in the HES was sufficiently reliable to be used for epidemiological research. High accuracy has also been noted for the diagnosis of stroke: appropriately selected codes

have consistently yielded PPVs above 70% and often above 90% (Woodfield et al., 2015). Furthermore, mental health diagnoses have demonstrated a PPV of 76%, with highest PPVs for psychotic and affective disorders (Davis et al., 2016).

Validation studies of diagnoses of neurological conditions using linked data have revealed great heterogeneity in coding accuracy among different disorders (St Germaine-Smith et al., 2012). For dementia, the results have generally been encouraging. A systematic review that explored studies comparing dementia coding from routinely collected data to expert-led reference standards (Wilkinson et al., 2018) found PPVs for all-cause dementia between 33% and 100%, with the majority above 75%. The sensitivities were generally lower between 21% and 86%. The authors noted the large heterogeneity between studies and recommended separate validation analyses for each sample for which linked data was to be used. The same research group undertook such a validation analysis for dementia in UK Biobank (Wilkinson et al., 2019). For 120 participants in UK Biobank with at least one dementia code from either primary care, hospital inpatient episodes, or mortality data, they compared the coded diagnoses to diagnoses based on expert adjudication of medical records. They determined the PPVs for all-cause dementia to be 86.8%, 87.3%, and 80% for primary care, inpatient, and mortality data, respectively. Moreover, using only clinical codes (as opposed to administrative codes that mostly include specialist referral codes), the PPV for all-cause dementia in primary care rose from 86.8% to 90.1%, with only a small loss of identified cases. The PPVs were lower across all sources for dementia subtypes.

Less is known about the quality of the prescribing data in UK Biobank. However, based on what is known about the nature of the data and its extraction procedures (UK Biobank, 2019b), several assumptions can be made. First, the prescriptions represent only those medicines prescribed within primary care and thus do not include drugs prescribed within secondary hospital care, over-the-counter drugs, and supplements. Moreover, it is unknown whether the medications were ever dispensed or taken by the participants. Using UK Biobank prescribing data implicitly assumes that the individuals that all prescribed drugs were dispensed and taken by patients, which is almost certainly not the case. Second, a strong

motivation for the switch from paper to electronic prescribing was regular prescribing, as it reduced the workload and obviated repeated writing of identical notes. Thus, while prescribing data is likely to be complete for regular prescriptions, this may not be the case for acute prescriptions (e.g., analgesics to treat pain after an acute injury). However, it is not known to what extent these issues introduce systematic bias to the data.

Primary care prescriptions in the UK have been often used for research. For example, the Clinical Practice Research Datalink (CPRD) has grown from a dataset established already in 1987 to contain anonymised GP medical records of over 11 million individuals from 674 practices in the UK (Herrett et al., 2015). The dataset is considered reliable and of high quality for use in research (Wolf et al., 2019). Considering that both the CPRD and UK Biobank contain the same type of data collected in analogous ways, it would be reasonable to assume that both resources exhibit relatively similar levels of accuracy.

However, whereas the CPRD undertakes data validation and quality checks that comprise over 900 individual actions before they are released for use in research (Wolf et al., 2019), only minimal data curation is performed on the raw prescriptions data in UK Biobank (UK Biobank, 2019b). Thus, UK Biobank data may include duplicates, missing or incorrect information, and do not provide researchers with information about the data quality relating to individual participants. Moreover, when duplicates are present, it is unclear whether they are valid (i.e., if several prescriptions were indeed issued) or false (e.g., if the printer in the practice didn't work the first time and a second prescription had to be issued).

To my knowledge, one paper has been published that explored the accuracy of UK Biobank prescription data. Darke et al. (2022) compared the presence of prescriptions in the record within the last 90 days with self-reports from the first UK Biobank assessment. They found the concordance to be lower than was the case for primary care diagnoses. Moreover, the concordance varied between different classes of drugs. For example, for TPP, the data provider that accounts for over 70% of all prescriptions in UK Biobank, the overlap with self-reported prescriptions was 92.1% for antihypertensives, 94.1% for statins, 86.6% for atypical antipsychotics, but only 52.3% for corticosteroids.

3.4.3 Data cleaning

UK Biobank provides ICD- and Read codes for most disorders used as outcomes or control variables in the analyses presented in this thesis. These are self-reported illnesses under data field 20002, primary-care diagnoses under data field 42039, and inpatient diagnoses under data fields 41270, 41271, 41280, and 41281. Summary diagnoses from all three sources are available under data categories 42 and 1712. UK Biobank also provide tables to convert between ICD-, Read-, and self-report codes in data codings 609, 1834, 1835, and 1836. Thus, these resources required minimal cleaning and preparation before their inclusion in the analyses.

For primary care prescriptions data in UK Biobank, only record-level access is available as data field 42039. Because of lack of prior data curation performed on these data, the latter required extensive cleaning before their use in analyses. The details of the procedure might differ between different analyses (i.e., between chapters 4-6); further details are available as Supplementary Figures 1 (p. 176) and 3 in chapters 5 and 6, respectively. Additionally, the code used for all the steps of the data-cleaning process was uploaded to UK Biobank and is available in public repositories on Github (https://github.com/JuM24?tab=repositories). The points below briefly describe the steps included in the data-cleaning process:

- Supplementation of empty prescriptions (i.e., no text in prescription content) with codes from the Read_v2-reader (supplied by UK Biobank).
- For drugs that were classified as anticholinergic by at least one scale, I substituted the brand names with generic drug names. The brand names were identified using the BNF website (https://bnf.nice.org.uk/drugs/), and potentially previously used brand names were identified with a custom web search.
- Removal of rows still missing prescriptions (even after Read-code supplementation), rows without dates, rows with invalid dates. Dates were considered invalid when they were recorded for the period before a participant's birth, after their death, or after data extraction.

- Identification of drugs that were rated as anticholinergic by at least one scale and assignment of anticholinergic potency scores according to each scale. All drugs not rated by any scale were given the potency rating of 0. Additionally, a potency rating of 0 was assigned to all otic, ophthalmic, nasal, respiratory, and topical drugs which were identified by the presence of words such as ‘topical’, ‘cream’, ‘eye drop’, ‘gel’, ‘spray’, etc.
- Assignment of drug dose and quantity to individual prescriptions. This could not be easily automated and largely had to be done manually for each anticholinergic drug.
- Separation of combination drugs into individual compounds. Some anticholinergics are combination drugs, the individual components of which can also exhibit anticholinergic potency and may be prescribed in isolation. For each such combination drug that contained an anticholinergic substance, the prescription was divided into different component compounds and only the anticholinergic compounds were scored according to each anticholinergic scale. The non-anticholinergic components of anticholinergic combination drugs received a potency score of 0.
- Assignment of DDDs to each drug and normalisation of units to “mg” or “ml/mg”.
- Creation of new data frames that displayed: (1) separate columns for each drug class, (2) participant-years as units of observations, and (3) participants as observations.
- Preparation for analyses: merging of the prescription datasets listed in the previous step with confounder variables, removal of outliers, calculation of the cumulative AChB, and testing for the prerequisites required for modelling.

3.4.4 Characteristics of linked prescriptions in UK Biobank

The following section briefly describes the main characteristics of the prescriptions in UK Biobank. I focus specifically on aspects of data quality and potential issues when interpreting the results. Further information on demographic variables of the sample and characteristics of anticholinergic drugs – including their prevalence and longitudinal trends – are presented in chapter 4.

Prescription data were initially available for 222,122 participants, 44 of which have since declined to continue participating in UK Biobank, thus yielding 222,078 participants and a total of 57,696,514 individual prescriptions. As mentioned before, the data had undergone minimal curation by UK Biobank; potential issues or missing data and their prevalence in the sample are presented in **Table 13**.

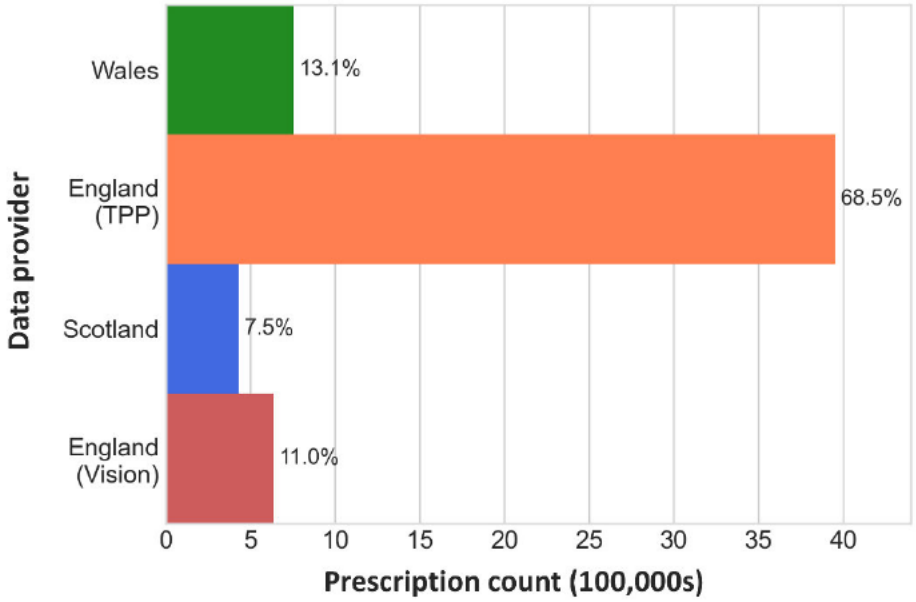
Most prescriptions were provided by England (TPP), with less than a third of prescriptions sourced from other data providers or other nations of the UK (**Figure 11**). Most of the sample was associated with a single data provider throughout the study period, with only 1,470 participants (6.6%) having switched data providers. However, these participants contributed 12.9% of prescriptions to the sample, likely due to their longer presence in the study.

Table 13: Potential issues and missing data among linked prescriptions in UK Biobank.

Potential issue	**n (% of total sample)**
Duplicate	1,460,763 (2.5%)
Prescription date in the future	105 ($1.8x10^{-4}$%)
Prescription dates before date of birth	7 ($1.2x10^{-5}$%)
Missing date of prescription	7,162 (0.012%)
Missing prescription content	7,533,498 (13.1%)
Missing read code	42,798,693 (74.2%)
Missing BNF code	13,933,160 (24.25%)
Missing dmd code	51,345,813 (89.0%)
Missing quantity information	7,582,851 (13.1%)
Changed data providers*	7,414,923 (12.9%)

*Number of prescriptions prescribed to individuals that were associated with different data providers at different time points during the study period.

Figure 11: Proportions of prescriptions provided by individual data providers. The x-axis displays the numbers (in 100,000s) of prescriptions for Wales, England (TPP), Scotland, and England (Vision). Next to each bar is displayed the percentage of participants in the sample that were associated with each data provider.



The earliest recorded prescriptions for participants in UK Biobank date as far as the year 1945, but less than 0.002% of total prescriptions in the dataset were prescribed before the year 1990. Prescriptions were extracted in September 2017 for Wales, August 2016 for England (TPP), May 2017 for Scotland, and June 2017 for England (Vision), with the number of prescriptions rising steadily until then. This rise was likely not only due to an increase in prescriptions in the sample, but also increased ascertainment. Different approaches were used to extract the data from the computer systems of different suppliers (UK Biobank, 2019b). This makes it impossible to determine the exact cut-off until which data are available. Indeed, for most data providers, substantial decreases in prescription numbers occur before the reported dates of data extraction (**Figure 12**). UK Biobank has noted for each data provider the range of dates for which a reduction was observed in the average number of records compared to previous years. It is unclear whether the reduction in sample size during that period is systematic and might bias analyses based on that data. Thus, I included in my analyses only drugs prescribed before that period. While the range of dates with reduced numbers of observations differed between data providers, I chose the earliest such period

(30th of May 2016) as the cut-off point for prescriptions in my analyses. **Figure 13** depicts the timeline for all relevant variables used in my analyses.

Figure 12: Number of yearly prescriptions for each data provider. The black dashed line indicates the month of data extraction. The red dotted line depicts the earliest date across all data providers (May 2016) after which a marked reduction in prescriptions was observed. The red shaded area represents the period excluded from data analysis. Note the sharp jump in prescriptions for Scotland after the year 2012, likely due to a system-wide block of missing records prior to that year.

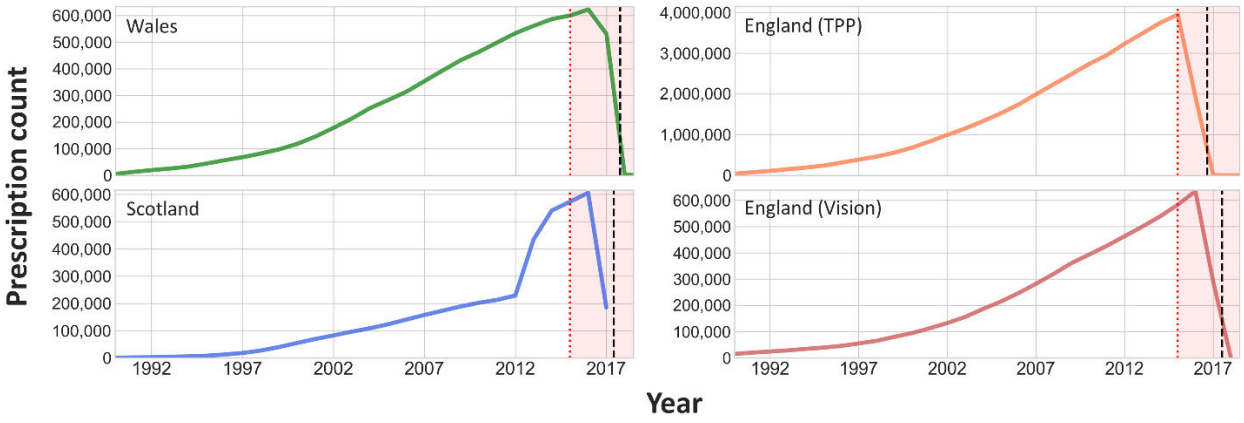
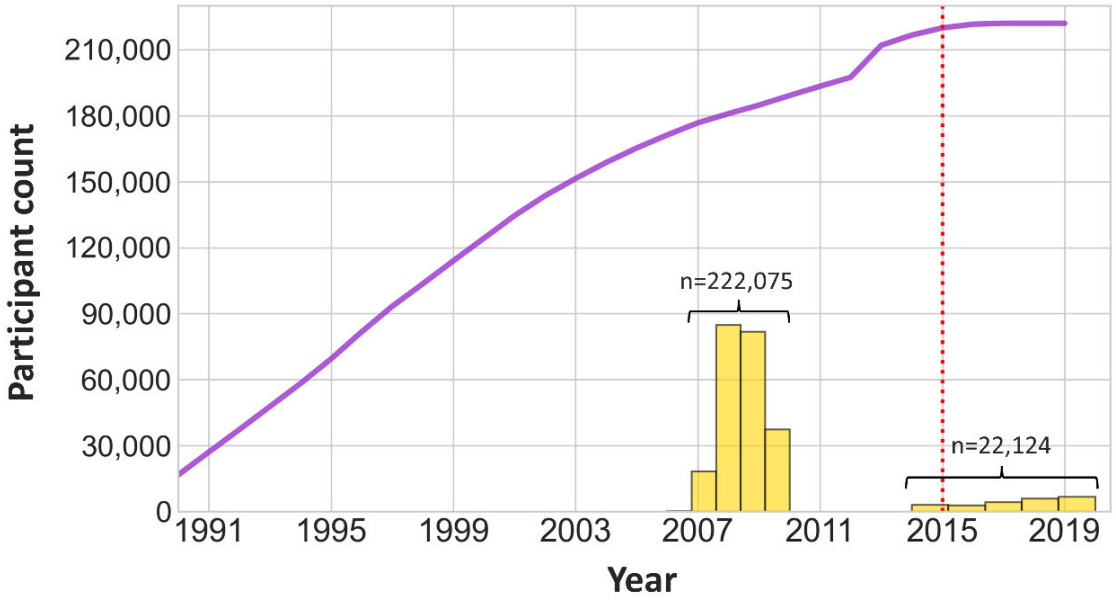


Figure 13: Timeline for analyses. The purple line depicts the number of participants in UK Biobank with at least one prescribed drug. The two histograms depict the yearly numbers of participants in the prescription subsample that underwent baseline assessments (left histogram) and imaging assessments (right histogram). The red line indicates the cut-off time point for prescriptions. Inpatient or primary care diagnoses are available for the entire duration of the study period.



4 Anticholinergic prescribing trends in the UK

4.1 Introduction

When studying the effects of environmental factors on health and disease, the prevalence of those factors plays an important role in estimating the value of potential interventions. However, as described in section 1.4.1, previous estimates for older, community-dwelling adults suggested prevalence rates of anticholinergic prescribing anywhere between 10% and 66% (Byrne et al., 2018; Fadare et al., 2021; Fox et al., 2011; Gnjidic, Bell, et al., 2012; Gnjidic, Hilmer, et al., 2012; Hilmer et al., 2009; Machado-Alba et al., 2016; Rémillard, 1996; Rhee et al., 2018; Richardson et al., 2015; Shmuel et al., 2021; Sumukadas et al., 2014). One reason for this variance in estimates of prevalence rates may be that studies use different anticholinergic scales that do not always exhibit substantial overlap in the included drugs and score the same drugs differently. Furthermore, different countries report different longitudinal trends in anticholinergic prescribing, with the trend for the UK unclear. To my knowledge, the only study that had previously characterised the longitudinal change in anticholinergic use in the UK, found anticholinergic use in older people in Tayside, Scotland, to be higher in 2010 (n=67,608) when compared to 1995 (n=73,465) (Sumukadas et al., 2014). However, that study measured the exposure on only two occasions and was limited both geographically and in terms of age of participants.

In this chapter, I present my analysis of the prevalence of AChB in the UK and its longitudinal trend over the course of ~25 years. For this I used continuous data on primary-care prescribing in >220,000 participants across the UK. Because I used a single sample to capture these outcomes, any observed linear change may be due to the ageing of the sample (age-effect), differences between birth cohorts (cohort-effect), or changes inherent to the time periods under study (period-effect). As explained later in this chapter, this Age-Period-Cohort (APC) problem is a well-known issue across scientific disciplines and cannot be resolved analytically. In this analysis, I used different plots of age- period- and cohort- effects to appraise the contributions to observed longitudinal trends in the data. Two important observations about

the analysis strategy are worth noting. First, my main objective was to ascertain whether the observed change in AChB can be explained purely by the ageing of the sample, or whether modifications in prescribing contributed to this change independently of ageing. Prescribing modifications could result from cohort or period effects. For example, people born at different times may be differently inclined to visit their physician or to request medicines (cohort effect). On the other hand, changes in guidelines or the appearance of new drugs may have made physicians more likely to prescribe drugs (period effect). Finally, these two causes may both drive prescribing modifications (cohort- and period effects). My aim was not to disentangle period- and cohort effects but in resolving whether any period- or cohort effects occurred in the data. Second, due to the impossibility of mathematically resolving linear APC-effects, my goal was to appraise the relative contribution by plotting the effects and interpreting the changes based on prior assumptions. This approach does not allow for hard numerical estimates but enables the evaluation of a broad linear trend.

This study was published in the British Journal of Clinical Pharmacology in August 2021 (Mur et al., 2021) and was presented as a poster at the GSA Annual Scientific meeting Online (November 4th – 7th 2020). It is available in full in section 4.2.

4.2 Increase in anticholinergic burden from 1990 to 2015: Age-period-cohort analysis in UK Biobank

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ORIGINAL ARTICLE

Increase in anticholinergic burden from 1990 to 2015: Age-period-cohort analysis in UK biobank

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Background: The use of prescription drugs with anticholinergic properties has been associated with multiple negative health outcomes in older people. Moreover, recent evidence suggests that associated adverse effects may occur even decades after stopping anticholinergic use. Despite the implicated importance of examining longitudinal patterns of anticholinergic prescribing for different age groups, few such data are available.

Methods: We performed an age-period-cohort (APC) analysis to study trends in an aggregate measure of anticholinergic burden between the years 1990 and 2015, utilising data from >220 000 UK Biobank participants with linked prescription data from primary care.

Results: Anticholinergic burden in the sample increased up to 9-fold over 25 years and was observed for both period and age effects across most classes of drugs. The greatest increase was seen in the prescribing of antidepressants. Female sex, lower education and greater deprivation were associated with greater anticholinergic burden.

Conclusions: The increase in anticholinergic prescribing is mostly due to an increase in polypharmacy and is attributable to both ageing of participants and period-related changes in prescribing practices. Research is needed to clarify the implications of rising anticholinergic use for public health and to contextualise this rise in light of other relevant prescribing practices.

KEYWORDS
anticholinergic drugs, drug prescribing, general practice, polypharmacy

1 | INTRODUCTION

Medicines with anticholinergic properties – antagonists to muscarinic receptors in the nervous system – are found among various classes of drugs.[1] Several anticholinergic drugs are listed in the American Geriatrics Society Beers Criteria for Potentially Inappropriate Medication[2] and the STOPP/START criteria for potentially inappropriate prescribing.[3] Age is the strongest predictor of polypharmacy, with the odds of taking 10 or more medicines doubling in every decade of life.[4] Moreover, due to the age-associated decline in the ability to metabolise drugs, older people are more sensitive to the side effects

This is a secondary investigation of an existing cohort study and therefore did not have a Principal Investigator.

of drugs.[5] This is especially pertinent in the case of anticholinergic compounds, which are commonly prescribed and whose side effects are well documented.[1] Anticholinergic burden in older adults is associated with reduced physical and cognitive ability,[6,7] impaired ability to perform activities of daily living,[8] increased risks of falls,[9] dementia[10] and mortality.[11] The association with dementia has been observed even when the anticholinergic exposure occurred decades prior to diagnosis.[10]

Several tools to assess inappropriate prescribing have been developed in the last few decades.[12] Subsequently, inappropriate prescribing in older people declined from 45.5% to 40.8% between 2006/2007 and 2009/2010 in the United States,[13] and from 32.2% to 28.3% between 1996 and 2005 in the UK.[14] However, older adults remain exposed to anticholinergic drugs,[15] the prevalence of which has remained stable in the United States,[16] but has increased by 3% in the UK from 1995 to 2010[17] and by 12.5% in Finland from 2007 to 2017.[18] While some studies have found associations between anticholinergic use and demographic factors,[16,17,19] these variables are rarely examined in detail. Moreover, it is not known whether these potential group differences persist over time.

The study of temporal changes of prescribing practices with in-depth assessment of age-period-cohort (APC) effects necessitates longitudinal designs. Cross-sectional studies[15] or repeated cross-sectional studies[17,18] have explored the extent of anticholinergic use in European countries,[17,18] but the last year of sampling in the UK was in 2010.[17] Moreover, they either lack longitudinal data or rely on participants from a relatively limited geographic area and within a narrow age range. In this paper, we address those limitations by using a large national sample from UK Biobank to characterise longitudinal prescribing patterns of anticholinergic drugs in 1990-2015.

What is already known about this subject

- Anticholinergic burden has been associated with reduced physical and cognitive ability, and an increased risk of dementia and all-cause mortality. Extant epidemiological studies in Europe suggest an increase in anticholinergic prescribing over time, but focus on limited geographic areas, utilise cross-sectional designs or focused on individuals in older age, despite the potential importance of anticholinergic exposure throughout life.

What this study adds

- We performed an age-period-cohort analysis of changes in anticholinergic burden in >220 000 participants from UK Biobank, using GP electronically prescribed longitudinal data from 1990 to 2015. Anticholinergic burden in the UK has increased across several age groups and classes of prescription drugs. The increase was related to both ageing of the underlying sample as well as period-related changes in prescribing.

2 | METHODS

2.1 | Hypotheses

We based our hypotheses on previous cross-sectional studies in the UK that showed greater polypharmacy[4] and anticholinergic burden[17] in 2010 when compared to 1995, and increased polypharmacy in older individuals.[4] We hypothesised that anticholinergic burden increased as a function of both period and age. Additionally, we hypothesised that anticholinergic burden was higher in women and in less educated individuals, as had been reported before.[15,17]

2.2 | Sample

UK Biobank is a prospective study of >500 000 participants aged 37-73 years, recruited in 22 assessment centres throughout the UK in 2006-10.[20] To ensure a representative sample in the given age range, eligible participants for the study were identified through general practice registers and invited by post. The assessments consisted of touch-screen questionnaires, computer-assisted interviews, measures of physical function and the collection of blood, saliva and urine. Primary care prescriptions were available for ~230 000 participants to May 2017 for Scotland, to September 2017 for Wales and to August 2017 for England. The data were provided to UK Biobank by region-specific data providers and include, among other information, the dates of prescriptions, names of drugs prescribed and drug codes. The latter include BNF codes provided by the British National Formulary, which provides prescribing guidance on medicines (https://www.bng.org/), Read v2- and CTV3-codes provided by the Terminology Reference Data Update Distribution (TRUD) service (https://isd.digital.nhs.uk/), and dmd + d codes provided by the National Health Service (NHS) (https://www.nhs.uk/). The drug code systems are used as dictionaries for medicines.

2.3 | Assignment of anticholinergic burden and drug class

Several resources allow for the identification of drugs with anticholinergic properties and provide a score of anticholinergic potency for each drug. These anticholinergic burden scales derive the lists of drugs from different sources, utilise different methods to assign the scores and validate the resulting tools in different populations and on different outcome measures. Previous studies have compared various existing anticholinergic scales and have generally reported poor overlap among them.[21–23] For the purposes of our study, we identified

multiple scales[24–33] from a systematic review[34]; apart from two[30,33] all had a four-point (0-3) scoring system of anticholinergic potency, where a lower score corresponds to lower anticholinergic potency (Supporting Information Table S1). We derived a meta-scale (Supporting Information Table S2) by calculating the mean anticholinergic burden across all nine original scales that had rated a drug. Thus, scales that scored a drug (even if that score was zero) were included in the computation for that drug, while scales that did not score the drug were not. All prescriptions of medicines with ophthalmic, otic, nasal or topical routes of administration were assigned an anticholinergic score of zero, as has been done before.[29,31,34,35]

For prescription entries that did not list any drugs (ie, for which the column indicating the name of the drug was blank), drug codes were used to supplement them. A series of steps was taken to exclude incomplete data or low number of individuals (Supporting Information Figures S1 and S2). Drugs were classified based on the Anatomical Therapeutic Chemical (ATC) Classification System (https://www.whocc.no), representing (1) the anatomical target, (2) the therapeutic subgroup, (3) the pharmacological subgroup, (4) the chemical subgroup and (5) the chemical substance. For example, metformin (5) affects the alimentary tract and metabolism (1), treats diabetes (2), lowers blood-glucose (3) and is a biguanide (4). Not all classes were equally represented in the sample. To allow for comparability of frequency of occurrence, we classified anticholinergic drugs into classes that do not all correspond to the same level in the ATC hierarchy (see number in parentheses): "drugs for acid disorders" (3), "analgesics" (2), "antidepressants" (3), "antithrombotic drugs" (2), "cardiovascular drugs" (1), "drugs for diabetes" (2), "gastrointestinal drugs" (2), "psycholeptics" (2), "respiratory drugs" (1) and "urological drugs" (3). A final class of "other drugs" was constructed that contained drugs that primarily due to their low prevalence individually contributed relatively little to the total anticholinergic burden. These included anticonvulsants, antibiotics, anti-Parkinsonian drugs, corticosteroids, immunosuppressants, anti-inflammatory drugs, muscle relaxants and anti-diarrhoeal drugs.

2.4 | Statistical approach

To enable longitudinal analyses, the original format of the data was transformed into two different formats that reflected for each participant the monthly and yearly anticholinergic burden, respectively. These period-based anticholinergic burden scores were calculated by summing the anticholinergic burden of all prescriptions in that period (Supporting Information Figure S3 and Text S1). When individual-level data are collected longitudinally, changes can be due to age, period or cohort effects.[36] Because the three effects are colinear (age period cohort), they cannot all be included in a regression analysis, as holding two terms constant keeps the third term constant as well.[37] While there have been attempts to estimate the unique contributions among the three effects,[38,39] no solution has been widely adopted. Hidden assumptions can have a strong effect on the interpretation of the APC effect,[40] and in our analysis we make the following assumptions. First, age is probably positively associated with anticholinergic burden due to the positive association between polypharmacy and age.[4] Second, we assume that birth cohort does not play a role in the above association, ie, we are only interested in whether a potential longitudinal change in anticholinergic burden is due to the participants' age or due to changes in prescribing practices over time. For the analysis of APC effects, we ran three models, excluding one of the APC terms at a time (ie, its effect was assumed to be zero). Thus, anticholinergic burden was modelled as a function of either period and cohort (period-cohort model), age and cohort (age-cohort model) or age and period (age-period model). This three-model approach represents the same process – the change in anticholinergic burden in the sample – from three different perspectives and allows for an appraisal of possible drivers of the observed trend. For example, assuming that the effect of birth cohort is zero, positive effects for both period and cohort in the period-cohort model, and a positive effect of period, but a negative effect of age in the age-period model demonstrates that anticholinergic burden (a) increases with time across cohorts, (b) is higher in younger cohorts in a given period, (c) decreases with age and (d) is higher in recent periods across age groups. This would suggest that the anticholinergic burden increased with the time period but decreased with age. We additionally computed the above models by fitting separate intercepts and slopes: for the period-cohort and age-cohort models separate intercepts and slopes for each cohort, and for the age-period model separate intercepts and slopes for each period. For analyses of lifestyle and demographic factors, we fitted tobit linear models[41] to average monthly anticholinergic burden, adjusting for sex, education, physical activity, social deprivation, region, smoking, body mass index (BMI), frequency of alcohol consumption and age at assessment. Tobit models are models of censored regression, where the values that fall either above or below a certain value are censored. In our analysis, tobit models were censored from below at 0, effectively simulating zero inflation. For models with random effects, we used generalised linear mixed models (R package glmmTMB[42]); for all other models, we used Tobit regression (R package censReg). Due to the relative infrequency of anticholinergic drugs, anticholinergic burden was right-skewed and models were adjusted for zero inflation. The results are reported in unstandardised beta coefficients. The figures accompanying the analyses were generated based on the output of these analyses. Descriptive figures depicting longitudinal changes were based on generalised additive smoothing; whenever the latter is the case, it is explicitly indicated. Data cleaning and statistical analyses were performed in Python version 3.7.4 and R versions 3.4.1 and 3.6.3.

Several covariates were ascertained during or immediately prior to the participants' recruitment to UK Biobank. These included sex (male *vs* female [ref.]), education (graduate degree, no graduate degree [ref.]), alcohol consumption (1, daily or almost daily [ref.]; 2 three or four times a week; 3, once or twice a week; 4, one to three times a month *vs* 5, only on special occasions; 6, never), smoking status (current smoker, past smoker, never smoker [ref.]), BMI and physical activity (strenuous, moderate, mild [ref.])[43] and the Townsend Index of Socioeconomic Deprivation[44] (range −6.3-11.0). The latter is derived from different variables available from census data and

calculated to yield *z* scores. These are then summed and provide with an index ranging from −12 to 12, with higher values indicating greater deprivation. Region (Scotland, Wales, England [ref.]) was derived by combining data providers so that all prescriptions issued in England, Scotland and Wales were classified under the same category.

Each model was run in three iterations: basic models were unadjusted; basic-adjusted models included sex, data provider, education and socioeconomic deprivation. Data providers were specific to each prescription and available longitudinally. Sex, education and deprivation were assumed constant (ie, treated as time-invariant covariates) within individuals: over 90% of UK Biobank participants reported the same educational attainment at reassessment, within-person stability of deprivation has been reported previously.[45] Fully adjusted models were additionally controlled for smoking, alcohol consumption frequency, BMI, and physical activity. While these covariates were available only cross-sectionally, they are important in health and disease. Outlier observations – for anticholinergic burden and for polypharmacy, observations five or more interquartile ranges beyond the median (without accounting for zero-values), for BMI values lower than 15 and greater than 50 – were removed prior to analysis. This resulted in the removal of at most (depending on iteration) 347 297 data points (~0.1% of the sample) for the data with monthly anticholinergic burden, 42 425 data points (1.3% of the sample) for the data with yearly anticholinergic burden and 13 836 data points (6.2% of the sample) for the individual lifestyle and demographic data.

A sensitivity analysis was conducted that was limited to the period from 2000 to 2015. This was done due to the relatively low level of ascertainment in the sample before that period (Supporting Information Figure S2).

2.5 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY, and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20.[46]

3 | RESULTS

The 220 867 participants were born between 1938 and 1969 (Supporting Information Figure S4). Individuals were being added to the database of prescriptions throughout the sampling period (1990-2015), but the demographic structure of the sample (Table 1) remained relatively stable over time. However, it is unclear how demographic variables changed within individuals over time.

3.1 | Anticholinergic prescribing

Of 248 drugs on the meta-scale, 201 (81.0%) were found in the sample and constituted 25.0% of all prescriptions. A total of 199 652 participants (90.4%) were prescribed at least one anticholinergic drug and 28 525 (13.2%) participants were prescribed anticholinergic drugs every year during the prescribing period. Among previously published scales, anticholinergic prescriptions constituted 2.5-23.1% of all prescriptions (Table 2) and anticholinergic burden according to each scale exhibited an increasing trend over time (Figure 1A). Depending on the scale used, anticholinergic burden increased

TABLE 1 Demographic characteristics of the sample at the time of recruitment to UK Biobank

Variable	Level	Median (IQR) or n (%)	Missing
Sex	Female	121 286 (54.9)	0
	Male	99 581 (45.1)	
Education	Graduate degree	69 745 (32.0)	2816
	No graduate degree	148 306 (68.0)	
Deprivation		−2.2 (4.1)	319
Alcohol consumption	Daily or almost daily	43 269 (19.6)	559
	Three or four times a week	50 753 (23.0)	
	Once or twice a week	57 934 (26.3)	
	Once to three times a month	24 891 (11.3)	
	Only special occasions	25 274 (11.5)	
	Never	18 187 (8.3)	
Smoking	Current smoker	23 069 (10.5)	1144
	Previous smoker	75 955 (34.6)	
	Non-smoker	120 699 (54.9)	
Physical activity	Strenuous	21 618 (10.6)	16 225
	Moderate	129 874 (63.4)	
	Light	53 150 (26.0)	
BMI		26.9 (5.8)	1332

between 3- and 9-fold from 1990 to 2015. Most anticholinergic prescriptions were for antidepressants, which accounted for 32.5% of the total anticholinergic burden (Table 3 and Figure 1B). The anticholinergic burden for each drug class increased with time (Figure 1C).

3.2 | Age-period-cohort analysis

In the basic period-cohort model, anticholinergic burden was positively associated with period and negatively associated with cohort. In the basic age-cohort model, anticholinergic burden was positively associated with age and with cohort. In the basic age-period model, anticholinergic burden was positively associated with age and with period. The same trends were observed in the basic-adjusted and fully adjusted models (Supporting Information Table S3). These results indicate that greater anticholinergic burden relates to both ageing and later period. That is, in a given period, older individuals experience a higher anticholinergic burden than younger individuals in the same period. Moreover, in recent periods, individuals will experience a higher anticholinergic burden than individuals of the same age did in the past. For example, the average yearly anticholinergic burden of a 50-year-old was 2.32 in 2000, 2.92 in 2007 and 3.67 in 2015, while the average yearly anticholinergic burden of a 60-year-old was 3.06 in 2000, 3.94 in 2007 and 5.12 in 2015. The trends persisted when the outcome was the number of prescribed anticholinergic drugs (Supporting Information Table S4). The proportion of drugs with different anticholinergic potencies remained stable over time (Supporting Information Figure S5). Thus, the increase in anticholinergic burden was likely due to a general increase in anticholinergic prescribing, rather than a relative increase in the prescribing of stronger anticholinergic drugs.

In the mixed-effects models, anticholinergic burden increased by 0.22 each year (SE 0.0012, $P < .001$). In the period-cohort model, earlier-born cohorts exhibited steeper slopes than later-born cohorts (n 32, correlation between slope and cohort r −0.97, SE 0.041, $P < .001$; Figure 2A). In the mixed-effects age-cohort model, earlier-born cohorts exhibited steeper slopes than later-born cohorts (n 32, correlation between slope and cohort r −0.97, SE 0.048, $P < .001$). In the mixed-effects age-period model, later periods exhibited steeper slopes than earlier periods (n 25, correlation between slope and period r 0.95, SE 0.064, $P < .001$).

When the change in anticholinergic burden was plotted for each drug class separately (Supporting Information Figure S6), the same pattern was observed for all drug classes except for drugs for acid disorders and cardiovascular drugs. For the former, an increase in anticholinergic burden over time was observed, but was similar across periods and cohorts, suggesting an effect of age, but without a prominent period effect. For cardiovascular drugs, we observed an increase in anticholinergic burden over time and a higher anticholinergic burden in earlier cohorts and later periods, suggesting a positive effect of age, but a negative period effect.

When the basic models were adjusted by the addition of the total number of prescribed drugs (Supporting Information Table S5), all effect sizes were greatly diminished, more so by the total number of prescribed drugs than by all other covariates combined. Furthermore, the effect of birth cohort was reversed in the period-cohort model and the effect of age was reversed in the age-period model. Thus, the period effect was retained, but the effect of age was reversed when adjusted for the number of prescribed drugs. These results possibly indicate that whereas overall anticholinergic burden has increased over time, and more so among older adults, anticholinergic drugs in the latter group comprise a relatively lower proportion of overall prescriptions when compared to younger individuals (Supporting Information Figure S7).

TABLE 2 Comparison of the number of drugs on each anticholinergic scale, the number of drugs on each scale that were prescribed in our sample, the percentages of all prescriptions in the sample that the drugs on the scales constituted and the increase in the mean yearly anticholinergic burden from 1990 to 2015

Scale	n drugs on the list	n drugs in the sample (%)	% total prescriptions	1990-2015 increase (%)
Han et al[24,35]	67	53 (79.1)	10.5	531
Ancelin et al[25]	27	21 (77.8)	2.5	464
Carnahan et al[26]	145	108 (74.5)	9.7	318
Chew et al[27]	39	33 (84.6)	11.3	697
Cancelli et al[28]	17	15 (88.2)	3.9	699
Rudolph et al[29]	69	62 (90.0)	5.9	374
Ehrt et al[30]	29	23 (79.3)	7.4	902
Sittironnarit et al[31]	49	42 (85.7)	12.5	533
Boustani et al[32] (2012)	99	85 (85.9)	12.3	478
Durán et al[33]	180	141 (78.3)	20.7	442
Kiesel et al[34]	165	141 (85.5)	23.2	525
Meta-scale	**248**	**201 (81.0)**	**25.0**	**432**

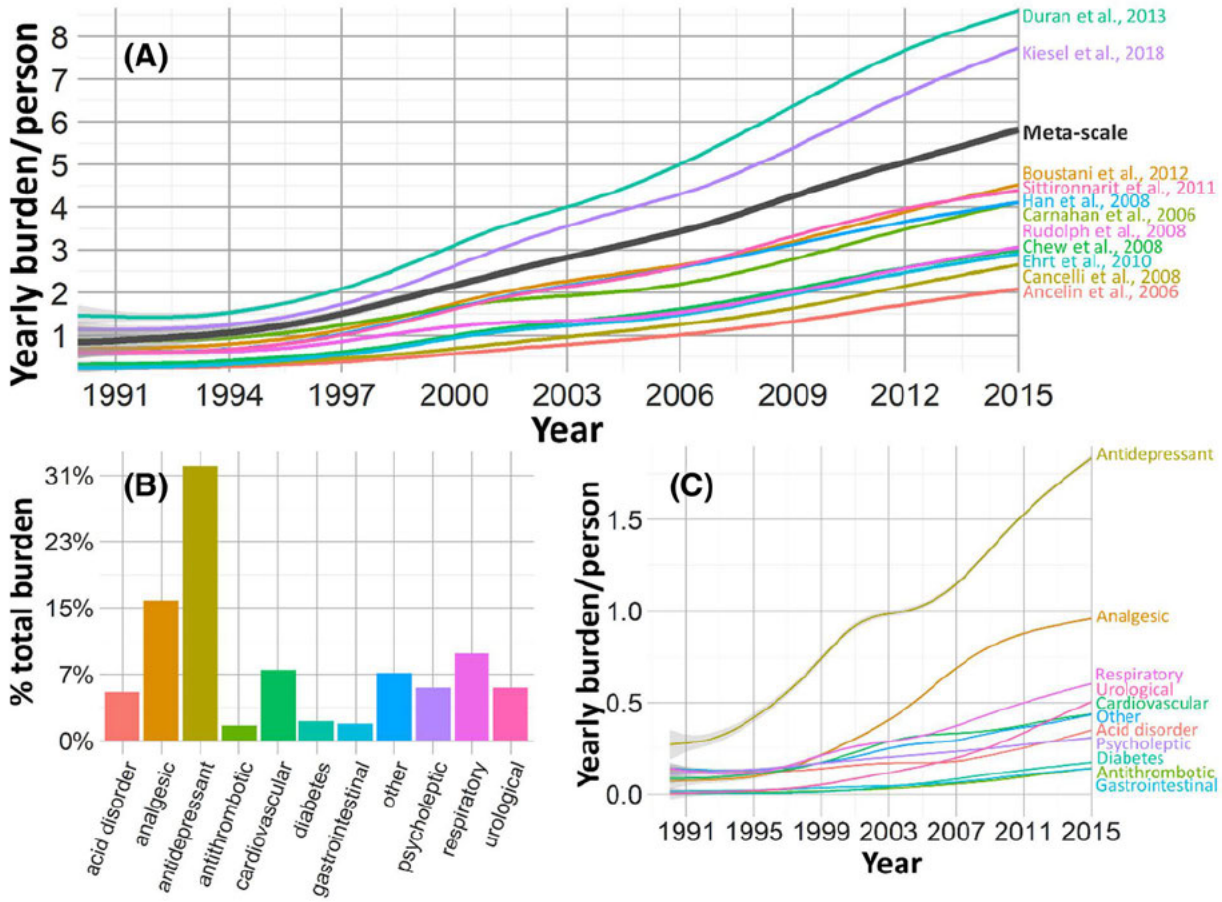

FIGURE 1 Anticholinergic burden over time based on different anticholinergic scales (A), percentage of anticholinergic burden in the sample due to each drug class (B) and the change in anticholinergic burden over time due to each drug class (C). The plots in (A) and (C) were generated using generalised additive smoothing

TABLE 3 Comparison of the number of anticholinergic drugs from different drug classes and their contributions to the total anticholinergic burden

Drug class	n	%	Number of drugs in class	Mean anticholinergic burden per drug	% total anticholinergic burden
Acid disorders	1 464 542	10.3	5	0.50	5.8
Analgesics	1 426 703	10.1	12	1.48	16.5
Antidepressants	2 678 379	18.9	27	1.54	32.4
Antithrombotics	546 311	3.8	2	0.41	1.8
Cardiovascular	2 006 594	14.1	16	0.53	8.4
Diabetes	785 940	5.5	1	0.38	2.3
Gastrointestinal	220 269	1.6	9	1.18	2.0
Psycholeptic	690 894	4.9	35	1.16	6.3
Respiratory	1 499 455	10.6	33	0.88	10.3
Urological	330 314	2.3	8	2.41	6.2
Other	2 545 293	17.9	57	0.40	8.0

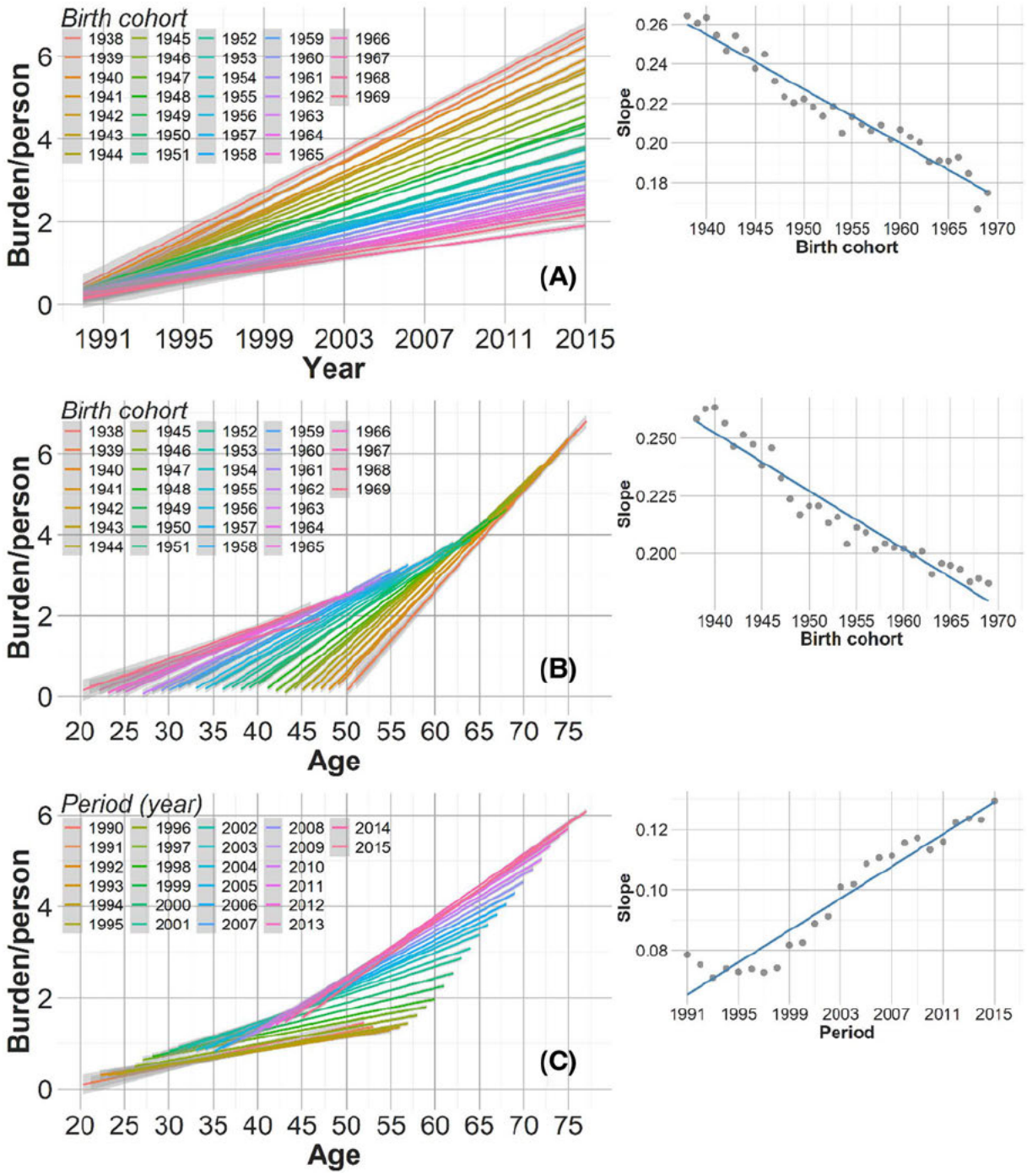

FIGURE 2 APC analysis with basic mixed models with random intercepts and slopes (left) and associations between slopes and different levels of predictors (right). (A) The period-cohort model with cohort as a random effect, (B) the age-cohort model with cohort as a random effect and (C) the age-period model with period as the random effect

3.3 | Anticholinergic burden and demographic factors

Higher anticholinergic burden was associated with female sex, lower educational attainment, greater deprivation, higher BMI, less frequent alcohol consumption and lower physical activity, and was greater in Scotland and Wales than in England (Table 4).

Examining each drug class separately, most effects remained (Supporting Information Table S6). However, anticholinergic burden due to antithrombotic drugs, cardiovascular drugs and drugs for diabetes was higher in males than in females. Moreover, regional differences in anticholinergic burden strongly depended on drug class. Deprivation was transformed into a binary categorical variable, with the median (~2.2) across all participants defining the groups. For

TABLE 4 Results of the model predicting monthly anticholinergic burden as a function of deprivation, smoking, BMI, sex, education, region, alcohol consumption, physical activity and age

Predictor	Level	Beta	SE	P
Deprivation		0.0058	2.6×10^{-4}	<.001
Smoking (*ref: non-smoker*)	Previous smoker	0.041	0.0016	<.001
	Current smoker	0.072	0.0027	<.001
BMI		0.010	1.7×10^{-4}	<.001
Sex	Male	−0.043	0.0015	<.001
Education	Graduate degree	−0.046	0.0016	<.001
Region (*ref: England*)	Scotland	0.047	0.0025	<.001
	Wales	0.029	0.0026	<.001
Alcohol consumption (*ref: daily or almost daily consumption*)	Three or four times a week	−0.004	0.0022	<.001
	Once or twice a week	0.014	0.0022	<.001
	Once to thrice a month	0.032	0.0028	<.001
	Special occasions only	0.066	0.0029	<.001
	Never	0.102	0.0033	<.001
Physical activity (*ref: mild or no physical activity*)	Moderate	−0.041	0.0018	<.001
	Strenuous	−0.072	0.0028	<.001

region, sex, education and deprivation, we then plotted anticholinergic burden as a function of period for different levels of predictor variables. Supporting Information Figure S8 illustrates the association between the above predictors and anticholinergic burden.

3.4 | Sensitivity analyses

When the observation period was restricted to prescriptions after 1999, the trends above were again observed for all models except for when polypharmacy was used as covariate (Supporting Information Tables S7-10, Text S2 and Figure S9). There, period was negatively associated with anticholinergic burden in the period cohort and in the age-period model. Age was negatively associated with anticholinergic burden in both the age cohort and the age-period model. Birth cohort was positively associated with anticholinergic burden in the period-cohort model, but negatively associated with anticholinergic burden in the age-cohort model. Thus, when accounting for polypharmacy, the sensitivity analysis supports an age-related decrease in anticholinergic burden, but does not support a period-related increase in anticholinergic burden.

4 | DISCUSSION

In a large longitudinal study of prescription drugs with anticholinergic properties, we showed that the anticholinergic burden in the UK is increasing, and older individuals continue to have the highest anticholinergic burden. Age-related increases in anticholinergic burden can be explained by polypharmacy in older adults. Indeed, when accounting for polypharmacy and period, anticholinergic burden decreases with age, possibly demonstrating proportionate deprescribing of anticholinergic drugs in older age. We also find associations between higher anticholinergic burden and various demographic and lifestyle factors, including female sex, less education and greater socioeconomic deprivation.

4.1 | Anticholinergic burden over time

Anticholinergic burden increased in all APC models. Throughout time periods and across birth cohorts, ageing was associated with greater anticholinergic burden. Moreover, across age groups and birth cohorts, anticholinergic burden has increased in recent years. Finally, at a given age, later-born cohorts experienced a greater anticholinergic burden than earlier-born cohorts, while in a given period, later-born cohorts experienced a smaller anticholinergic burden than earlier-born cohorts.

Because of the collinearity of age, period and cohort (age period cohort), they cannot all be included in a regression analysis, as holding two terms constant keeps the third term constant as well.[37] Some argue that the APC problem cannot be completely resolved[40] and that results from APC-based models should be based on well-founded and clearly communicated assumptions. In the present paper we assumed no cohort effects and predicted anticholinergic burden to increase with ageing. Based on current knowledge on polypharmacy and anticholinergic burden, the following conclusions can be drawn from our results. First, due to increased multimorbidity and polypharmacy in older individuals,[4] age contributed to the trend. When intercept and slope were modelled separately in mixed models with random effects, cohort was negatively associated with the slope, suggesting not only a greater anticholinergic burden, but also a more

rapid accumulation of burden in older individuals. Second, as previously reported,[4] individuals are now being prescribed more drugs than in the past. The increase in anticholinergic burden could be caused by a new generation of patients who either demand more or who are diagnosed with more maladies. Alternatively, the increase could be related to changes in prescribing practices due to societal changes or changes in medical training. Regardless of the underlying causes, people in the UK are being increasingly prescribed anticholinergic drugs.

The increases in anticholinergic burden could be related to an increase in general polypharmacy and not an increase in specifically anticholinergic prescribing. Indeed, when the models were adjusted for the number of prescriptions, the changes in anticholinergic burden were greatly diminished. Furthermore, earlier-born individuals exhibited a lower anticholinergic burden across periods and across age groups than those born later. Moreover, across age groups, anticholinergic burden was higher in later periods than in earlier periods. While correcting for polypharmacy had no effect on the trend of the age-cohort model, it changed the direction of birth cohort and age in the period-cohort model and the age-period model, respectively. Later-born individuals exhibited a higher anticholinergic burden, and this burden was positively associated with period, but negatively associated with age. The failure to exactly replicate these results when the period was restricted to 2000-2015 indicates that the relationship between polypharmacy and anticholinergic burden is complex and warrants more detailed study. While the results indicate that medical practitioners have been mitigating the increase in polypharmacy by deprescribing anticholinergic drugs in older people, this group nevertheless experienced the highest burden. Furthermore, older people experienced a greater anticholinergic burden in 2015 than at any point in the preceding 25 years.

4.2 | Demographic- and lifestyle factors

Anticholinergic use has been linked with some demographic and lifestyle factors.[16,17,19] In our study, female sex, lower education, higher socioeconomic deprivation, higher BMI, lower frequency of alcohol consumption, lower physical activity and being prescribed in Scotland or Wales (compared to England) were associated with a higher anticholinergic burden. Certain groups do require a greater number of medications but medical professionals may prescribe more to certain groups, independent of underlying medical conditions.

Interestingly, greater alcohol consumption was associated with decreased anticholinergic burden. Individuals who take many medications may reduce their alcohol consumption to reduce the risk of drug interactions or to reduce the impact of existing disease.

4.3 | Strengths and limitations

The present study used a very large, well-characterised sample and utilised primary care electronic prescription data over a wide period. However, we recognise several limitations. First, while visual inspection of anticholinergic burden across different scales did reveal a common upward trend, our newly computed meta-scale was not previously validated and estimates a higher anticholinergic burden than most other scales. Second, we did not include longitudinal data on over-the-counter drugs and dietary supplements. Considering the availability in the UK of over-the-counter anticholinergic medicines, especially histamines, the computed anticholinergic burden was likely an underestimate across all scales. Third, while our assumption that topical, ophthalmic, otic and nasal drugs do not have anticholinergic effects is common in the literature,[29,31,34,35] we are not aware of conclusive evidence to support it. Fourth, estimates of prevalence and statistical inferences are dependent on the underlying sample and UK Biobank is not representative of the UK population. On average, participants in the study are less likely to be obese, to smoke, have fewer health conditions and live in socioeconomically less deprived areas.[47] Thus, differences in anticholinergic burden and period-dependent disparities are possibly greater in real populations. Fifth, our analysis of the effects of demographic and lifestyle factors on anticholinergic burden assessed the correlation between the average value of a metric that changes with time (anticholinergic burden) and cross-sectional data (eg, BMI, smoking, alcohol consumption and physical activity), which was ascertained towards the end of the period in question when participants were of different ages. We also modelled deprivation and educational attainment as time-invariant covariates. Thus, our results cannot clarify the exact nature of potential temporal relationships. Finally, we did not have data on the oldest people, who represent the group most at risk of anticholinergic effects.

5 | CONCLUSION AND FUTURE DIRECTIONS

Prescribing drugs involves balancing their medicinal value with potential harms. Moreover, exhaustive longitudinal studies are required to fully determine all their effects. However, besides well-documented side effects,[48] exposure has been linked to an increased frequency of falls,[9] reduced physical, cognitive and functional ability,[6–8] and increased risks of dementia[10] and all-cause mortality.[11] Thus, anticholinergic drugs ought to be prescribed sparingly and the use of alternatives strongly considered. An understanding of temporal prescribing trends in a population may help to guide prescribing and stimulate further research. Our work represents an overview and future studies should describe prescribing trends and their relationship to age groups, and demographic and lifestyle characteristics in greater detail. There is also evidence of differences between drug classes in the association between anticholinergic burden and health outcomes.[10] Identifying distinct anticholinergic trends for individual drug classes for different groups could help to further improve prescribing guidelines. Additionally, future work should attempt to identify the causes for the increase in anticholinergic prescribing, and more precisely quantify the potential implications for important life outcomes, including brain and cognitive health, and dementia. Finally, decreases in potentially inappropriate prescribing have been reported even when

the same population experienced increases in polypharmacy and in anticholinergic use.[49] Thus, increases in anticholinergic burden should not be considered in isolation, but in the context of other prescribing practices.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to disclose.

CONTRIBUTORS

J.M. designed the study, cleaned and analysed the data, interpreted the results, generated the figures and wrote the first draft of the manuscript. All authors assisted with the data analysis strategy, contributed to the editing of the manuscript and approved the final version.

DATA AVAILABILITY STATEMENT

All data are available via UK Biobank. The code used for cleaning and modelling the data is available on GitHub (https://github.com/JuM24/Anticholinergic-trends-UK-Biobank).

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REFERENCES

1. Collamati A, Martone AM, Poscia A, et al. Anticholinergic drugs and negative outcomes in the older population: from biological plausibility to clinical evidence. *Aging Clin Exp Res*. 2016;28(1):25-35.
2. Fick DM, Semla TP, Steinman M, et al. American Geriatrics Society 2019 Updated AGS Beers Criteria® for Potentially Inappropriate Medication Use in Older Adults. *J Am Geriatr Soc*. 2019;67(4):674-694.
3. O'Mahony D, O'Sullivan D, Byrne S, O'Connor MN, Ryan C, Gallagher P. STOPP/START criteria for potentially inappropriate prescribing in older people: Version 2. *Age Ageing*. 2015;44(2):213-218.
4. Guthrie B, Makubate B, Hernandez-Santiago V, Dreischulte T. The rising tide of polypharmacy and drug-drug interactions: Population database analysis 1995-2010. *BMC Med*. 2015;13(1):1-10.
5. McLachlan AJ, Hilmer SN, Le Couteur DG. Variability in response to medicines in older people: Phenotypic and genotypic factors. *Clin Pharmacol Ther*. 2009;85(4):431-433.
6. Tannenbaum C, Paquette A, Hilmer S, Holroyd-Leduc J, Carnahan R. Re: A systematic review of amnestic and non-amnestic mild cognitive impairment induced by anticholinergic, antihistamine, GABAergic and opioid drugs. *Drugs Aging*. 2012;29(8):239-658.
7. Landi F, Russo A, Liperoti R, et al. Anticholinergic drugs and physical function among frail elderly population. *Clin Pharmacol Ther*. 2007;81(2):235-241.
8. Salahudeen MS, Duffull SB, Nishtala PS. Anticholinergic burden quantified by anticholinergic risk scales and adverse outcomes in older people: A systematic review. *BMC Geriatr*. 2015;15(1):31. https://doi.org/10.1186/s12877-015-0029-9
9. Cardwell K, Hughes CM, Ryan C. The association between anticholinergic medication burden and health related outcomes in the 'oldest old': A systematic review of the literature. *Drugs Aging*. 2015;32(10):835-848.
10. Richardson K, Fox C, Maidment I, et al. Anticholinergic drugs and risk of dementia: Case-control study. *BMJ*. 2018;361:1-12.
11. Ruxton K, Woodman RJ, Mangoni AA. Drugs with anticholinergic effects and cognitive impairment, falls and all-cause mortality in older adults: A systematic review and meta-analysis. *Br J Clin Pharmacol*. 2015;80(2):209-220.
12. Dimitrow MS, Airaksinen MSA, Kivelä SL, Lyles A, Leikola SNS. Comparison of prescribing criteria to evaluate the appropriateness of drug treatment in individuals aged 65 and older: A systematic review. *J Am Geriatr Soc*. 2011;59(8):1521-1530.
13. Davidoff AJ, Miller GE, Sarpong EM, Yang E, Brandt N, Fick DM. Prevalence of potentially inappropriate medication use in older adults using the 2012 Beers criteria. *J Am Geriatr Soc*. 2015;63(3):486-500.
14. Carey IM, De Wilde S, Harris T, et al. What factors predict potentially inappropriate primary care prescribing in older people? Analysis of uk primary care patient record database. *Drugs Aging*. 2008;25(8):693-706.
15. Byrne CJ, Walsh C, Cahir C, Ryan C, Williams DJ, Bennett K. Anticholinergic and sedative drug burden in community-dwelling older people: A national database study. *BMJ Open*. 2018;8(7):1-8.
16. Rhee TG, Choi YC, Ouellet GM, Ross JS. National prescribing trends for high-risk anticholinergic medications in older adults. *J Am Geriatr Soc*. 2018;66(7):1382-1387.
17. Sumukadas D, McMurdo MET, Mangoni AA, Guthrie B. Temporal trends in anticholinergic medication prescription in older people: Repeated crosssectional analysis of population prescribing data. *Age Ageing*. 2013;1-7.
18. Aalto UL, Roitto HM, Finne-Soveri H, Kautiainen H, Pitkälä KH. Temporal trends in the use of anticholinergic drugs among older people living in long-term care facilities in Helsinki. *Drugs Aging*. 37(1):27-34. https://doi.org/10.1007/s40266-019-00720-6
19. Grande G, Tramacere I, Vetrano DL, et al. Role of anticholinergic burden in primary care patients with first cognitive complaints. *Eur J Neurol*. 2017;24(7):950-955.
20. Sudlow C, Gallacher J, Allen N, et al. UK Biobank: An open access resource for identifying the causes of a wide range of complex diseases of middle and old age. *PLoS Med*. 2015;12(3):1-10.
21. Salahudeen MS, Hilmer SN, Nishtala PS. Comparison of anticholinergic risk scales and associations with adverse health outcomes in older people. *J Am Geriatr Soc*. 2015;63(1):85-90.

22. Villalba-Moreno AM, Alfaro-Lara ER, Pérez-Guerrero MC, Nieto-Martín MD, Santos-Ramos B. Systematic review on the use of anticholinergic scales in poly pathological patients. *Arch Gerontol Geriatr*. 2016;62:1-8. https://doi.org/10.1016/j.archger.2015.10.002
23. Welsh TJ, van der Wardt V, Ojo G, Gordon AL, Gladman JRF. Anticholinergic drug burden tools/scales and adverse outcomes in different clinical settings: A systematic review of reviews. *Drugs Aging*. 35(6):523-538. https://doi.org/10.1007/s40266-018-0549-z
24. Han L, McCusker J, Cole M, Abrahamowicz M, Primeau F, Élie M. Use of medications with anticholinergic effect predicts clinical severity of delirium symptoms in older medical inpatients. *Arch Intern Med*. 2001;161(8):1099-1105.
25. Ancelin ML, Artero S, Portet F, Dupuy AM, Touchon J, Ritchie K. Non-degenerative mild cognitive impairment in elderly people and use of anticholinergic drugs: Longitudinal cohort study. *Br Med J*. 2006;332(7539):455-458.
26. Carnahan RM, Lund BC, Perry PJ, Pollock BG, Gulp KR. The anticholinergic drug scale as a measure of drug-related anticholinergic burden: Associations with serum anticholinergic activity. *J Clin Pharmacol*. 2006;46(12):1481-1486.
27. Chew ML, Mulsant BH, Pollock BG, et al. Anticholinergic activity of 107 medications commonly used by older adults. *J Am Geriatr Soc*. 2008;56(7):1333-1341.
28. Cancelli I, Valentinis L, Merlino G, Valente M, Gigli GL. Drugs with anticholinergic properties as a risk factor for psychosis in patients affected by Alzheimer's disease. *Clin Pharmacol Ther*. 2008;84(1):63-68.
29. Rudolph JL, Salow MJ, Angelini MC, McGlinchey RE. The anticholinergic risk scale and anticholinergic adverse effects in older persons. *Arch Intern Med*. 2008;168(5):508-513.
30. Ehrt U, Broich K, Larsen JP, Ballard C, Aarsland D. Use of drugs with anticholinergic effect and impact on cognition in Parkinson's disease: A cohort study. *J Neurol Neurosurg Psychiatry*. 2010;81(2):160-165.
31. Sittironnarit G, Ames D, Bush AI, et al. Effects of anticholinergic drugs on cognitive function in older Australians: Results from the AIBL study. *Dement Geriatr Cogn Disord*. 2011;31(3):173-178.
32. Boustani M, Campbell N, Munger S, Maidment I, Fox C. Impact of anticholinergics on the aging brain: A review and practical application. *Aging Health*. 2008;4(3):311-320.
33. Durán CE, Azermai M, Stichele RHV. Systematic review of anticholinergic risk scales in older adults. *Eur J Clin Pharmacol*. 2013;69(7):1485-1496.
34. Kiesel EK, Hopf YM, Drey M. An anticholinergic burden score for German prescribers: Score development. *BMC Geriatr*. 2018;18(1):1-11.
35. Han L, Agostini JV, Allore HG. Cumulative anticholinergic exposure is associated with poor memory and executive function in older men. *J Am Geriatr Soc*. 2008;56(12):2203-2210.
36. Suzuki E. Time changes, so do people. *Soc Sci Med*. 2012;75(3):452-456. Available from: https://doi.org/10.1016/j.socscimed.2012.03.036
37. Bell A, Jones K. The impossibility of separating age, period and cohort effects. *Soc Sci Med*. 2013;93:163-165. Available from: https://doi.org/10.1016/j.socscimed.2013.04.029
38. Yang Y, Schulhofer-Wohl S, Fu WJ, Land KC. The intrinsic estimator for age-period-cohort analysis: what it is and how to use it. *Am J Sociol*. 2008;113(6):1697-1736.
39. Fosse E, Winship C. Bounding analyses of age-period-cohort effects. *Demography*. 2019;56(5):1975-2004.
40. Bell A. Age period cohort analysis: A review of what we should and shouldn't do. *Ann Hum Biol*. 2020;47(2):208-217.
41. Tobin J. Estimation of relationships for limited dependent variables. *Econometrica*. 1958;26(1):24-36.
42. Brooks ME, Kristensen K, van Benthem KJ, et al. glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *R J*. 2017;9(2):378-400.
43. Hanlon P, Quinn TJ, Gallacher KI, et al. Assessing risks of polypharmacy involving medications with anticholinergic properties. *Ann Fam Med*. 2020;18(2):148-155.
44. Townsend P. Deprivation. *J Soc Policy*. 1987;16(2):125-146.
45. Kontopantelis E, Mamas MA, Van Marwijk H, et al. Geographical epidemiology of health and overall deprivation in England, its changes and persistence from 2004 to 2015: A longitudinal spatial population study. *J Epidemiol Community Health*. 2018;72(2):140-147.
46. Alexander SPH, Christopoulos A, Davenport AP, et al. The Concise Guide to PHARMACOLOGY 2019/20: G protein-coupled receptors. *Br J Pharmacol*. 2019;176(S1):S21-S141.
47. Fry A, Littlejohns TJ, Sudlow C, et al. Comparison of sociodemographic and health-related characteristics of UK Biobank participants with those of the general population. *Am J Epidemiol*. 2017;186(9):1026-1034.
48. Tune LE. Anticholinergic effects of medication in elderly patients. *J Clin Psychiatry*. 2001;62:11-14.
49. Hovstadius B, Petersson G, Hellström L, Ericson L. Trends in inappropriate drug therapy prescription in the elderly in Sweden from 2006 to 2013: Assessment using national indicators. *Drugs Aging*. 2014;31(5):379-386.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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4.3 Conclusion

This chapter has presented an analysis of APC effects in UK Biobank and the prevalence rates of AChB according to different anticholinergic scales. I found AChB attributable to different drug classes and between different demographic groups to vary. Moreover, I found that the increase in AChB in the UK was not solely due to the ageing of the UK Biobank sample but was likely also due to period- or cohort effects.

4.3.1 Supplementary material

This section contains the complete Supplementary material that accompanies the manuscript presented in this chapter.

Supplementary Table S1: Anticholinergics scales identified in the present study. We considered anticholinergic scales that were available as complete lists of drugs, scored each drug for its anticholinergic potency, and did not utilize dosage. Grey shading indicates that the scale was not considered for further analysis. For two scales[1,2], updated versions were used (Aging Brain Care, 2012; Carnahan, 2014, personal communication on 21.10.2019). One scale[3] was modified to include newer drugs from the UK market as has been done before[4]. Most scales use a four-point (0-3) scoring system of anticholinergic potency, where 0 indicates no anticholinergic effect and 3 indicates a strong anticholinergic effect. One study[5] used a two-point system, where 2 and 1 indicated strong and weak anticholinergic scores, respectively. Some drugs from this scale were categorised as "drugs with improbable or no anticholinergic action". For our analyses, the drugs in the latter category were scored with 1, while the scores of other drugs on the scale were increased by one point. Another study[6] used a five-point, 0-4 scale, which was changed to a 0-3 scale as has been done before[5,7].

Surname of first author	Scale name	Year of publication	Reason for exclusion
Summers[8]	Drug Risk Number (DRN)	1978	Outdated (based on the date of publication and on new scales developed on its basis).
Han[9,10]	Clinician-rated Anticholinergic Scale (CrAS)	2001	
Aizenberg[11]	Anticholinergic Burden Score (ABS)	2002	Publicly unavailable and no response from lead author to two email requests within a year.
Minzenberg[12]	n.a.	2004	Based on a reference compound.
Ancelin[13]	Anticholinergic Burden Classification (ABC) scale	2006	
Carnahan[1]	Anticholinergic Drug Scale (ADS)	2006	
Hilmer[14]	Drug Burden Index (DBI)	2007	Required information on drug dosage.
Chew[15]	Anticholinergic Activity Scale (AAS)	2008	
Cancelli[16]	n.a.	2008	
Rudolph[3]	Anticholinergic Risk Scale (ARS)	2008	
Ehrt[6]	Revised Anticholinergic Activity Scale (AAS-r)	2010	
Sittironnarit[17]	Anticholinergic Loading Scale (ALS)	2011	
Boustani[2]	Anticholinergic Cognitive Burden (ACB)	2008	
Whalley[18]	n.a.	2012	Unavailable in full.
Durán[5]	n.a.	2013	
Dauphinot[19]	Drug Burden Index, International Version (DBI-WHO)	2014	Required information on drug dosage.
Klamer[20]	MARANTE	2017	Required information on drug dosage.
Kiesel[7]	n.a.	2018	

Supplementary Table S2: The meta-scale, with generic drug names for all anticholinergic drugs (i.e., drugs with an anticholinergic score greater than 0) listed in the first column and their respective anticholinergic scores in the second column.

Drug name	Score
acepromazine	3
aceprometazine	3
aclidinium bromide	1
alimemazine	1.5
alprazolam	1
alverine	1.5
amantadine	1.25
aminophylline	1
amitriptyline	3
amoxapine	3
amoxicillin	0.125
ampicillin	0.5
aripiprazole	0.25
asenapine	1
atenolol	0.333333
atropine	3
azatadine	3
azathioprine	1
baclofen	1
barberry	1
belladonna	2.75
benazepril	0.5
benztropine	3
betaxolol	0.333333
biperiden	3
bisacodyl	0.25
bromocriptine	0.666667
brompheniramine	2
buclizine	3
bupropion	0.333333
captopril	0.4
carbamazepine	0.5
carbidopa	0.333333
carbidopa/levodopa	0.5
carbinoxamine	3
carisoprodol	1
cefalexin	0.125
cefamandole	1
cefoxitin	1
celecoxib	0.5
cephalotin	1
cetirizine	1.166667
chlordiazepoxide	1
chloroquine	1
chlorphenamine	3
chlorpromazine	2.833333
chlorprothixene	3
chlortalidone	0.5
ciclosporin	1
cimetidine	1.666667
citalopram	1
clemastine	3
clidinium	2
clindamycin	0.5
clomipramine	2.8
clonazepam	0.666667
clorazepate	1.666667
clotiapine	3
clozapine	2.833333
codeine	0.5
colchicine	1
corticosterone	1
cortisone	0.5
cyclizine	3
cyclobenzaprine	1.75
cycloserine	1
cyproheptadine	2.2
darifenacin	2.666667
desipramine	2.5
desloratadine	0.5
dexamethasone	0.333333
dexbrompheniramine	3
dexchlorpheniramine	3
dextromethorphan	0.5
diazepam	0.75
dicycloverine	2.4
digitoxin	1
digoxin	1.0625
dimenhydrinate	3
dimetindene	1

diphenhydramine	2.8
diphenoxylate	0.166667
diphenoxylate/atropine	3
dipyridamole	0.25
disopyramide	0.75
domperidone	0.5
donepezil	0.1
dosulepin	2.333333
doxepine	2.857143
doxylamine	2
duloxetine	0.166667
emepronium	3
entacapone	0.5
ephedrine	1
ergotamine	1
escitalopram	0.75
estazolam	1
etoricoxib	1
famotidine	0.25
fentanyl	0.5
fesoterodine	2.666667
fexofenadine	1
flavoxate	2.666667
flunitrazepame	1
flunizepam	1
fluoxetine	1
fluphenazine	2
flurazepam	1
fluvoxamine	1
furosemide	0.9375
gentamicin	1
glycopyrronium	3
guaifenesin	0.5
haloperidol	0.857143
homatropine	3
hydralazine	0.5
hydrocodone	0.833333
hydrocortisone	0.5
hydroxyzine	2.4
hyoscine butylbromide	3
hyoscine hydrobromide	3
hyoscyamine	3
iloperidone	1
imipramine	3

ipratropium	1.25
isosorbide	1
ketamine	3
ketorolac	0.5
ketotifen	1
lansoprazole	0.3
levocetirizine	0.5
levofloxacin	0.166667
levomepromazine	2.2
lithium	1
lofepramine	1
loperamide	0.833333
loratadine	1
lorazepam	0.333333
loxapine	2
lumiracoxib	1
maprotiline	1.5
meclizine	2.25
mesoridazine	2
metformin	0.375
methadone	1.5
methocarbamol	1.25
methotrexate	0.5
methscopolamine	3
methylprednisolone	0.333333
metoclopramide	0.4
metoprolol	0.333333
midazolam	0.5
mirtazapine	0.75
molindone	1
morphine	0.5
nalbuphine	1
naratriptan	1
nefazodone	0.5
nefopam	2
neomycin	1
nifedipine	0.428571
nitrazepam	0.333333
nizatidine	0.333333
nortriptyline	2.5
olanzapine	2.333333
opipramol	3
orphenadrine	2.833333
oxazepam	0.2

oxcarbazepine	1
oxitropium	2
oxybutynin	2.444444
oxycodone	1
paliperidone	0.5
pancuronium	1
paracetamol/codeine	2
paracetamol/codeine/caffeine	2
paroxetine	2
pericyazine	2.5
perphenazine	1.857143
pethidine	1
phenelzine	0.5
phenindamine	3
pheniramine	3
phenobarbital	1
phenyltoloxamine	3
phenytoin	0.25
pimozide	2
piperacillin	0.5
pramipexole	0.333333
prednisolone	0.333333
prednisone	0.666667
procainamide	0.5
prochlorperazine	1.4
procyclidine	3
promazine	2.5
promethazine	3
propantheline	3
propiverine	2.5
propoxyphene	0.5
protriptyline	3
pseudoephedrine	1
pyrilamine	3
quetiapine	1.666667
quinidine	0.666667
ranitidine	1.285714
reboxetine	0.5
risperidone	0.666667
rotigotine	1
selegiline	0.25
sertraline	0.4
solifenacin	2
sumatriptan	0.5

temazepam	0.75
theophylline	1.428571
thioridazine	3
thiothixene	1.5
tiagabine	1
tiotropium	0.333333
tizanidine	1.5
tobramycin	1
tolterodine	2.8
topiramate	0.25
tramadol	1.25
tranylcypromine	1
trazodone	0.6
triamcinolone	0.5
triamterene	0.666667
triazolam	1
trifluoperazine	2.666667
triflupromazine	2
trihexyphenidyl	3
trimethobenzamide	3
trimipramine	2.666667
triprolidine	2.5
tropatepine	3
trospium	2.666667
valproate	0.25
vancomycin	0.5
venlafaxine	0.4
warfarin	0.428571
ziprasidone	0.333333
zolmitriptan	0.5
zuclopenthixol	1

Supplementary Table S3: Age-period-cohort (APC) analyses for the basic models (top), basic-adjusted models (middle), and the fully-adjusted models (bottom). Each model assumes that one of the APC-terms is zero. Unstandardized regression coefficients (beta) are reported.

Model	Age (years)			Period (months)			Birth cohort		
	beta	SE	p	beta	SE	p	beta	SE	p
Period-cohort				0.0089	9.7×10^{-6}	<0.001	-0.057	9.3×10^{-5}	<0.001
Age-cohort	0.11	1.2×10^{-4}	<0.001				0.050	1.4×10^{-4}	<0.001
Age-period	0.057	9.3×10^{-5}	<0.001	0.0042	1.2×10^{-5}	<0.001			

Model	Age (years)			Period (months)			Birth cohort		
	beta	SE	p	beta	SE	p	beta	SE	p
Period-cohort				0.0094	9.6×10^{-6}	<0.001	-0.065	8.9×10^{-5}	<0.001
Age-cohort	0.11	1.1×10^{-4}	<0.001				0.047	1.4×10^{-4}	<0.001
Age-period	0.065	8.9×10^{-5}	<0.001	0.0039	1.1×10^{-5}	<0.001			

Model	Age (years)			Period (months)			Birth cohort		
	beta	SE	p	beta	SE	p	beta	SE	p
Period-cohort				0.0093	9.5×10^{-6}	<0.001	-0.065	8.9×10^{-5}	<0.001
Age-cohort	0.11	1.1×10^{-4}	<0.001				0.046	1.4×10^{-4}	<0.001
Age-period	0.065	8.9×10^{-5}	<0.001	0.0039	1.1×10^{-5}	<0.001			

Supplementary Table S4: APC analysis for the fully-adjusted models, with the monthly number of prescribed anticholinergic drugs as the outcome. Each model assumes that one of the APC-terms is zero. Unstandardized regression coefficients (beta) are reported.

Model	Age (years)			Period (months)			Birth cohort		
	beta	SE	p	beta	SE	p	beta	SE	p
Period-cohort				0.0095	9.2×10^{-6}	<0.001	-0.067	8.5×10^{-5}	<0.001
Age-cohort	0.11	1.1×10^{-4}	<0.001				0.047	1.3×10^{-4}	<0.001
Age-period	0.067	8.6×10^{-5}	<0.001	0.0039	1.1×10^{-5}	<0.001			

Supplementary Table S5: APC-analysis for the basic models, with total number of prescribed drugs as a covariate. Each model assumes that one of the APC-terms is zero. Unstandardized regression coefficients (beta) are reported.

Model	Age (years)			Period (months)			Birth cohort		
	beta	SE	p	beta	SE	p	beta	SE	p
Period-cohort				2.2×10^{-4}	6.9×10^{-6}	<0.001	0.0029	6.4×10^{-6}	<0.001
Age-cohort	0.0027	8.3×10^{-5}	<0.001				0.0055	1.0×10^{-4}	<0.001
Age-period	-0.0029	6.4×10^{-5}	<0.001	4.6×10^{-4}	8.3×10^{-6}	<0.001			

Supplementary Table S6: Results of the models predicting monthly anticholinergic burden due to different drug classes as a function of deprivation, smoking, BMI, sex, education, region, alcohol consumption, physical activity, and age.

Antidepressant				
Predictor	**Level**	**Beta**	**SE**	**p**
Deprivation		0.002	3.2×10^{-4}	<0.001
Smoking (*ref: non-smoker*)	Previous smoker	0.039	0.0020	<0.001
	Current smoker	0.099	0.0032	<0.001
BMI		0.004	2.0×10^{-4}	<0.001
Sex	Male	-0.117	0.0019	<0.001
Education	graduate degree	-0.045	0.0020	<0.001
Region (*ref: England*)	Scotland	-0.033	0.0032	<0.001
	Wales	0.020	0.0032	<0.001
Alcohol consumption (*ref: daily or almost daily consumption*)	Three or four times a week	-0.011	0.0028	<0.001
	Once or twice a week	0.007	0.0028	<0.001
	Once to thrice a month	0.026	0.0034	1.1×10^{-2}
	Special occasions only	0.044	0.0035	<0.001
	Never	0.060	0.0040	<0.001
Physical activity (*ref: mild or no physical activity*)	Moderate	-0.031	0.0021	<0.001
	Strenuous	-0.073	0.0036	<0.001

Acid disorders				
Predictor	**Level**	**Beta**	**SE**	**p**
Deprivation		0.001	1.11×10^{-4}	<0.001
Smoking (*ref: non-smoker*)	Previous smoker	0.011	6.87×10^{-4}	<0.001
	Current smoker	0.012	0.0011	<0.001
BMI		0.002	6.95×10^{-5}	<0.001
Sex	Male	-0.002	6.49×10^{-4}	0.0025
Education	graduate degree	-0.020	7.00×10^{-4}	<0.001
Region (*ref: England*)	Scotland	-0.024	0.0011	<0.001
	Wales	0.005	0.0011	<0.001
Alcohol consumption (*ref: daily or almost daily consumption*)	Three or four times a week	-2.0×10^{-4}	9.5×10^{-4}	0.83
	Once or twice a week	0.005	9.36×10^{-4}	<0.001
	Once to thrice a month	0.008	0.0012	<0.001
	Special occasions only	0.013	0.0012	<0.001
	Never	0.022	0.0014	<0.001
Physical activity (*ref: mild or no physical activity*)	Moderate	-0.008	7.27×10^{-4}	<0.001
	Strenuous	-0.018	0.0012	<0.001

Analgesic				
Predictor	**Level**	**Beta**	**SE**	**p**
Deprivation		0.004	$1.77x10^{-4}$	<0.001
Smoking (*ref: non-smoker*)	Previous smoker	0.023	0.0011	<0.001
	Current smoker	0.048	0.0018	<0.001
BMI		0.006	$1.12x10^{-4}$	<0.001
Sex	Male	-0.028	0.0011	<0.001
Education	graduate degree	-0.044	0.0011	<0.001
Region (*ref: England*)	Scotland	0.036	0.0016	<0.001
	Wales	-0.014	0.0018	<0.001
Alcohol consumption (*ref: daily or almost daily consumption*)	Three or four times a week	$-2.47x10^{-4}$	0.0016	0.87
	Once or twice a week	0.011	0.0015	<0.001
	Once to thrice a month	0.020	0.0019	<0.001
	Special occasions only	0.033	0.0019	<0.001
	Never	0.043	0.0022	<0.001
Physical activity (*ref: mild or no physical activity*)	Moderate	-0.015	0.0012	<0.001
	Strenuous	-0.035	0.0020	<0.001

Antithrombotic				
Predictor	**Level**	**Beta**	**SE**	**p**
Deprivation		$4.1x10^{-4}$	$5.3x10^{-4}$	0.44
Smoking (*ref: non-smoker*)	Previous smoker	0.014	0.0032	<0.001
	Current smoker	0.005	0.0055	0.327
BMI		0.008	$3.3x10^{-4}$	<0.001
Sex	Male	0.104	0.0033	<0.001
Education	graduate degree	-0.007	0.0033	0.034
Region (*ref: England*)	Scotland	-0.011	0.0052	0.039
	Wales	0.033	0.0049	<0.001
Alcohol consumption (*ref: daily or almost daily consumption*)	Three or four times a week	-0.011	0.0044	<0.001
	Once or twice a week	-0.006	0.0043	0.139
	Once to thrice a month	$-1.4x10^{-4}$	0.0057	0.980
	Special occasions only	0.011	0.0057	0.047
	Never	0.026	0.0063	<0.001
Physical activity (*ref: mild or no physical activity*)	Moderate	-0.017	0.0034	<0.001
	Strenuous	-0.026	0.0061	<0.001

Diabetes				
Predictor	**Level**	**Beta**	**SE**	**p**
Deprivation		0.006	4.2×10^{-4}	<0.001
Smoking (*ref: non-smoker*)	Previous smoker	0.020	0.0027	<0.001
	Current smoker	0.024	0.0043	<0.001
BMI		0.019	2.9×10^{-4}	<0.001
Sex	Male	0.111	0.0028	<0.001
Education	graduate degree	-0.009	0.0028	0.001
Region (*ref: England*)	Scotland	-0.015	0.0043	0.001
	Wales	0.011	0.0042	0.009
Alcohol consumption (*ref: daily or almost daily consumption*)	Three or four times a week	0.010	0.0041	0.013
	Once or twice a week	0.042	0.0039	<0.001
	Once to thrice a month	0.068	0.0047	<0.001
	Special occasions only	0.100	0.0047	<0.001
	Never	0.129	0.0050	<0.001
Physical activity (*ref: mild or no physical activity*)	Moderate	-0.034	0.0027	<0.001
	Strenuous	-0.088	0.0057	<0.001

Cardiovascular				
Predictor	**Level**	**Beta**	**SE**	**p**
Deprivation		0.002	3.1×10^{-4}	<0.001
Smoking (*ref: non-smoker*)	Previous smoker	0.020	0.0019	<0.001
	Current smoker	0.017	0.0031	<0.001
BMI		0.012	1.9×10^{-4}	<0.001
Sex	Male	0.030	0.0018	<0.001
Education	graduate degree	-0.035	0.0020	<0.001
Region (*ref: England*)	Scotland	3.3×10^{-4}	0.0029	0.91
	Wales	0.013	0.0030	<0.001
Alcohol consumption (*ref: daily or almost daily consumption*)	Three or four times a week	-0.001	0.0026	0.61
	Once or twice a week	0.004	0.0026	0.14
	Once to thrice a month	0.008	0.0033	0.02
	Special occasions only	0.029	0.0033	<0.001
	Never	0.038	0.0037	<0.001
Physical activity (*ref: mild or no physical activity*)	Moderate	-0.029	0.0020	<0.001
	Strenuous	-0.070	0.0038	<0.001

Psycholeptic				
Predictor	**Level**	**Beta**	**SE**	**p**
Deprivation		0.001	1.0×10^{-4}	<0.001
Smoking (*ref: non-smoker*)	Previous smoker	0.007	6.3×10^{-4}	<0.001
	Current smoker	0.015	0.0010	<0.001
BMI		0.001	6.4×10^{-5}	<0.001
Sex	Male	-0.027	6.1×10^{-4}	<0.001
Education	graduate degree	-0.007	6.3×10^{-4}	<0.001
Region (*ref: England*)	Scotland	-0.005	0.0010	<0.001
	Wales	0.006	0.0010	<0.001
Alcohol consumption (*ref: daily or almost daily consumption*)	Three or four times a week	-0.004	8.8×10^{-4}	<0.001
	Once or twice a week	-0.001	8.6×10^{-4}	0.48
	Once to thrice a month	0.004	0.0011	<0.001
	Special occasions only	0.009	0.0011	<0.001
	Never	0.017	0.0012	<0.001
Physical activity (*ref: mild or no physical activity*)	Moderate	-0.007	6.7×10^{-4}	<0.001
	Strenuous	-0.013	0.0011	<0.001

Gastrointestinal				
Predictor	**Level**	**Beta**	**SE**	**p**
Deprivation		0.001	1.1×10^{-4}	<0.001
Smoking (*ref: non-smoker*)	Previous smoker	0.006	7.2×10^{-4}	<0.001
	Current smoker	0.007	0.0012	<0.001
BMI		3.3×10^{-4}	7.2×10^{-5}	<0.001
Sex	Male	-0.025	7.0×10^{-4}	<0.001
Education	graduate degree	-0.013	7.4×10^{-4}	<0.001
Region (*ref: England*)	Scotland	-0.007	0.0011	<0.001
	Wales	0.002	0.0011	0.03
Alcohol consumption (*ref: daily or almost daily consumption*)	Three or four times a week	-0.001	0.0010	0.46
	Once or twice a week	0.006	0.0010	<0.001
	Once to thrice a month	0.009	0.0012	<0.001
	Special occasions only	0.015	0.0012	<0.001
	Never	0.021	0.0014	<0.001
Physical activity (*ref: mild or no physical activity*)	Moderate	-0.008	7.5×10^{-4}	<0.001
	Strenuous	-0.015	0.0013	<0.001

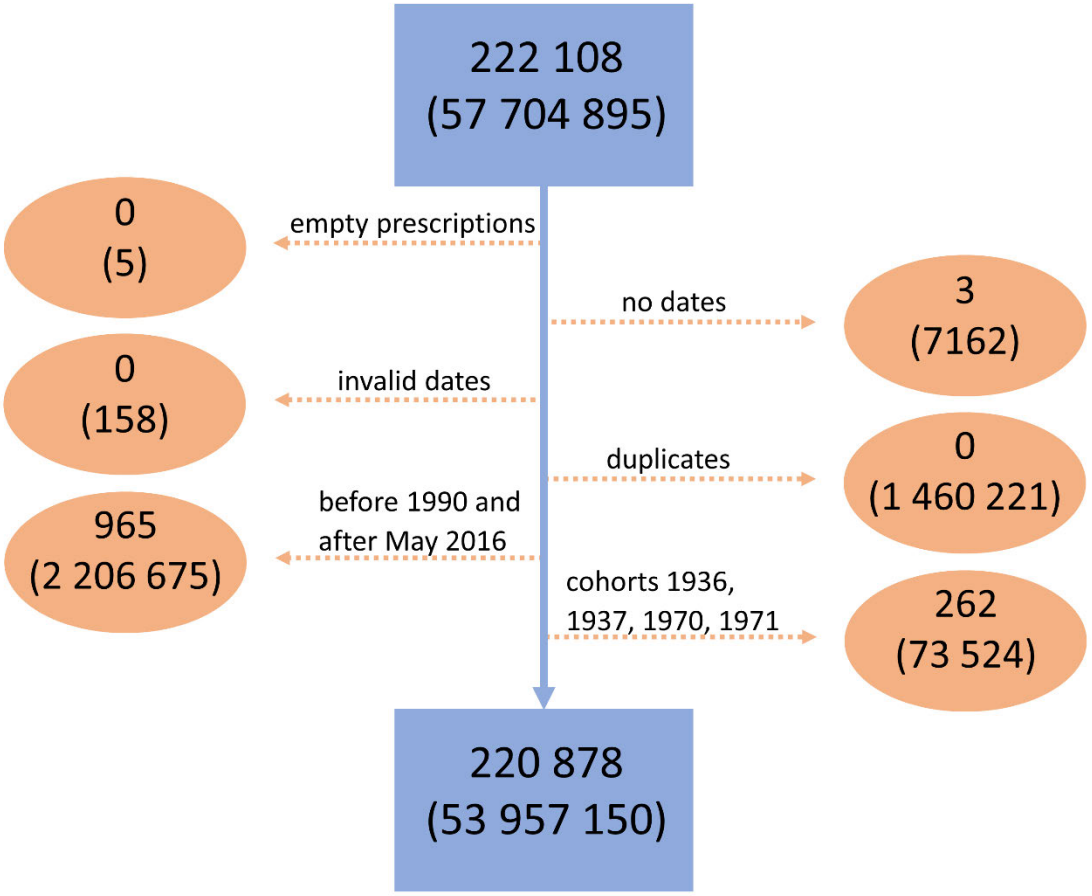
Urological				
Predictor	**Level**	**Beta**	**SE**	**p**
Deprivation		0.003	$7.2x10^{-4}$	<0.001
Smoking (*ref: non-smoker*)	Previous smoker	0.023	0.0044	<0.001
	Current smoker	0.008	0.0075	0.31
BMI		0.007	$4.4x10^{-4}$	<0.001
Sex	Male	-0.081	0.0043	<0.001
Education	graduate degree	-0.038	0.0046	<0.001
Region (*ref: England*)	Scotland	0.019	0.0067	0.005
	Wales	0.003	0.0072	0.70
Alcohol consumption (*ref: daily or almost daily consumption*)	Three or four times a week	0.015	0.0064	0.02
	Once or twice a week	0.045	0.0062	<0.001
	Once to thrice a month	0.060	0.0077	<0.001
	Special occasions only	0.080	0.0077	<0.001
	Never	0.090	0.0086	<0.001
Physical activity (*ref: mild or no physical activity*)	Moderate	-0.007	0.0047	0.12
	Strenuous	-0.029	0.0084	<0.001

Respiratory				
Predictor	**Level**	**Beta**	**SE**	**p**
Deprivation		0.002	$1.2x10^{-4}$	<0.001
Smoking (*ref: non-smoker*)	Previous smoker	0.010	$7.7x10^{-4}$	<0.001
	Current smoker	0.015	0.0012	<0.001
BMI		0.002	$7.7x10^{-5}$	<0.001
Sex	Male	-0.025	$7.3x10^{-4}$	<0.001
Education	graduate degree	-0.015	$7.7x10^{-4}$	<0.001
Region (*ref: England*)	Scotland	-0.018	0.0012	<0.001
	Wales	0.030	0.0012	<0.001
Alcohol consumption (*ref: daily or almost daily consumption*)	Three or four times a week	-0.002	0.0011	0.074
	Once or twice a week	0.003	0.0010	0.006
	Once to thrice a month	0.004	0.0013	<0.001
	Special occasions only	0.013	0.0013	<0.001
	Never	0.023	0.0015	<0.001
Physical activity (*ref: mild or no physical activity*)	Moderate	-0.007	$8.2x10^{-4}$	<0.001
	Strenuous	-0.015	0.0013	<0.001

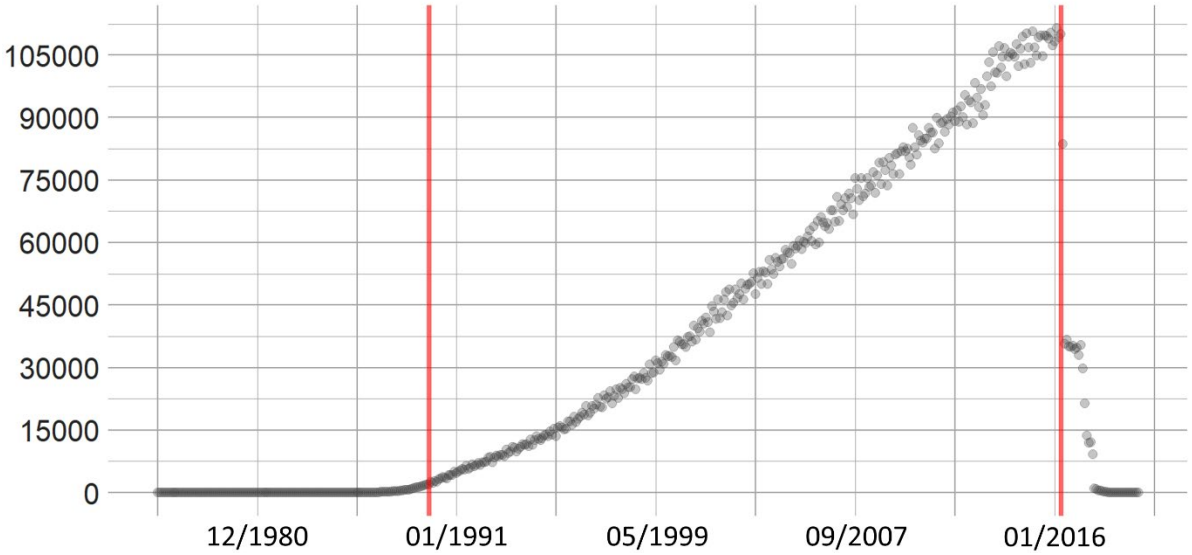
Other				
Predictor	**Level**	**Beta**	**SE**	**p**
Deprivation		$2.0x10^{-4}$	$6.4x10^{-5}$	0.001
Smoking (*ref: non-smoker*)	Previous smoker	0.004	$3.9x10^{-4}$	<0.001
	Current smoker	0.006	$6.4x10^{-4}$	<0.001
BMI		0.001	$4.0x10^{-5}$	<0.001
Sex	Male	-0.002	$3.7x10^{-4}$	<0.001
Education	graduate degree	-0.006	$3.9x10^{-4}$	<0.001
Region (*ref: England*)	Scotland	-0.006	$6.1x10^{-4}$	<0.001
	Wales	0.004	$6.2x10^{-4}$	<0.001
Alcohol consumption (*ref: daily or almost daily consumption*)	Three or four times a week	-0.001	$5.4x10^{-4}$	0.15
	Once or twice a week	0.001	$5.3x10^{-4}$	0.039
	Once to thrice a month	0.002	$6.7x10^{-4}$	0.011
	Special occasions only	0.005	$6.9x10^{-4}$	<0.001
	Never	0.012	$7.9x10^{-4}$	<0.001
Physical activity (*ref: mild or no physical activity*)	Moderate	-0.004	$4.2x10^{-4}$	<0.001
	Strenuous	-0.007	$6.8x10^{-4}$	<0.001

Supplementary Figure S1: Prescriptions without drug and Read-code were removed. We removed prescriptions without recorded dates, prescriptions dated before or at participants' dates of birth or after the participants' dates of death, and duplicate prescriptions (= the same prescription issued to the same individual on the same day). We removed prescriptions issued after the date on which sampling was terminated for any data provider and all months for which the number of recorded participants was lower than 10% of the monthly median over the entire sampling period (median=20,647), resulting in the removal of prescriptions prior to and including December 1989 and those after and including June 2016. For mixed-models analyses, we additionally removed birth cohorts 1936, 1937, 1970, and 1971 due to low numbers of individuals (1, 182, 81, 1, respectively; median=6,487). The final sample consisted of 53,956,916 prescriptions issued to 220,867 individuals.

Depiction of the data cleaning procedure. The top numbers refer to the numbers of participants, the lower numbers in brackets refer to the numbers of prescriptions in the sample. The top and bottom blue box indicate the numbers of participants/prescriptions before and after data cleaning, respectively. Each orange oval represents a step in data cleaning; the steps were performed sequentially from top to the bottom.



Supplementary Figure S2: The removal of the period prior to and including December 1989 and after and including June 2016. The x-axis represents months in the original dataset, the y axis represents the number of participants that were issued a prescription each month. The vertical red lines indicate December 1989 and June 2016; the period between those dates was retained for further analysis.



Supplementary Text S1: Description of the process of data-preparation.

In the raw data, the unit of observation was a prescription. For longitudinal analyses, the unit of observation was transformed into a participant-period combination, with the anticholinergic burden for each participant the sum over the anticholinergic burden of all prescriptions for that participant in that period. Monthly anticholinergic burden was the outcome for all models except mixed-effects models, where – for computational parsimony – it was yearly anticholinergic burden. For analyses of lifestyle- and demographic factors, the data were transformed so that individual participants were units of observations. Person-time was calculated by subtracting the date of the participant's appearance in the dataset from (1) the participant's date of death or (2) the last date in the dataset (whichever came first). For each participant we then summed their anticholinergic burden across their prescriptions and divided it by their person-time. We removed participants with a person-time of <12 months (n=11).

Supplementary Figure S3: Imaginary example illustrating the data transformation process. The *prescriptions data frame* represents the format of the raw data after the computation and addition of anticholinergic scores. Each row is a single prescription and the "AC-burden" is the anticholinergic score associated with a given prescription. The *id-period data frame* represents the format used for the analyses of longitudinal trends; in the example below, the period is a year. The *id data frame* represents the format used for the analysis of the association between demographic- and lifestyle factors and anticholinergic burden. In this table, each row is a participant and the anticholinergic burden is the average anticholinergic burden in a given period (year in the example below) for that participant.

prescription data frame

id	drug	date	AC-burden
003	C	21.03.2001	1
002	A	02.06.2003	0.5
001	A	01.04.2003	3
001	B	06.08.2003	2
003	C	18.12.2003	3
003	D	11.01.2004	0
002	E	08.06.2004	1

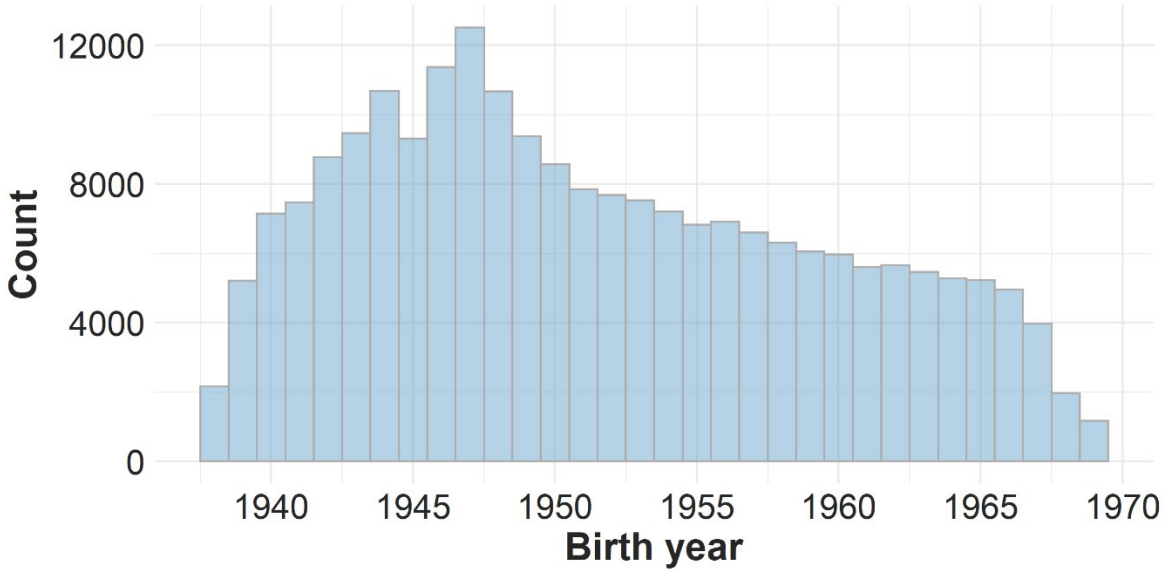
id-period data frame

id-year	AC-burden
001-2003	5
001-2004	0
002-2003	0.5
002-2004	1
003-2001	1
003-2002	0
003-2003	3
003-2004	0

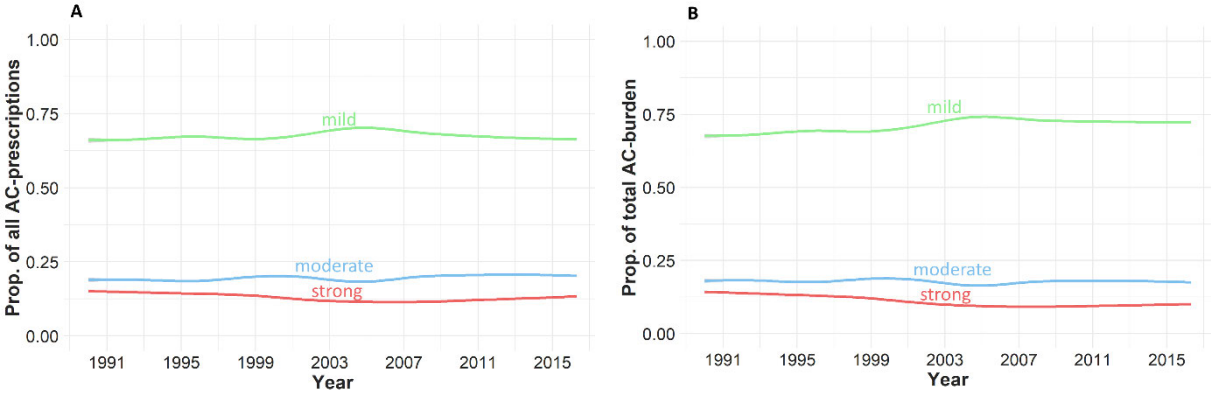
id data frame

id	(yearly) AC-burden
001	2.5
002	0.75
003	2.0

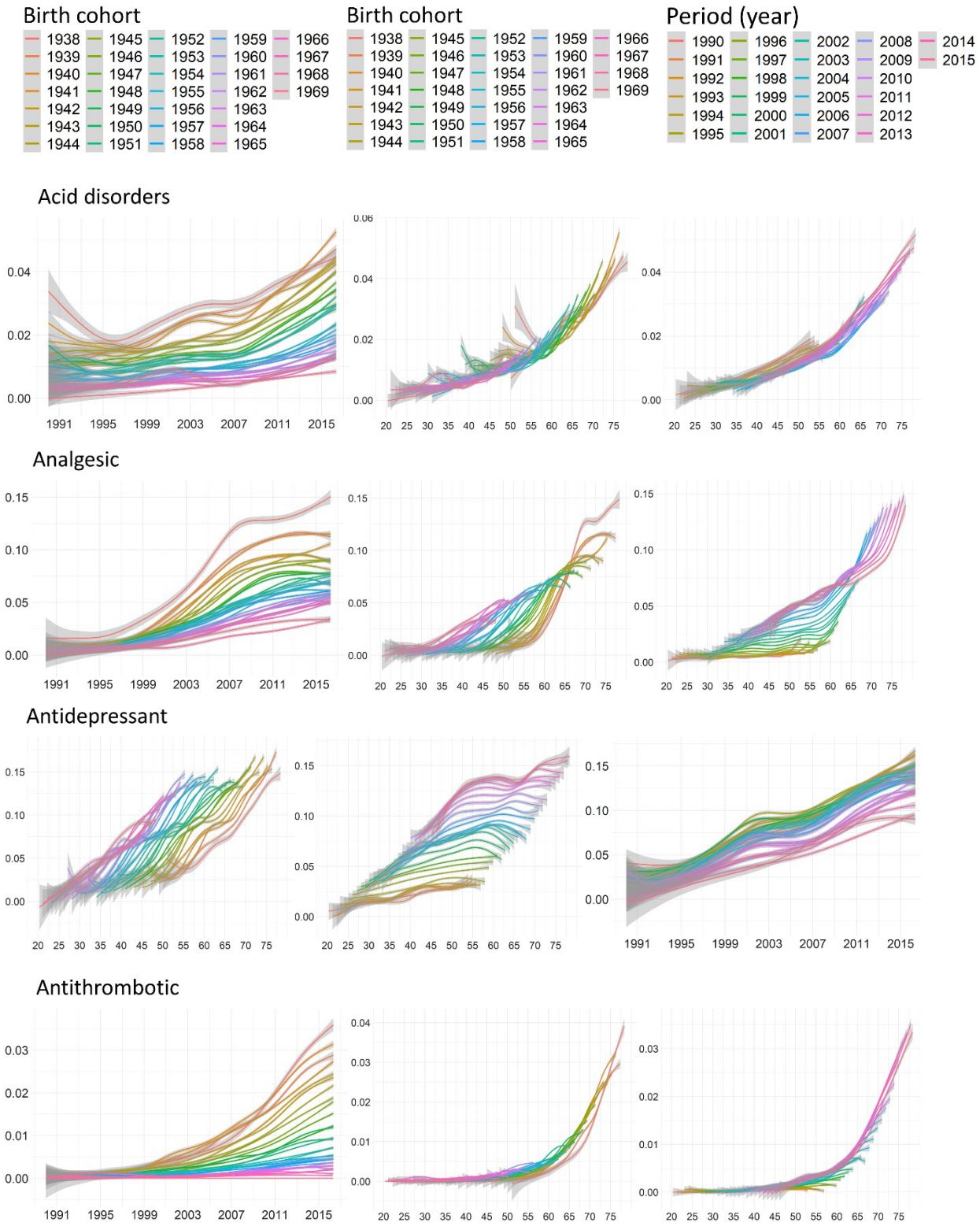
Supplementary Figure S4: Distribution of birth cohorts in the sample.



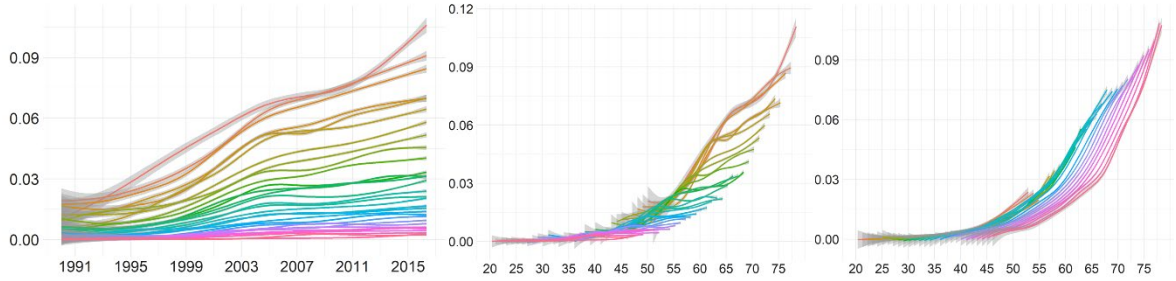
Supplementary Figure S5: Changes in the proportions of anticholinergic burden due to the different categories of drugs (**A**) and in the proportions of the total numbers of prescribed drugs from each category (**B**). The categories refer to mild (greater than 0, equal to or lower than 1), moderate (greater than 1, equal to or lower than 2), and strong (greater than 2) anticholinergic activity.



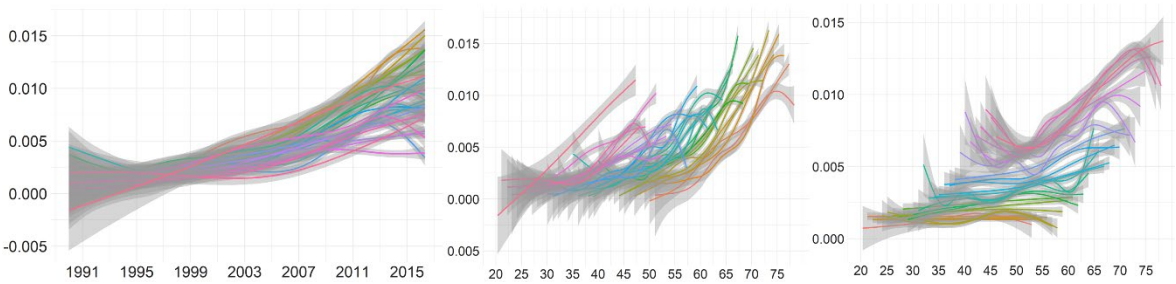
Supplementary Figure S6: Plots of anticholinergic burden for each drug class. Displayed are the period-cohort model with cohort as a random effect (left column), the age-cohort model with cohort as a random effect (middle column), and the age-period model with period as the random effect (right column). To increase accuracy, the plotting was done by using the id-month data frame (with monthly anticholinergic burden as the outcome). The plots were generated using generalised additive model smoothing.



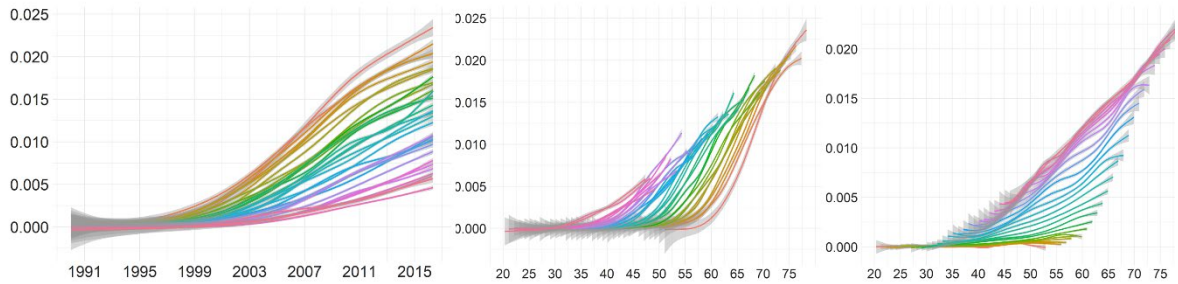
Cardiovascular



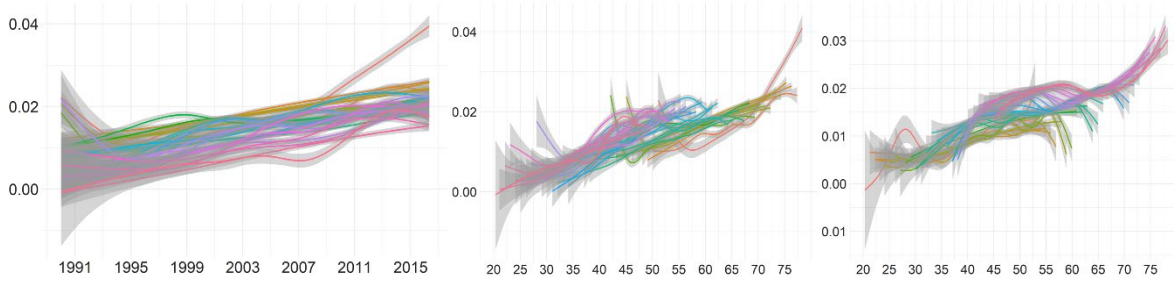
Gastrointestinal



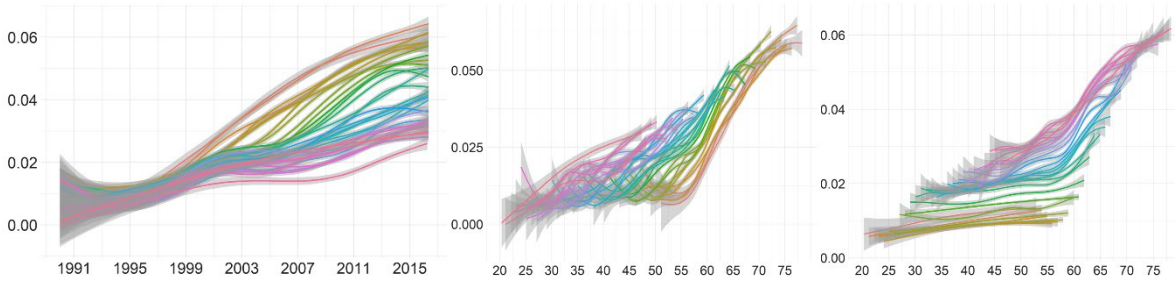
Diabetes



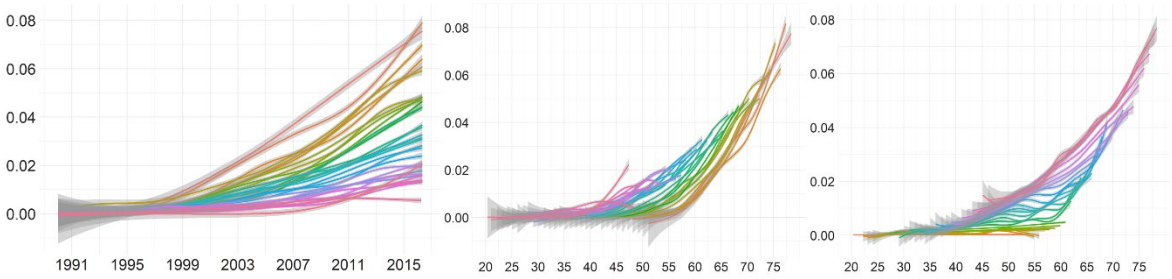
Psycholeptic



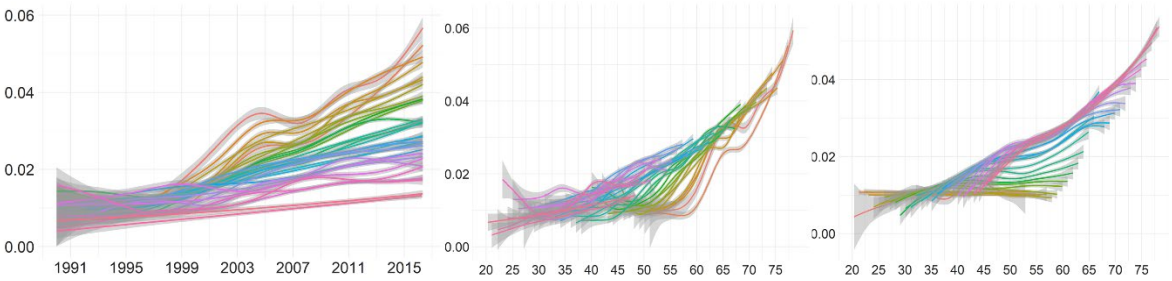
Respiratory



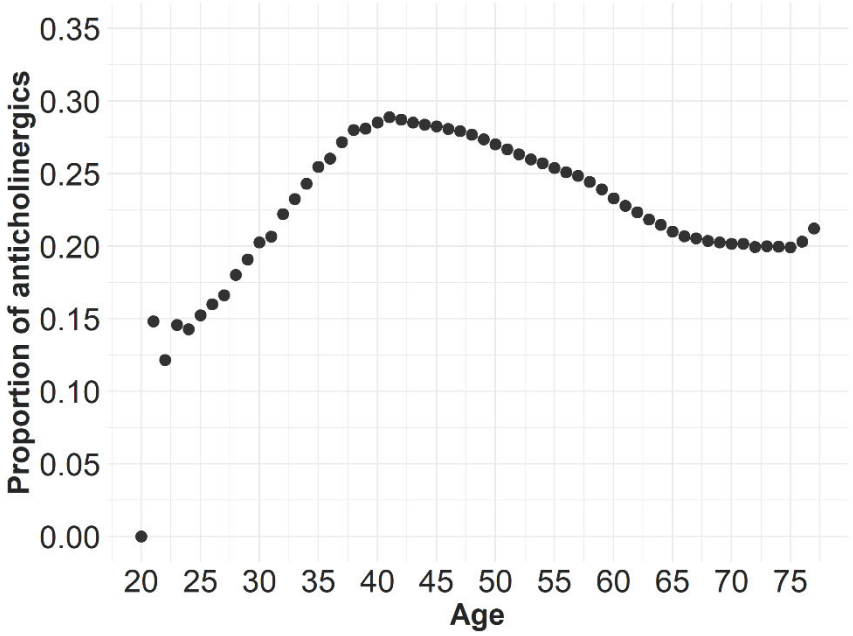
Urological



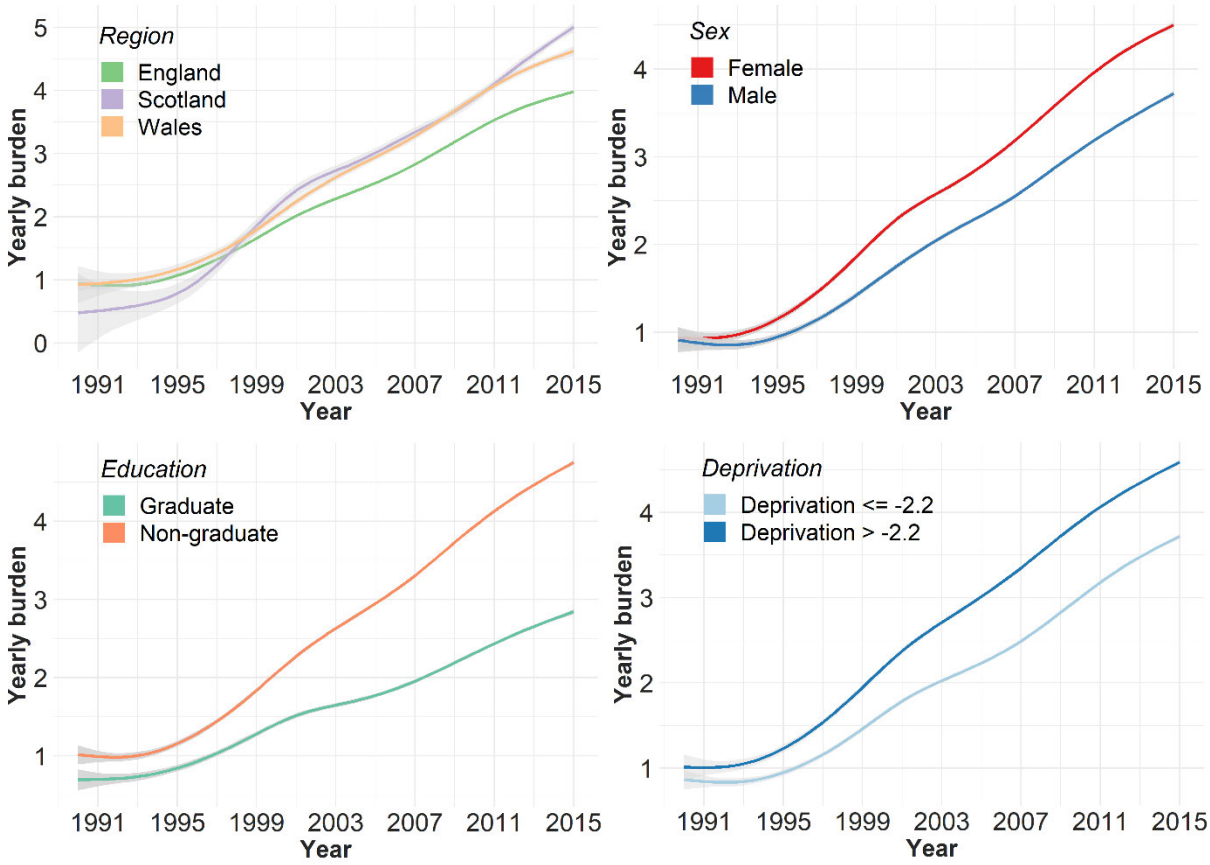
Other



Supplementary Figure S7: Plot of the number of prescribed anticholinergic drugs as a proportion of all drugs for each age group across the entire prescribing period. The x-axis represents the rounded age at time of prescription, the y-axis represents the ratio of the number of prescribed anticholinergic drugs and the number of all prescribed drugs.



Supplementary Figure S8: Temporal changes in anticholinergic burden for different levels of predictor variables. The plots were generated using generalised additive model smoothing. Shading indicates 95%



Sensitivity analyses

Supplementary Table S7: Age-period-cohort (APC) analyses for the basic models (top), basic-adjusted models (middle), and the fully-adjusted models (bottom). Each model assumes that one of the APC-terms is zero. Unstandardized regression coefficients (beta) are reported.

Model	Age (years)			Period (months)			Birth cohort		
	beta	SD	p	beta	SE	p	beta	SE	p
Period-cohort				0.0082	1.3x10	<0.001	-0.067	$9.4x10^{-4}$	<0.001
Age-cohort	0.098	$1.6x10^{-4}$	<0.001				0.031	$1.8x10^{-4}$	<0.001
Age-period	0.068	$9.4x10^{-5}$	<0.001	0.0026	$1.5x10^{-5}$	<0.001			

Model	Age (years)			Period (months)			Birth cohort		
	beta	SD	p	beta	SE	p	beta	SE	p
Period-cohort				0.0081	$1.3x10^{-5}$	<0.001	-0.067	$9.5x10^{-5}$	<0.001
Age-cohort	0.097	$1.6x10^{-4}$	<0.001				0.030	$1.8x10^{-4}$	<0.001
Age-period	0.067	$9.5x10^{-5}$	<0.001	0.0025	$1.5x10^{-5}$	<0.001			

Model	Age (years)			Period (months)			Birth cohort		
	beta	SD	p	beta	SE	p	beta	SE	p
Period-cohort				0.0078	$1.3x10^{-5}$	<0.001	-0.059	$9.8x10^{-5}$	<0.001
Age-cohort	0.094	$1.6x10^{-4}$	<0.001				0.035	$1.8x10^{-4}$	<0.001
Age-period	0.059	$9.8x10^{-5}$	<0.001	0.0029	$1.5x10^{-5}$	<0.001			

Supplementary Table S8: APC analysis for the fully-adjusted models, with the monthly number of prescribed anticholinergic drugs as the outcome. Each model assumes that one of the APC-terms is zero. Unstandardized regression coefficients (beta) are reported.

Model	Age (years)			Period (months)			Birth cohort		
	beta	SD	p	beta	SE	p	beta	SE	p
Period-cohort				0.0095	9.2×10^{-6}	<0.001	-0.067	8.5×10^{-5}	<0.001
Age-cohort	0.11	1.1×10^{-4}	<0.001				0.047	1.3×10^{-4}	<0.001
Age-period	0.067	8.6×10^{-5}	<0.001	0.0039	8.6×10^{-5}	<0.001			

Supplementary Table S9: APC-analysis for the basic models, with total number of prescribed drugs as a covariate. Each model assumes that one of the APC-terms is zero. Unstandardized regression coefficients (beta) are reported.

Model	Age (years)			Period (months)			Birth cohort		
	beta	SD	p	beta	SE	p	beta	SE	p
Period-cohort				-9.7×10^{-4}	9.6×10^{-6}	<0.001	0.0043	6.8×10^{-5}	<0.001
Age-cohort	-0.012	1.2×10^{-4}	<0.001				-0.0072	1.3×10^{-4}	<0.001
Age-period	-0.0043	1.1×10^{-5}	<0.001	-6.1×10^{-4}	1.1×10^{-5}	<0.001			

Supplementary Text S2: The results of mixed-effects models for the period from 2000 to 2015.

Mixed-effects models

Anticholinergic burden increased by 0.23 per year (SE=0.0043, p<0.001).

Period-cohort model; correlation between slope and birth cohort: -0.93, 0.065, p<0.001, n=32
Age-cohort model; correlation between slope and birth cohort: -0.96, 0.047, p<0.001, n=32
Age-period model; correlation between slope and period: 0.99, 0.031, p<0.001, n=16

Supplementary Table S10: Results of the models predicting monthly anticholinergic burden due to different drug classes as a function of deprivation, smoking, BMI, sex, education, region, alcohol consumption, physical activity, and age.

Total				
Predictor	**Level**	**Beta**	**SE**	**p**
Deprivation		0.0062	$3.0x10^{-4}$	<0.001
Smoking (*ref: non-smoker*)	Previous smoker	0.0463	0.0019	<0.001
	Current smoker	0.0835	0.003	<0.001
BMI		0.0115	$1.90x^{10-4}$	<0.001
Sex	Male	-0.0505	0.0018	<0.001
Education	graduate degree	-0.054	0.0018	<0.001
Region (*ref: England*)	Scotland	0.0501	0.0028	<0.001
	Wales	0.038	0.003	<0.001
Alcohol consumption (*ref: daily or almost daily consumption*)	Three or four times a week	-0.0056	0.0025	0.0269
	Once or twice a week	0.0167	0.0025	<0.001
	Once to thrice a month	0.0339	0.0032	<0.001
	Special occasions only	0.076	0.0033	<0.001
	Never	0.1166	0.0038	<0.001
Physical activity (*ref: mild or no physical activity*)	Moderate	-0.0463	0.002	<0.001
	Strenuous	-0.0823	0.0032	<0.001

Antidepressant				
Predictor	**Level**	**Beta**	**SE**	**p**
Deprivation		0.0024	3.8x10-04	<0.001
Smoking (*ref: non-smoker*)	Previous smoker	0.0455	0.0024	<0.001
	Current smoker	0.114	0.0037	<0.001
BMI		0.0048	2.4x10-04	<0.001
Sex	Male	-0.1355	0.0023	<0.001
Education	graduate degree	-0.0535	0.0024	<0.001
Region (*ref: England*)	Scotland	-0.027	0.0037	<0.001
	Wales	0.0269	0.0038	<0.001
Alcohol consumption (*ref: daily or almost daily consumption*)	Three or four times a week	-0.0128	0.0033	<0.001
	Once or twice a week	0.0081	0.0033	0.0126
	Once to thrice a month	0.0287	0.0041	<0.001
	Special occasions only	0.0513	0.0041	<0.001
	Never	0.0704	0.0047	<0.001
Physical activity (*ref: mild or no physical activity*)	Moderate	-0.038	0.0025	<0.001
	Strenuous	-0.085	0.0042	<0.001

Analgesic				
Predictor	**Level**	**Beta**	**SE**	**p**
Deprivation		0.0046	$2.1x10^{-4}$	<0.001
Smoking (*ref: non-smoker*)	Previous smoker	0.0263	0.0013	<0.001
	Current smoker	0.0571	0.0021	<0.001
BMI		0.0072	$1.3x10^{-4}$	<0.001
Sex	Male	-0.0319	0.0012	<0.001
Education	graduate degree	-0.0511	0.0013	<0.001
Region (*ref: England*)	Scotland	0.0406	0.0019	<0.001
	Wales	-0.0161	0.0021	<0.001
Alcohol consumption (*ref: daily or almost daily consumption*)	Three or four times a week	$-8.9x10^{-4}$	0.0018	0.6272
	Once or twice a week	0.0136	0.0018	<0.001
	Once to thrice a month	0.022	0.0022	<0.001
	Special occasions only	0.038	0.0023	<0.001
	Never	0.0503	0.0026	<0.001
Physical activity (*ref: mild or no physical activity*)	Moderate	-0.018	0.0014	<0.001
	Strenuous	-0.0414	0.0024	<0.001

Acid disorders				
Predictor	**Level**	**Beta**	**SE**	**p**
Deprivation		$6.0x10^{-4}$	$1.3x10^{-4}$	<0.001
Smoking (*ref: non-smoker*)	Previous smoker	0.0117	$7.8x10^{-4}$	<0.001
	Current smoker	0.0129	0.0013	<0.001
BMI		0.0023	$7.9x10^{-5}$	<0.001
Sex	Male	-0.0038	$7.4x10^{-4}$	<0.001
Education	graduate degree	-0.0215	$7.9x10^{-4}$	<0.001
Region (*ref: England*)	Scotland	-0.0261	0.0013	<0.001
	Wales	0.0074	0.0012	<0.001
Alcohol consumption (*ref: daily or almost daily consumption*)	Three or four times a week	$-3.1x10^{-4}$	0.0011	0.7752
	Once or twice a week	0.0059	0.0011	<0.001
	Once to thrice a month	0.0088	0.0013	<0.001
	Special occasions only	0.0151	0.0014	<0.001
	Never	0.0242	0.0015	<0.001
Physical activity (*ref: mild or no physical activity*)	Moderate	-0.0082	$8.2x10^{-4}$	<0.001
	Strenuous	-0.0193	0.0014	<0.001

Antithrombotic				
Predictor	**Level**	**Beta**	**SE**	**p**
Deprivation		$2.6x10^{-4}$	$6.2x10^{-4}$	0.6784
Smoking (*ref: non-smoker*)	Previous smoker	0.0157	0.0037	<0.001
	Current smoker	0.0084	0.0063	0.1851
BMI		0.0089	$3.8x10^{-4}$	<0.001
Sex	Male	0.1194	0.0038	<0.001
Education	graduate degree	-0.0062	0.0039	0.1077
Region (*ref: England*)	Scotland	-0.0106	0.006	0.0747
	Wales	0.0407	0.0057	<0.001
Alcohol consumption (*ref: daily or almost daily consumption*)	Three or four times a week	-0.0126	0.0051	0.0131
	Once or twice a week	-0.0084	0.005	0.094
	Once to thrice a month	$-6.9x10^{-4}$	0.0066	0.9162
	Special occasions only	0.012	0.0066	0.0671
	Never	0.0314	0.0072	<0.001
Physical activity (*ref: mild or no physical activity*)	Moderate	-0.0194	0.004	<0.001
	Strenuous	-0.03	0.0071	<0.001

Diabetes				
Predictor	**Level**	**Beta**	**SE**	**p**
Deprivation		0.0069	$4.6x10^{-4}$	<0.001
Smoking (*ref: non-smoker*)	Previous smoker	0.023	0.003	<0.001
	Current smoker	0.0262	0.0048	<0.001
BMI		0.0216	$3.2x10^{-4}$	<0.001
Sex	Male	0.1224	0.0031	<0.001
Education	graduate degree	-0.0111	0.0032	<0.001
Region (*ref: England*)	Scotland	-0.0181	0.0047	<0.001
	Wales	0.0137	0.0047	0.0034
Alcohol consumption (*ref: daily or almost daily consumption*)	Three or four times a week	0.0109	0.0046	0.0175
	Once or twice a week	0.0458	0.0044	<0.001
	Once to thrice a month	0.0751	0.0053	<0.001
	Special occasions only	0.1113	0.0052	<0.001
	Never	0.1443	0.0056	<0.001
Physical activity (*ref: mild or no physical activity*)	Moderate	-0.0377	0.003	<0.001
	Strenuous	-0.0983	0.0064	<0.001

Cardiovascular				
Predictor	**Level**	**Beta**	**SE**	**p**
Deprivation		0.003	$3.6x10^{-4}$	<0.001
Smoking (*ref: non-smoker*)	Previous smoker	0.023	0.0022	<0.001
	Current smoker	0.0231	0.0037	<0.001
BMI		0.0138	$2.3x10^{-4}$	<0.001
Sex	Male	0.0394	0.0021	<0.001
Education	graduate degree	-0.04	0.0024	<0.001
Region (*ref: England*)	Scotland	0.0037	0.0035	0.2882
	Wales	0.0171	0.0036	<0.001
Alcohol consumption (*ref: daily or almost daily consumption*)	Three or four times a week	-0.0023	0.0031	0.4608
	Once or twice a week	0.0026	0.0031	0.4082
	Once to thrice a month	0.0079	0.0039	0.044
	Special occasions only	0.0319	0.0039	<0.001
	Never	0.0457	0.0044	<0.001
Physical activity (*ref: mild or no physical activity*)	Moderate	-0.0347	0.0023	<0.001
	Strenuous	-0.0863	0.0045	<0.001

Gastrointestinal				
Predictor	**Level**	**Beta**	**SE**	**p**
Deprivation		0.0012	$1.5x10^{-4}$	<0.001
Smoking (*ref: non-smoker*)	Previous smoker	0.0081	$9.4x10^{-4}$	<0.001
	Current smoker	0.0087	0.0015	<0.001
BMI		$4.1x10^{-4}$	$9.3x10^{-5}$	<0.001
Sex	Male	-0.0297	$9.1x10^{-4}$	<0.001
Education	graduate degree	-0.016	$9.6x10^{-4}$	<0.001
Region (*ref: England*)	Scotland	-0.0053	0.0014	<0.001
	Wales	0.0034	0.0015	0.022
Alcohol consumption (*ref: daily or almost daily consumption*)	Three or four times a week	$2.5x10^{-5}$	0.0013	0.9852
	Once or twice a week	0.0085	0.0013	<0.001
	Once to thrice a month	0.0117	0.0016	<0.001
	Special occasions only	0.0209	0.0016	<0.001
	Never	0.0277	0.0018	<0.001
Physical activity (*ref: mild or no physical activity*)	Moderate	-0.0103	$9.7x10^{-4}$	<0.001
	Strenuous	-0.0194	0.0017	<0.001

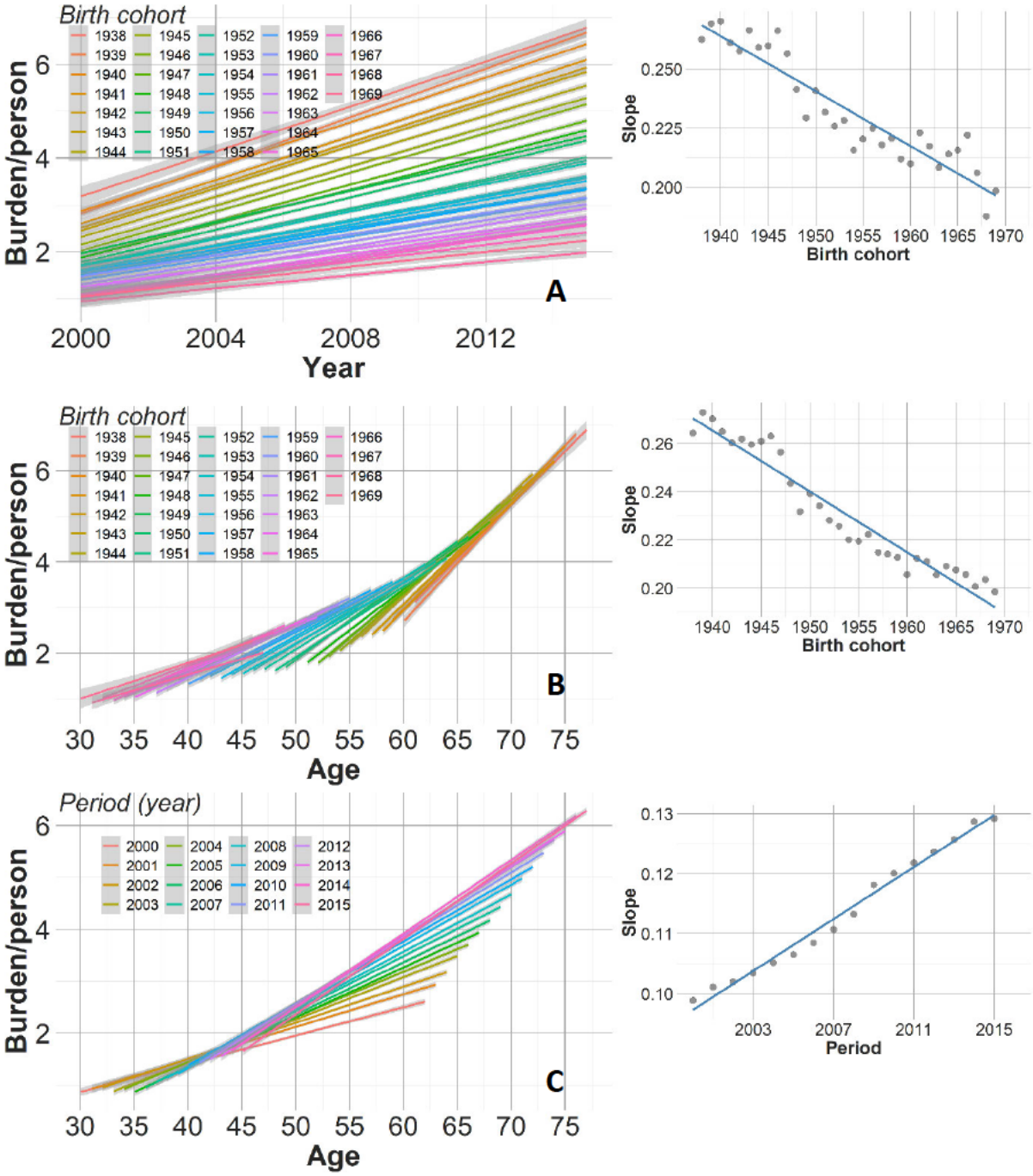
Psycholeptic				
Predictor	**Level**	**Beta**	**SE**	**p**
Deprivation		0.0012	$1.2x10^{-4}$	<0.001
Smoking (*ref: non-smoker*)	Previous smoker	0.0078	$7.7x10^{-4}$	<0.001
	Current smoker	0.0187	0.0012	<0.001
BMI		$7.5x10^{-4}$	$7.8x10^{-5}$	<0.001
Sex	Male	-0.0315	$7.4x10^{-4}$	<0.001
Education	graduate degree	-0.0082	$7.7x10^{-4}$	<0.001
Region (*ref: England*)	Scotland	-0.0023	0.0012	0.0524
	Wales	0.0091	0.0012	<0.001
Alcohol consumption (*ref: daily or almost daily consumption*)	Three or four times a week	-0.0047	0.0011	<0.001
	Once or twice a week	$-8.5x10^{-4}$	0.0011	0.4198
	Once to thrice a month	0.0053	0.0013	<0.001
	Special occasions only	0.0111	0.0013	<0.001
	Never	0.0224	0.0015	<0.001
Physical activity (*ref: mild or no physical activity*)	Moderate	-0.0085	$8.1x10^{-4}$	<0.001
	Strenuous	-0.0152	0.0014	<0.001

Respiratory				
Predictor	**Level**	**Beta**	**SE**	**p**
Deprivation		0.002	$1.4x10^{-4}$	<0.001
Smoking (*ref: non-smoker*)	Previous smoker	0.0126	$8.9x10^{-4}$	<0.001
	Current smoker	0.0187	0.0014	<0.001
BMI		0.0025	$9.0x10^{-5}$	<0.001
Sex	Male	-0.0283	$8.5x10^{-4}$	<0.001
Education	graduate degree	-0.0183	$9.0x10^{-4}$	<0.001
Region (*ref: England*)	Scotland	-0.019	0.0014	<0.001
	Wales	0.0383	0.0014	<0.001
Alcohol consumption (*ref: daily or almost daily consumption*)	Three or four times a week	-0.002	0.0012	0.104
	Once or twice a week	0.0035	0.0012	0.0035
	Once to thrice a month	0.0048	0.0015	0.0018
	Special occasions only	0.0155	0.0016	<0.001
	Never	0.0267	0.0018	<0.001
Physical activity (*ref: mild or no physical activity*)	Moderate	-0.008	$9.5x10^{-4}$	<0.001
	Strenuous	-0.0178	0.0016	<0.001

Urological				
Predictor	**Level**	**Beta**	**SE**	**p**
Deprivation		0.0036	$8.6x10^{-4}$	<0.001
Smoking (*ref: non-smoker*)	Previous smoker	0.0277	0.0053	<0.001
	Current smoker	0.0035	0.009	0.6951
BMI		0.0079	$5.3x10^{-4}$	<0.001
Sex	Male	-0.0929	0.0051	<0.001
Education	graduate degree	-0.0445	0.0055	<0.001
Region (*ref: England*)	Scotland	0.0334	0.0079	<0.001
	Wales	0.0111	0.0086	0.1968
Alcohol consumption (*ref: daily or almost daily consumption*)	Three or four times a week	0.0152	0.0077	0.0488
	Once or twice a week	0.0521	0.0075	<0.001
	Once to thrice a month	0.0695	0.0092	<0.001
	Special occasions only	0.0931	0.0092	<0.001
	Never	0.105	0.0103	<0.001
Physical activity (*ref: mild or no physical activity*)	Moderate	-0.0067	0.0056	0.2292
	Strenuous	-0.0329	0.01	0.001

Other				
Predictor	**Level**	**Beta**	**SE**	**p**
Deprivation		$2.9x10^{-4}$	$7.3x10^{-5}$	<0.001
Smoking (*ref: non-smoker*)	Previous smoker	0.0051	$4.5x10^{-4}$	<0.001
	Current smoker	0.0079	$7.3x10^{-4}$	<0.001
BMI		$8.5x10^{-4}$	$4.6x10^{-5}$	<0.001
Sex	Male	-0.0021	$4.2x10^{-4}$	<0.001
Education	graduate degree	-0.0072	$4.5x10^{-4}$	<0.001
Region (*ref: England*)	Scotland	-0.0062	$6.9x10^{-4}$	<0.001
	Wales	0.0046	$7.1x10^{-4}$	<0.001
Alcohol consumption (*ref: daily or almost daily consumption*)	Three or four times a week	-0.0011	$6.1x10^{-4}$	0.0627
	Once or twice a week	0.0014	$6.1x10^{-4}$	0.0205
	Once to thrice a month	0.0021	$7.7x10^{-4}$	0.0065
	Special occasions only	0.0067	$7.9x10^{-4}$	<0.001
	Never	0.014	$9.0x10^{-4}$	<0.001
Physical activity (*ref: mild or no physical activity*)	Moderate	-0.0044	$4.8x10^{-4}$	<0.001
	Strenuous	-0.0088	$7.8x10^{-4}$	<0.001

Supplementary Figure S9: APC-analysis with basic mixed models with random intercepts and slopes (**left**) and associations between slopes and different levels of predictors (**right**). **A** depicts the period-cohort model with cohort as a random effect, **B** depicts the age-cohort model with cohort as a random effect, and **C** depicts the age-period model with period as the random effect.



Supplementary references

1. Carnahan RM, Lund BC, Perry PJ, et al. The anticholinergic drug scale as a measure of drug-related anticholinergic burden: Associations with serum anticholinergic activity. J Clin Pharmacol 2006; 46: 1481–1486.
2. Boustani M, Campbell N, Munger S, et al. Impact of anticholinergics on the aging brain: A review and practical application. Aging health 2008; 4: 311–320.
3. Rudolph JL, Salow MJ, Angelini MC, et al. The anticholinergic risk scale and anticholinergic adverse effects in older persons. Arch Intern Med 2008; 168: 508–513.
4. Sumukadas D, McMurdo MET, Mangoni AA, et al. Temporal trends in anticholinergic medication prescription in older people: Repeated crosssectional analysis of population prescribing data. Age Ageing 2013; 0: 1–7.
5. Durán CE, Azermai M, Stichele RHV. Systematic review of anticholinergic risk scales in older adults. Eur J Clin Pharmacol 2013; 69: 1485–1496.
6. Ehrt U, Broich K, Larsen JP, et al. Use of drugs with anticholinergic effect and impact on cognition in Parkinson's disease: A cohort study. J Neurol Neurosurg Psychiatry 2010; 81: 160–165.
7. Kiesel EK, Hopf YM, Drey M. An anticholinergic burden score for German prescribers: Score development. BMC Geriatr 2018; 18: 1–11.
8. Aldrugh S, Sardana M, Henninger N, et al. Atrial fibrillation, cognition and dementia: A review. J Cardiovasc Electrophysiol 2017; 28: 958–965.
9. Han L, McCusker J, Cole M, et al. Use of medications with anticholinergic effect predicts clinical severity of delirium symptoms in older medical inpatients. Arch Intern Med 2001; 161: 1099–1105.
10. Han L, Agostini J V., Allore HG. Cumulative anticholinergic exposure is associated with poor memory and executive function in older men. J Am Geriatr Soc 2008; 56: 2203–2210.
11. Aizenberg D, Sigler M, Weizman A, et al. Anticholinergic burden and the risk of falls among elderly psychiatric inpatients: A 4-year case-control study. Int Psychogeriatrics 2002; 14: 307–310.
12. Minzenberg MJ, Poole JH, Benton C, et al. Assocation of Anticholinergic Load with Impairment of Complex Attention and Memory in Schizophrenia. Am J Psychiatry 2004; 161: 116–124.
13. Ancelin ML, Artero S, Portet F, et al. Non-degenerative mild cognitive impairment in elderly people and use of anticholinergic drugs: Longitudinal cohort study. Br Med J 2006; 332: 455–458.
14. Hilmer SN. Calculating and using the durg burden index score in reasearch and practice. Expert Rev Clin Pharmacol 2018; 11: 1053–1055.
15. Chew ML, Mulsant BH, Pollock BG, et al. Anticholinergic activity of 107 medications commonly used by older adults. J Am Geriatr Soc 2008; 56: 1333–1341.
16. Cancelli I, Valentinis L, Merlino G, et al. Drugs with anticholinergic properties as a risk factor for psychosis in patients affected by Alzheimer's disease. Clin Pharmacol Ther 2008; 84: 63–68.
17. Sittironnarit G, Ames D, Bush AI, et al. Effects of anticholinergic drugs on cognitive function in older Australians: Results from the AIBL study. Dement Geriatr Cogn Disord 2011; 31: 173–178.
18. Whalley LJ, Sharma S, Fox HC, et al. Anticholinergic drugs in late life: Adverse effects on cognition but not on progress to dementia. J Alzheimer's Dis 2012; 30: 253–261.
19. Dauphinot V, Faure R, Omrani S, et al. Exposure to anticholinergic and sedative drugs, risk of falls, and mortality: An elderly inpatient, multicenter cohort. J Clin Psychopharmacol 2014; 34: 565–570.
20. Klamer TT, Wauters M, Azermai M, et al. A Novel Scale Linking Potency and Dosage to Estimate Anticholinergic Exposure in Older Adults: the Muscarinic Acetylcholinergic Receptor ANTagonist Exposure Scale. Basic Clin Pharmacol Toxicol 2017; 120: 582–590.

5 AChB and dementia in UK Biobank

5.1 Introduction

The study presented in section 4.2 explored the demographic characteristics and longitudinal trends of anticholinergic prescribing in the UK. I found AChB to have substantially increased in the sample from the year 1991 to 2015. People across age groups exhibited higher AChB compared to those same age groups in past periods. This indicated that the observed increase was not solely due to ageing of the sample, but also due to period/cohort effects.

If long-term anticholinergic drugs contribute to adverse outcomes, the increase in their use might pose a threat to public health. Indeed, as described in section 2.4.4.3, prior work indicates a robust relationship between anticholinergic use and the risk of falls and fractures in older people. However, for many other health outcomes, including dementia, such a clear relationship has not been clearly established. Although many analyses exist on the topic, the results vary from study to study. The heterogeneity of previous findings could be due to a variety of factors. One factor could be the use of different anticholinergic scales that are used to ascertain AChB. Next, the effect of AChB may be different in different drug classes, as some recent reports have demonstrated (Coupland et al., 2019; Joung et al., 2019; Richardson et al., 2018). Additionally, many studies have not corrected for polypharmacy, or for disorders that may be causing the need for anticholinergic prescribing (Taylor-Rowan et al., 2021).

In this chapter, I examined the association between AChB and dementia in UK Biobank, while exploring some of the potential sources of previous inconsistencies. I compared 13 different anticholinergic scales and performed analyses of the relationship between AChB and dementia between different drug classes. The relatively wide age range of the sample enabled the exploration of associations between long-term use of anticholinergics in middle-age and late-life dementia.

This study was published in Alzheimer's & Dementia: Translational Research & Clinical Interventions in April 2022 (Mur et al., 2022) and presented as an oral presentation at the

25th Nordic Congress of Gerontology (June 2nd – 5th 2021). It is available in full in section 5.2. The supplementary material for this work is available in section 5.3.1 and at https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9005668/. A corrigendum for this article (due to an error in the abstract) was submitted on the 2nd of November 2022 and is currently pending online publication.

5.2 Association between anticholinergic burden and dementia in UK Biobank

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RESEARCH ARTICLE

Association between anticholinergic burden and dementia in UK Biobank

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Abstract

Background: Previous studies on the relationship between anticholinergic drugs and dementia have reported heterogeneous results. This variability could be due to different anticholinergic scales and differential effects of distinct classes of drugs.

Methods: Using Cox proportional hazards models, we computed the association between annual anticholinergic burden (AChB) and the risk of dementia in UK Biobank with linked general practitioner prescription records between the years 2000 and 2015 (n = 171,775).

Results: AChB according to most anticholinergic scales (standardized odds ratio range: 1.027–1.125) and the slope of the AChB trajectory (hazard ratio = 1.094; 95% confidence interval: 1.068–1.119) were predictive of dementia. However, the association between AChB and dementia held only for some classes of drugs, especially antidepressants, antiepileptics, and diuretics.

Discussion: The heterogeneity in previous findings may partially be due to different effects for different classes of drugs. Future studies should establish differences in more detail and further examine the practicality of a general measure of AChB relating to the risk of dementia.

KEYWORDS
anticholinergic drugs, cohort study, dementia, prescriptions drugs, primary care

1 | INTRODUCTION

The number of people with dementia is predicted to increase in the UK by 50% from the year 2016 to 2040 and worldwide from 50 million today to 152 million in 30 years.[1] Considering the lack of treatment options, the specification of risk factors to reduce the incidence of the disease is crucial. It is estimated that ≈40% of risk factors for dementia are preventable[1] and that the decreases in the incidence of dementia in some countries are partially attributable to reductions in some of these risk factors.[2]

Anticholinergic drugs block muscarinic acetylcholine receptors in the nervous system, which are important in the innervation of brain areas involved in cognitive function and in the pathophysiology of Alzheimer's disease (AD).[3] Due to their mechanism of action, sustained use of these medicines might impair cognitive function later in life. Anticholinergic burden (AChB)—a measure of anticholinergic drug use—has indeed been linked to an increased risk of cognitive impairment and dementia in older people.[4,5] Recent studies have focused on the long-term effects of anticholinergic drugs when taken before advanced age: both Coupland et al.[6] and Richardson et al.[7] studied the

associations between anticholinergic use in middle-aged patients from general practices in the UK and the risk of late-life dementia. For certain anticholinergic drugs, these studies reported an increased rate of dementia after their use decades before the diagnosis. This suggests a potential for AChB as a marker for cognitive decline or as a causative risk factor. In other words, AChB could be indicative of comorbidities that themselves affect cognition or could—through the drugs' mechanism of action—contribute to cognitive decline as an independent risk factor. However, the status of anticholinergic medication in dementia prevention is unclear, as several recent reviews on the topic report heterogeneous findings.[4,8,9]

The variability in previous findings can be partially explained by differences in study design, the characteristics of the samples, the covariates in the models, and the choice of anticholinergic scales that assign drugs their anticholinergic potency. There is no widely accepted procedure to score anticholinergic potency[10] and anticholinergic scales were constructed in distinct regions and contexts, and validated in different samples. Additionally, likely due to their propensity to cross the blood-brain barrier, drugs differ in their ability to cause effects in the central nervous system as opposed to the periphery. Because anticholinergic scales are constructed with different outcomes in mind, they will not all place the same focus on centrally acting compounds. The above differences between scales lead to poor agreement between them and to uncertainty when choosing a scale for medical practice or research.[11,12]

Moreover, the associations between anticholinergic drugs and dementia may hold only for some classes of drugs. Recent studies exploring class-based associations reported effects especially for antidepressants, urological drugs, and antipsychotics.[6,7] Thus, while general recommendations of (de-)prescribing of anticholinergic drugs are often made,[5,6,13] they might not always be appropriate. This is especially the case because drugs are prescribed to manage underlying conditions that themselves decrease the quality of life and in cases when drug alternatives that exhibit fewer side effects are unavailable.

To elucidate the proposed association between AChB and dementia, well-powered replication studies and detailed inspections of the effects of different anticholinergic scales and drug classes are necessary. In this paper, the aims were threefold: (1) compare different anticholinergic scales in their propensity to predict dementia, (2) study the association between AChB at baseline and the longitudinal evolution of AChB and dementia, and (3) compare AChB due to different classes of drugs and the risk of dementia.

RESEARCH IN CONTEXT

1. Systematic Review: The authors used recent systematic reviews and manual search on PubMed to explore the extant literature. While there is research on the topic, the results are heterogeneous and only two studies probe the association between anticholinergic drug use in middle life and dementia in older age.
2. Interpretation: In this cohort study of 2124 participants diagnosed with dementia and 169,651 controls from UK Biobank, anticholinergic burden was associated with dementia risk across most scales used. However, only some drug classes were associated with dementia, especially antidepressants, antiepileptics, and diuretics. Anticholinergic potency did not show a clear relationship with dementia risk.
3. Future Directions: The relationships between various anticholinergic drugs and dementia should be clarified, and broad recommendations spanning several drug classes re-evaluated.

2 | METHODS

2.1 | Hypotheses

We expected AChB to be positively associated with dementia across anticholinergic scales and the association to be stronger than the association between dementia and polypharmacy. Furthermore, we anticipated the increase of AChB over time to be positively associated with dementia. Finally, based on previous studies,[6,7] we hypothesized that AChB due to antidepressants, antihistamines, antiepileptics, urological, and antipsychotic drugs would show a positive association with dementia. The association between other classes of drugs and dementia and the analysis of latencies between AChB and dementia was not based on prior hypotheses.

2.2 | Sample

UK Biobank is a prospective study of > 500,000 participants that were recruited across the UK from 2006 to 2010.[14] For ≈230,000 of these participants, primary-care electronic prescription entries are available until September 2017. The entries contain the drugs prescribed, dates of prescriptions, and Read codes (https://isd.digital.nhs.uk[15]) that act as dictionaries for medicines. Diagnoses were obtained from two sources: (1) primary care electronic prescription records and (2) inpatient records. The former are prescriptions written on the computer by the primary care provider, while the latter are prescriptions issued during hospital stays. Dementia diagnoses and diagnoses used as covariates (see below) were ascertained using both primary care (UK Biobank field 42040) and hospital (UK Biobank fields 41270 and 41271) records (Table S1 in supporting information). In cases of multiple entries for a disorder, we retained the earliest record.

2.3 | Anticholinergic burden and drug class

Eleven anticholinergic scales[16 26] were chosen as previously identified[27] and two[28,29] were identified through a recent

systematic review.[30] All anticholinergic scales used in this study, including full names and potential reasons for exclusion from the analyses, are listed in Table S2 in supporting information. One scale[25] was modified to include newer drugs as before;[31] for two scales,[17,19] updated versions were used (Aging Brain Care;[32] Carnahan, 2014, personal communication on October 21, 2019). For one scale,[21] drugs classified by the authors as having "improbable anticholinergic action" were assigned an anticholinergic burden of 0.5 (between "no anticholinergic potency" and "weak anticholinergic potency") as has been done before.[27]

Using the British National Formulary (https://bnf.nice.org.uk[33]), brand names of anticholinergic drugs in the sample were substituted with generic names. Combination prescriptions containing several anticholinergic compounds were separated into multiple entries, each containing a single anticholinergic compound.

Each prescription was assigned anticholinergic scores based on the ratings from anticholinergic scales. Prescriptions of drugs with ophthalmic, otic, nasal, or topical routes of administration were assigned an anticholinergic score of 0, as before.[23 26] In the analysis comparing anticholinergic scales, for each scale, AChB was estimated by four separate means. First, the total yearly number of anticholinergic drugs was determined (count-based scale). Second, each drug was assigned the anticholinergic value as listed in the anticholinergic scale and the values were summed for each year (value-based scale). Third, a standardized dosage was calculated for each prescription by dividing the prescribed dose by the defined daily dose (DDD, https://www.whocc.no[34]) and then multiplying it by the anticholinergic score (dosage-adjusted scale). Fourth, the quantity of the prescribed drug (e.g., volume or number of tablets) was accounted for by multiplying the prescribed dose with the quantity, divided by the DDD, and then multiplied by the anticholinergic score (quantity-adjusted scale). To compare anticholinergic scales, separate models were run for each scale, resulting in 52 models. Additionally, two separate models were run for which polypharmacy was the main predictor. For the dosage-adjusted and quantity-adjusted scales, years in which any anticholinergic prescription was missing information on dosage or quantity, respectively, were removed for that participant (751 observations for the dosage-adjusted scale and 8008 observations for the quantity-adjusted scale). For all other analyses (i.e., when anticholinergic classes were not compared to one another), the scale by Dúran et al.[21] was used to calculate AChB, as it exhibited the strongest association with the risk of dementia (see below). Each drug was assigned to a class based on the Anatomical Therapeutic Chemical (ATC) classification system (https://www.whocc.no/atc ddd index/[34]; Table S3 in supporting information) and to a group of anticholinergic potency (groups 0, 0.5, 1, 2; a higher value indicates a greater presumed anticholinergic potency) according to the anticholinergic scale by Dúran et al.[21]

2.4 | Covariates and statistical analysis

The predictor in most models was the cumulative AChB in year 0 (the sum of anticholinergic scores of prescriptions for a participant). Due to the low ascertainment of prescriptions in the early years of sampling,[27] year 0 was for each participant defined as the first full year of having been included in the prescriptions' register after the year 1999.

Because the rate of dementia increases with age, participants younger than 60 years at the time of diagnosis or at the end of the prescriptions sampling period (June 30, 2020)—whichever came first—were excluded from the analyses. Additionally, participants who before year 0 or within a cut-off period after year 0, had been diagnosed with dementia or prescribed a cholinesterase inhibitor (donepezil, galantamine, or rivastigmine) or memantine were excluded from the analyses. For all analyses in the main text, the cut-off period above was 1 year. Based on comments by the reviewers, we varied this cut-off and repeated the analysis on the association between AChB according to the scale by Dúran et al.[21] and dementia for every possible value of this cut-off (1 year to 20 years; Figure S4 in supporting information). People diagnosed with certain disorders are more likely to develop dementia. For this reason, we also excluded participants diagnosed at any point with Parkinson's disease, Huntington disease, Creutzfeldt-Jacob disease, or multiple sclerosis from our analyses. Finally, the prescribing period after the year 2015 was incomplete[27] and was removed. The data cleaning process is described in Figure S1 in supporting information.

Models were adjusted for age at year 0, sex (reference: female), data provider (region-specific providers of prescriptions: The Phoenix Partnership [TPP] England, Vision England [reference], Vision/EMIS Health Scotland, Vision/EMIS Health Wales), education (binary; reference: no graduate degree), socioeconomic deprivation based on census data (scale range: −12 to 12; range in sample: −6.3 to 7.4; bigger number indicates greater deprivation),[35] body mass index (BMI in kg/m^2, categorized: < 18.5, 18.5–25 [reference], 25–30, 30–35, 35–40, > 40), self-reported smoking status (smoker, non-smoker [reference], former smoker), self-reported alcohol consumption frequency (daily or almost daily [reference], three or four times a week, once or twice a week, one to three times a month, only on special occasions, never), self-reported physical activity (mild [reference], moderate, strenuous),[36] number of comorbidities (number of all unique diagnosis codes) by year 0, depression by year 0 (reference: no depression), stroke by year 0 (reference: no stroke), diabetes by year 0 (reference: no diabetes), hypercholesterolemia by year 0 (reference: no hypercholesterolemia), hypertension by year 0 (reference: no hypertension), apolipoprotein E (*APOE*) carrier status (reference: ε2), and polypharmacy. The latter was determined separately for each anticholinergic scale by subtracting the yearly number of anticholinergic drugs according to that scale from the total yearly drug count. *APOE* genotype was determined based on the nucleotides at single nucleotide polymorphism positions rs239358 and rs7412; *APOE* carrier status was denoted as ε3 for participants with the ε3/ε3, ε1/ε3, or ε2/ε4 haplotype, ε2 for participants with the ε2/ε2 or ε2/ε3 haplotype, and ε4 for participants with the ε3/ε4 or ε4/ε4 haplotype.

For the association between AChB and dementia, Cox proportional hazards models were used, and effects are expressed as hazard ratios (HRs) with accompanying 95% confidence intervals (CIs). For studying time-to-event latencies, logistic regression was used, and effects

are expressed in odds ratios (ORs). The association between the longitudinal evolution of AChB and dementia accounted for the competing risk of death and was assessed with the joint model for longitudinal and time-to-event data using the R library JM.[37] For all other analyses using only a single anticholinergic scale, the value-based scale by Durán et al.[21] was used, as it exhibited the strongest association with dementia. Models for which AChB was the main predictor were additionally controlled for polypharmacy. The two models for which polypharmacy was the main predictor differed from each other in the included covariates: (1) one was controlled for all covariates described above except for polypharmacy, and (2) the other (termed "polypharmacy plus") was additionally controlled for the total number of anticholinergic drugs (according to any anticholinergic scale).

Numerical values three or more standard deviations beyond the mean were defined as outliers and removed from the analytical sample prior to analysis. Due to zero inflation for AChB, the number of prescriptions, and the number of comorbidities, null values were removed before calculating means and standard deviations for outlier removal for these variables. Cases with missing values were removed prior to analysis and constituted up to 16.9% of the sample, depending on the model. When exploring the AChB attributable to different drug classes, only drug classes were included that were in year 0 prescribed to at least 10 participants that later developed dementia. The proportional hazards assumption was satisfied, but the assumption of linearity between the predictor and the log hazard was sometimes violated (Figure S2 in supporting information). In models in which that was the case, the covariates were transformed, and the type of transformation is indicated in the results. When a distinct model was run for each predictor, the Bonferroni correction was used. When all predictors were included in a single model, no adjustment for multiple comparisons was done. Numerical variables were scaled to have a mean of 0 and a standard deviation of 1. Results are reported as standardized effect sizes. All analyses were performed in R version 4.1.0 and Python 3.7.10. The code is available at https://github.com/JuM24/UKB-ACB-dementia.

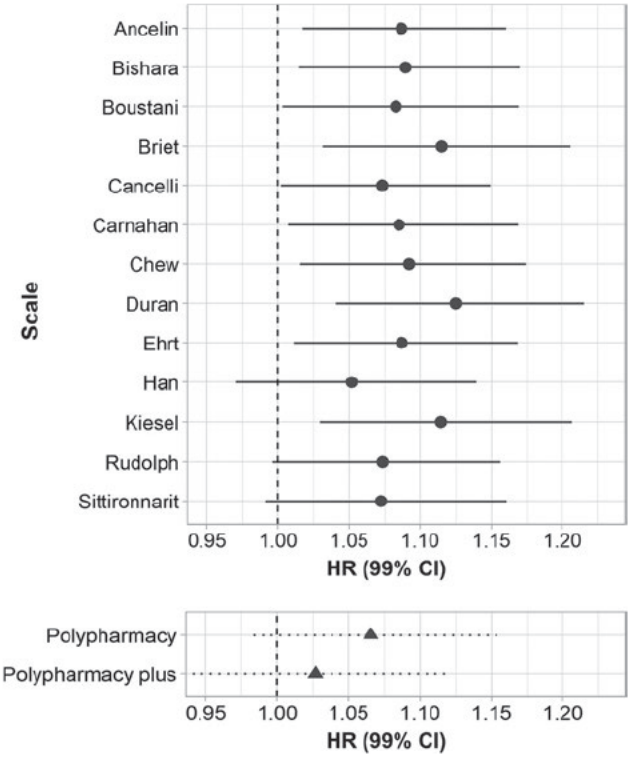

FIGURE 1 Hazard ratios (HRs) for the association between anticholinergic burden (top panel) or drug count (bottom panel) and dementia. The names on the y-axis of the top panel refer to the first names of the authors of the original anticholinergic scales; "polypharmacy plus" was additionally controlled for the total number of anticholinergic drugs. CI, confidence interval

3 | RESULTS

3.1 | Characteristics of the sample

After data cleaning, the final sample consisted of 171,775 participants. Among the participants, 2124 (1.2%) were diagnosed with dementia (Table S4 in supporting information), with diagnoses dating between July 2002 and June 2020. The median age of participants at year 0 was 55 years (Q1 = 49 years, Q3 = 59 years) and the median age of diagnosis with dementia was 72.6 years (interquartile range [IQR] = 7.2). The average follow-up—defined as the median number of years between year 0 and the year of censoring—was 20 years for participants without dementia (Q1 = 14 years, Q3 = 20 years) and 14 years for participants diagnosed with dementia (Q1 = 11 years, Q3 = 17 years). The characteristics of variables for year 0 are presented in Table 1 and Table S5 in supporting information. Depending on the scale used, anticholinergic drugs constituted between 2.5% and 21.8% of all prescriptions between the years 2000 and 2015, with 0.24 to 2.12 anticholinergic prescriptions per person in year 0 (Table S6 in supporting information). The characteristics of anticholinergic prescribing in UK Biobank have been described in greater detail elsewhere.[27]

3.2 | Anticholinergic scales comparison

Most anticholinergic scales showed positive associations with dementia and with greater effect size estimates than for general polypharmacy (Figure 1). HRs for standardized AChB ranged from 1.027 to 1.125 (count-based: median = 1.087, IQR = 0.044; value-based: median = 1.087, IQR = 0.019; dosage-adjusted: median = 1.078, IQR = 0.009; quantity-adjusted: median = 1.065, IQR = 0.032; Tables S7 and S8 in supporting information). The overlap in CIs was substantial both between scales and within scales; similar results were observed for models with log- and rank-inverse normally transformed predictors (Figure S3 in supporting information). The value-based scale by Durán et al.[21] exhibited the strongest association with dementia (Tables S7 and S9 in supporting information) and was used in all subsequent analyses. The effect of AChB on dementia was relatively invariant among the models with different exclusion cut-offs for the period of dementia diagnosis (Figure S4 in supporting information). When death was

TABLE 1 : Descriptive statistics of variables used in the models

Variable	Level	Median (IQR) or n (%)
Age		55 (10)
Sex	Female	94,310 (54.9)
Education	No graduate degree	118,191 (69.7)
Deprivation		2.3 (3.8)
Alcohol consumption	Daily or almost daily	35,989 (21.0)
	Three or four times a week	39,747 (23.2)
	Once or twice a week	43,815 (25.6)
	Once to three times a month	18,149 (10.6)
	Only special occasions	19,673 (11.5)
	Never	14,024 (8.2)
Smoking	Current smoker	16,412 (9.6)
	Previous smoker	63,372 (37.1)
	Non-smoker	91,091 (53.3)
Physical activity	Strenuous	13,577 (8.5)
	Moderate	103,121 (64.7)
	Light	42,777 (26.8)
BMI	<18.5	768 (0.45)
	18.5 25	3372 (2.0)
	25 30	51,649 (30.2)
	30 35	74,192 (43.4)
	35 40	31,807 (18.6)
	>40	9070 (5.3)
Data provider	England (Vision)	14,036 (8.2)
	Scotland	18,758 (10.9)
	England (TPP)	123,133 (71.7)
	Wales	15,848 (9.2)
Dementia diagnosis		2124 (1.2)
Prior depression		13,136 (7.6)
Prior stroke		1598 (0.9)
Prior diabetes		4034 (2.3)
Prior hypercholesterolemia		4901 (2.9)
Prior hypertension		16,152 (9.4)
Number of prior comorbidities		18 (40)
Total number of prescriptions*		3 (12)
APOE carrier	ε2	21,626 (12.9)
	ε3	102,740 (61.3)
	ε4	43,199 (25.8)

*The total number of prescriptions was used along the number of anticholinergic drugs to calculate the scale-specific non-anticholinergic drug count.
Abbreviations: *APOE*, apolipoprotein E; BMI, body mass index; IQR, interquartile range; TPP, The Phoenix Partnership.

modeled as a competing outcome, a one standard deviation increase in AChB was associated with a 12.0% (95% CI: 7.1%–17.2%) increase in the incidence of dementia, and a 6.0% (95% CI: 3.5%–8.5%) increase in the incidence of all-cause mortality.

3.3 | Time-to-event latency

We compared the risk of dementia occurring within 12 years, between 12 and 14 years, between 14 and 16 years, 16 and 18 years, or more

TABLE 2 : ORs for the risk of dementia within different time periods since the measurement of anticholinergic burden

Latency (years since 2000)	OR	95% CI	N cases
0 12	1.20	1.05 1.34	250
12 14	1.06	0.90 1.22	257
14 16	1.11	1.00 1.22	446
16 18	1.21	1.10 1.33	375
18 20.5	1.07	0.98 1.15	813

Abbreviations: CI, confidence interval; OR, odds ratio.

than 18 years (effectively 18–20.5) after year 0. ORs did not differ between most of the different latencies, nor was a pattern discernible in the relationship between latency and effect size (Table 2).

3.4 | Change in AChB and dementia

The estimate for the association between the individual longitudinal evolution of AChB and dementia was positive (HR = 1.094; 95% CI: 1.068–1.119). When the rate of dementia was modeled as a function of the individual longitudinal evolution of AChB in a competing risk model (competing risks: dementia, death), the effect was also positive (death: HR = 1.066, 95% CI = 1.042–1.089; dementia: HR = 1.056, 95% CI = 1.008–1.11).

3.5 | Drug classes and categories of AChB

Several drug classes exhibited a positive association between AChB and dementia, including drugs for treating the nervous-, gastrointestinal-, and cardiovascular systems (Figure 2; Tables S10, S11 in supporting information). The effect was strongest for antiepileptic drugs, antidepressants, and diuretics (furosemide). While many drugs exhibited a positive tendency for an association between AChB and dementia, the effect sizes were small, and the CIs mostly overlapped with HR = 1. When the individual yearly drug counts for each group of anticholinergic potency were used to predict dementia (Figure 3; Table S12 in supporting information), only the number of drugs with an anticholinergic potency of 1 was predictive of dementia.

4 | DISCUSSION

4.1 | Interpretation of the findings

In this study, we used electronic prescription data from 171,775 participants in UK Biobank to study the relationship between AChB and dementia risk. In line with our hypotheses, AChB was associated with dementia across most anticholinergic scales and the best effect estimate for most scales tended to be greater than that for polypharmacy. The data also supported our hypothesis that the trajectory of AChB over time was predictive of dementia, even after accounting for the competing risk of death. The hypotheses regarding class-specific effects were mostly upheld, with AChB due to antidepressants, antiepileptics, and antihistamines positively associated with dementia risk. However, the effects for antipsychotics and for urological drugs were not significant. We also found associations between additional classes of drugs and risk of dementia, especially high-ceiling diuretics (furosemide). Finally, the strength of the association between AChB and dementia remained unchanged, regardless of the latency between time of measurement and time of diagnosis.

Our results support an association between AChB and dementia across anticholinergic scales, a finding observed previously using self-reported medicine use in UK Biobank.[36] This relationship persisted after controlling for several covariates. Across most anticholinergic scales, AChB was a stronger predictor than the total number of prescribed drugs, suggesting that anticholinergic medicines may represent a risk factor distinct from polypharmacy. When applying the anticholinergic scale[21] that exhibited the strongest association with dementia, AChB also predicted all-cause mortality. Furthermore, not only cumulative AChB measured over 1 year, but the intra-individual longitudinal trajectory in AChB over the course of 15 years was associated with the risk of dementia. In other words, steeper slopes in the increase of AChB over time were associated with an increased risk of dementia.

However, despite the association between AChB and dementia, several caveats need consideration. First, in contrast to previous findings[6,7,38] suggesting a dose–response relationship, including dosage and quantity in the computation of AChB did not increase model precision or the strength of the association between AChB and dementia. The same was true for the inclusion of anticholinergic scores: simply counting anticholinergic drugs (as opposed to assigning a potency value or weighing by dosage) was equally predictive of dementia. Second, the association between AChB and dementia was limited to AChB attributable to certain classes of drugs. This is consistent with previous findings[6,7] that reported that AChB attributable to antidepressants, antihistamines, and antiepileptic drugs was associated with dementia; this consistency was not found for antipsychotics and urological drugs. Third, findings here and elsewhere[7] indicate that a higher anticholinergic potency of a drug does not always correspond to a higher risk of dementia.

The consistency in effect sizes for the association between AChB and dementia for different time-to-event latencies has been observed before[6,7] and suggests that the value of AChB as a potential marker of later cognitive decline does not vary with time. This could indicate the longitudinal consistency in differences in AChB between individuals. While some authors[7] understand this finding as strengthening the case for causality, it could also—along with the primary finding of an association between AChB and dementia—be explained by confounding by indication: dementia could be caused by the indication for which anticholinergic drugs were prescribed. Indeed, the drugs classes linked to dementia in our study and others[6,7] are used to treat cardiovascular problems, epilepsy, depression, and schizophrenia, which themselves correlate with neuroanatomical changes or may act as risk factors for

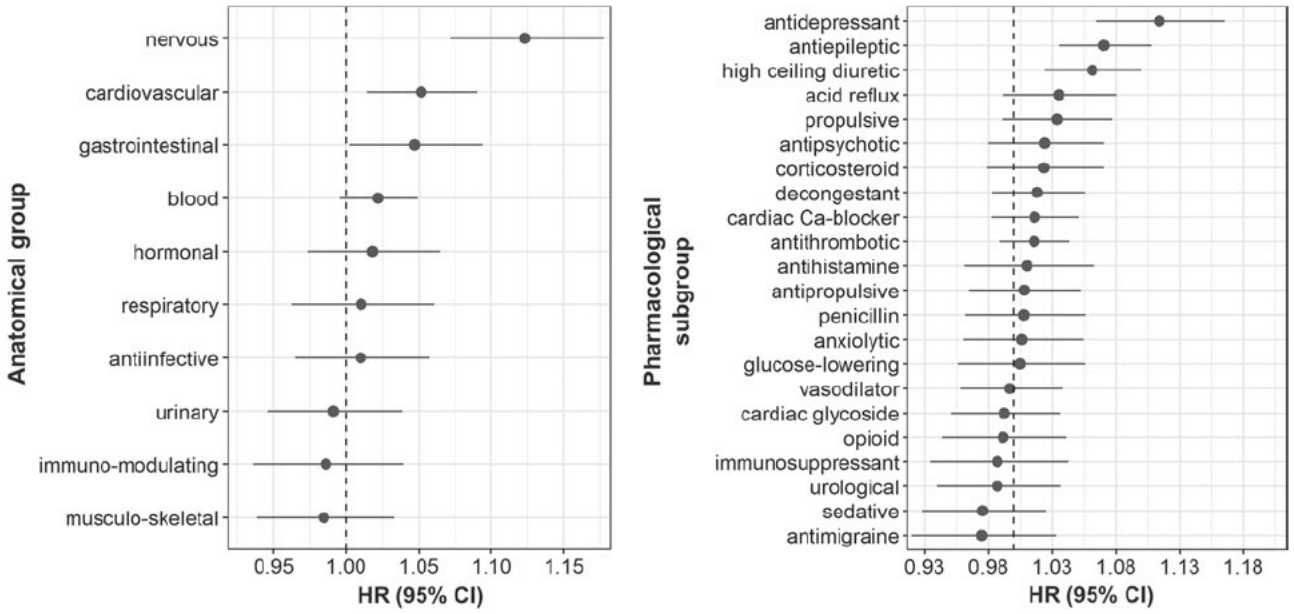

FIGURE 2 Hazard ratios (HRs) for the association between anticholinergic burden (rank-based inverse normal transformation) attributable to different classes of drugs and dementia. Left and right panels reflect the same data, but at different levels of granularity, with left panel representing the topmost level, and right panel the third level from the top according to the World Health Organization classification. CI, confidence interval

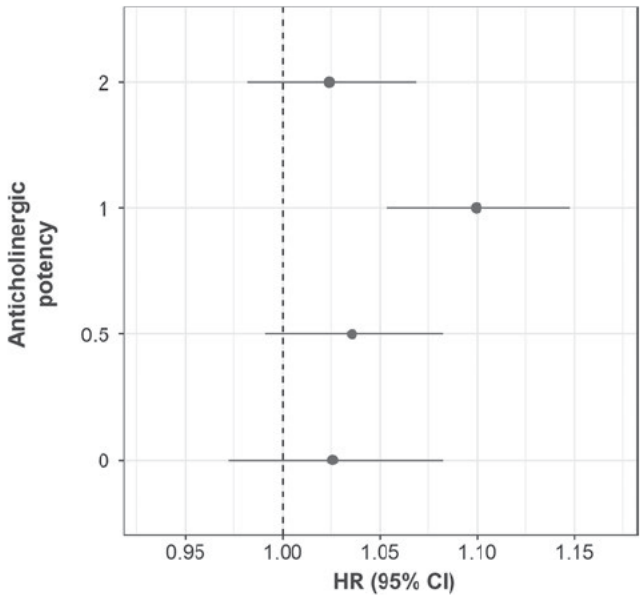

FIGURE 3 Hazard ratios (HRs) for the association between the numbers of anticholinergic drugs (rank-based inverse normal transformation) of different levels of potency and dementia. CI, confidence interval

dementia.[1,39 43] However, the lack of differences in effect size for various latencies does not preclude causality between AChB and dementia. As opposed to increasing the rate of cognitive decline (i.e., the slope of longitudinal cognitive function), the results could be explained by AChB producing a fixed degree of cognitive impairment (i.e., change the intercept of longitudinal cognitive function).

4.2 | Strengths and weaknesses

The main strengths of our study are the size of the sample, the depth of available data, and the high accuracy of UK Biobank for ascertainment of dementia.[44] Furthermore, our analyses examined AChB from multiple perspectives, including comparing different scales and drug classes. However, we acknowledge several limitations. The participants in UK Biobank are on average healthier and live in less deprived areas than the UK population.[45] Additionally, linked data do not include information on over-the-counter drugs and dietary supplements. Thus, AChB in the UK is likely higher than estimated in our study. Also, due to the low average age of the participants, UK Biobank has relatively few cases of dementia. Next, our analytical approach exhibits weaknesses. First, the dosages and quantities of medicines used in the calculation of the dosage- and quantity-adjusted scales required substantial manual cleaning and may not have been completely accurate. Second, the assumption of linearity between the predictor and the log hazard was sometimes not satisfied and transformations of the data were required to reliably run the models. Third, comparing the effects of different potencies of anticholinergic drugs, prescriptions with the highest potency were much less common than other groups of drugs. This could have affected the accuracy of our estimate.

5 | CONCLUSIONS AND FUTURE DIRECTIONS

Inconsistencies in the literature, uncertainty of dose–response- or potency–response relationships, a strong drug-class dependency, and the difficulty of excluding confounding by indication, have led

some[46] to suggest that a different common denominator—other than anticholinergic effect—is responsible for the observed association between anticholinergic drugs and dementia. If correct, the first goal should be the elucidation of the proposed association. Instead of studying the relationship of a general measure of AChB and cognitive decline, researchers could specify and describe the role of distinct classes of anticholinergic medicines—or even individual drugs.

Considering the role of the cholinergic system in the development of AD,[3] a biological underpinning for the effect of anticholinergic drugs in dementia is intuitive. However, further evidence is needed to determine the brain regions associated with the action of these drugs and the biological pathways likely involved in their proposed effects.

Finally, while previous studies assessed and/or compared anticholinergic scales,[12,30,47 50] questions about their relevance and potential utility remain unanswered. Scales are most often constructed based on expert opinions rooted in past practice and propound established views that might be dated. The contents of anticholinergic scales may certainly reflect a facet of inappropriate prescribing that could help in medical decision-making. However, their heterogeneity and lack of a clear potency–outcome relationship point to an urgent need for reappraisal.

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CONFLICTS OF INTEREST

REM has received consulting fees from the Epigenetic Clock Development Foundation and speaker fees from Illumina. TCR has received fees for medicolegal work from private solicitors. SRC has received speaker fees from the Society of Biological Psychiatry. GMT has received consulting fees for grants funded by the NIH. JM has nothing to disclose.

REFERENCES

1. Livingston G, Huntley J, Sommerlad A, et al. Dementia prevention, intervention, and care: 2020 report of the Lancet Commission. *The Lancet*. 2020;396(10248):413-446.
2. Wu YT, Beiser AS, Breteler MMB, et al. The changing prevalence and incidence of dementia over time - current evidence. *Nat Rev Neurol*. 2017;13(6):327-339. https://doi.org/10.1038/nrneurol.2017.63
3. Hampel H, Mesulam MM, Cuello AC, et al. The cholinergic system in the pathophysiology and treatment of Alzheimer's disease. *Brain*. 2018;141(7):1917-1933. https://doi.org/10.1093/brain/awy132
4. Taylor-Rowan M, Edwards S, Noel-Storr AH, et al. Anticholinergic burden (prognostic factor) for prediction of dementia or cognitive decline in older adults with no known cognitive syndrome. *Cochrane Database Syst Rev*. 2021;5:CD013540. https://doi.org/10.1002/14651858.CD013540.pub2
5. Zheng YB, Shi L, Zhu XM, et al. Anticholinergic drugs and the risk of dementia: A systematic review and meta-analysis. *Neurosci Biobehav Rev*. 2021;127:296-306. https://doi.org/10.1016/j.neubiorev.2021.04.031
6. Coupland CAC, Hill T, Dening T, Morriss R, Moore M, Hippisley-Cox J. Anticholinergic drug exposure and the risk of dementia: a nested case-control study. *JAMA Intern Med*. 2019;179(8):1084-1093. https://doi.org/10.1001/jamainternmed.2019.0677
7. Richardson K, Fox C, Maidment I, et al. Anticholinergic drugs and risk of dementia: case-control study. *BMJ*. 2018;361:k1315. https://doi.org/10.1136/bmj.k1315
8. Andre L, Gallini A, Montastruc F, et al. Association between anticholinergic (atropinic) drug exposure and cognitive function in longitudinal studies among individuals over 50 years old: a systematic review. *Eur J Clin Pharmacol*. 2019;75(12):1631-1644. https://doi.org/10.1007/s00228-019-02744-8
9. Pieper NT, Grossi CM, Chan WY, et al. Anticholinergic drugs and incident dementia, mild cognitive impairment and cognitive decline: a meta-analysis. *Age Ageing*. 2020;49(6):939-947. https://doi.org/10.1093/ageing/afaa090
10. Rudd KM, Raehl CL, Bond CA, Abbruscato TJ, Stenhouse AC. Methods for assessing drug-related anticholinergic activity. *Pharmacotherapy*. 2005;25(11):1592-1601. https://doi.org/10.1592/phco.2005.25.11.1592
11. Naples JG, Marcum ZA, Perera S, et al. Concordance between anticholinergic burden scales. *J Am Geriatr Soc*. 2015;63(10):2120-4. https://doi.org/10.1111/jgs.13647
12. Welsh TJ, van der Wardt V, Ojo G, Gordon AL, Gladman JRF. Anticholinergic drug burden tools/scales and adverse outcomes in different clinical settings: a systematic review of reviews. *Drugs Aging*. 2018;35(6):523-538. https://doi.org/10.1007/s40266-018-0549-z
13. Dmochowski RR, Thai S, Iglay K, et al. Increased risk of incident dementia following use of anticholinergic agents: a systematic literature review and meta-analysis. *Neurourol Urodyn*. 2021;40(1):28-37. https://doi.org/10.1002/nau.24536
14. Sudlow C, Gallacher J, Allen N, et al. UK biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. *PLoS Med*. 2015;12(3):e1001779. https://doi.org/10.1371/journal.pmed.1001779
15. NHS Digital Trud. Accessed February 9th 2022, 2022. https://isd.digital.nhs.uk
16. Ancelin ML, Artero S, Portet F, Dupuy AM, Touchon J, Ritchie K. Non-degenerative mild cognitive impairment in elderly people and use of anticholinergic drugs: longitudinal cohort study. *BMJ*. 2006;332(7539):455-9. https://doi.org/10.1136/bmj.38740.439664.DE
17. Boustani M, Campbell N, Munger S, Maidment I, Fox C. Impact of anticholinergics on the aging brain: a review and practical application. *Aging Health*. 2008;4(3):311-320. https://doi.org/10.2217/1745509X.4.3.311

18. Cancelli I, Valentinis L, Merlino G, Valente M, Gigli GL. Drugs with anticholinergic properties as a risk factor for psychosis in patients affected by Alzheimer's disease. *Clin Pharmacol Therapeut*. 2008;84(1):63-68. https://doi.org/10.1038/sj.clpt.6100435
19. Carnahan RM, Lund BC, Perry PJ, Pollock BG, Culp KR. The Anticholinergic Drug Scale as a measure of drug-related anticholinergic burden: associations with serum anticholinergic activity. *J Clin Pharmacol*. 2006;46(12):1481-6. https://doi.org/10.1177/0091270006292126
20. Chew ML, Mulsant BH, Pollock BG, et al. Anticholinergic activity of 107 medications commonly used by older adults. *J Am Geriatr Soc*. 2008;56(7):1333-41. https://doi.org/10.1111/j.1532-5415.2008.01737.x
21. Durán CE, Azermai M, Vander Stichele RH. Systematic review of anticholinergic risk scales in older adults. *Eur J Clin Pharmacol*. 2013;69(7):1485-96. https://doi.org/10.1007/s00228-013-1499-3
22. Ehrt U, Broich K, Larsen JP, Ballard C, Aarsland D. Use of drugs with anticholinergic effect and impact on cognition in Parkinson's disease: a cohort study. *J Neurol Neurosurg Psychiatry*. 2010;81(2):160-5. https://doi.org/10.1136/jnnp.2009.186239
23. Han L, McCusker J, Cole M, Abrahamowicz M, Primeau F, Élie M. Use of medications with anticholinergic effect predicts clinical severity of delirium symptoms in older medical inpatients. *Archi Intern Med*. 2001;161(8):1099-1105. https://doi.org/10.1001/archinte.161.8.1099
24. Kiesel EK, Hopf YM, Drey M. An anticholinergic burden score for German prescribers: score development. *BMC Geriatr*. 2018;18(1):239. https://doi.org/10.1186/s12877-018-0929-6
25. Rudolph JL, Salow MJ, Angelini MC, McGlinchey RE. The anticholinergic risk scale and anticholinergic adverse effects in older persons. *Arch Intern Med*. 2008;168(5):508-513. https://doi.org/10.1001/archinternmed.2007.106
26. Sittironnarit G, Ames D, Bush AI, et al. Effects of anticholinergic drugs on cognitive function in older Australians: results from the AIBL study. *Dement Geriatr Cogn Disord*. 2011;31(3):173-8. https://doi.org/10.1159/000325171
27. Mur J, Cox SR, Marioni RE, Muniz-Terrera G, Russ TC. Increase in anticholinergic burden from 1990 to 2015: age-period-cohort analysis in UK biobank. *Br J Clin Pharmacol*. 2021;https://doi.org/10.1111/bcp.15045
28. Bishara D, Harwood D, Sauer J, Taylor DM. Anticholinergic effect on cognition (AEC) of drugs commonly used in older people. *Int J Geriatr Psychiatry*. 2017;32(6):650-656. https://doi.org/10.1002/gps.4507
29. Briet J, Javelot H, Heitzman E, et al. The anticholinergic impregnation scale: towards the elaboration of a scale adapted to prescriptions in French psychiatric settings. *Thérapie*. 2017;72:427-437. https://doi.org/10.1016/j.therap.2016.12.010
30. Lisibach A, Benelli V, Ceppi MG, Waldner-Knogler K, Csajka C, Lutters M. Quality of anticholinergic burden scales and their impact on clinical outcomes: a systematic review. *Eur J Clin Pharmacol*. 2021;77(2):147-162. https://doi.org/10.1007/s00228-020-02994-x
31. Sumukadas D, McMurdo ME, Mangoni AA, Guthrie B. Temporal trends in anticholinergic medication prescription in older people: repeated cross-sectional analysis of population prescribing data. *Age Ageing*. 2014;43(4):515-21. https://doi.org/10.1093/ageing/aft199
32. Anticholinergic Cognitive Burden Scale: 2012 Update. 2012. www.agingbraincare.org
33. BNF. National Institute for Health and Care Excellence (NICE). Accessed February 9th 2022, 2022. https://bnf.nice.org.uk
34. ATC/DDD Index 2022. WHO Collaborating Centre for Drug Statistics Methodology. Updated December 14th 2021. Accessed February 9th 2022, 2022. https://www.whocc.no/atc_ddd_index/
35. Townsend P. Deprivation. *J Social Policy*. 1987;16(2):125-146. https://doi.org/10.1017/s0047279400020341
36. Hanlon P, Quinn TJ, Gallacher KI, et al. Assessing Risks of Polypharmacy Involving Medications With Anticholinergic Properties. *Ann Fam Med*. 2020;18(2):148-155. https://doi.org/10.1370/afm.2501
37. Dimitris R. *Joint Models for Longitudinal and Time-to-Event Data With Applications in R*. 1st ed. Chapman and Hall/CRC; 2012.
38. Gray SL, Anderson ML, Dublin S, et al. Cumulative use of strong anticholinergics and incident dementia: a prospective cohort study. *JAMA Intern Med*. 2015;175(3):401-7. https://doi.org/10.1001/jamainternmed.2014.7663
39. Diniz BS, Butters MA, Albert SM, Dew MA, Reynolds CF, 3rd. Late-life depression and risk of vascular dementia and Alzheimer's disease: systematic review and meta-analysis of community-based cohort studies. *Br J Psychiatry*. 2013;202(5):329-35. https://doi.org/10.1192/bjp.bp.112.118307
40. Fischer CE, Aguera-Ortiz L. Psychosis and dementia: risk factor, prodrome, or cause? *Int Psychogeriatr*. 2018;30(2):209-219. https://doi.org/10.1017/S1041610217000874
41. Haijma SV, Van Haren N, Cahn W, Koolschijn PC, Hulshoff Pol HE, Kahn RS. Brain volumes in schizophrenia: a meta-analysis in over 18 000 subjects. *Schizophr Bull*. 2013;39(5):1129-38. https://doi.org/10.1093/schbul/sbs118
42. Kempton MJ., Salvador Z, Munafo MR., et al. Structural neuroimaging studies in major depressive disorder. Meta analysis and comparison with bipolar disorder. *Archives of General Psychiatry*. 2011;68(7):675-690. https://doi.org/10.1001/archgenpsychiatry.2011.60
43. Sen A, Capelli V, Husain M. Cognition and dementia in older patients with epilepsy. *Brain*. 2018;141(6):1592-1608. https://doi.org/10.1093/brain/awy022
44. Wilkinson T, Schnier C, Bush K, et al. Identifying dementia outcomes in UK Biobank: a validation study of primary care, hospital admissions and mortality data. *Eur J Epidemiol*. 2019;34(6):557-565. https://doi.org/10.1007/s10654-019-00499-1
45. Fry A, Littlejohns TJ, Sudlow C, et al. Comparison of sociodemographic and health-related characteristics of UK Biobank Participants with those of the general Population. *Am J Epidemiol*. 2017;186(9):1026-1034. https://doi.org/10.1093/aje/kwx246
46. Andrade C. Anticholinergic drug exposure and the risk of dementia: there is modest evidence for an association but not for causality. *J Clin Psychiatry*. 2019;80(4)https://doi.org/10.4088/JCP.19f13000
47. Kersten H, Wyller TB. Anticholinergic drug burden in older people's brain - how well is it measured? *Basic Clin Pharmacol Toxicol*. 2014;114(2):151-9. https://doi.org/10.1111/bcpt.12140
48. Lozano-Ortega G, Johnston KM, Cheung A, et al. A review of published anticholinergic scales and measures and their applicability in database analyses. *Arch Gerontol Geriatr*. 2020;87:103885. https://doi.org/10.1016/j.archger.2019.05.010
49. Salahudeen MS, Duffull SB, Nishtala PS. Anticholinergic burden quantified by anticholinergic risk scales and adverse outcomes in older people: a systematic review. *BMC Geriatr*. 2015;15:31. https://doi.org/10.1186/s12877-015-0029-9
50. Salahudeen MS, Hilmer SN, Nishtala PS. Comparison of anticholinergic risk scales and associations with adverse health outcomes in older people. *J Am Geriatr Soc*. 2015;63(1):85-90. https://doi.org/10.1111/jgs.13206

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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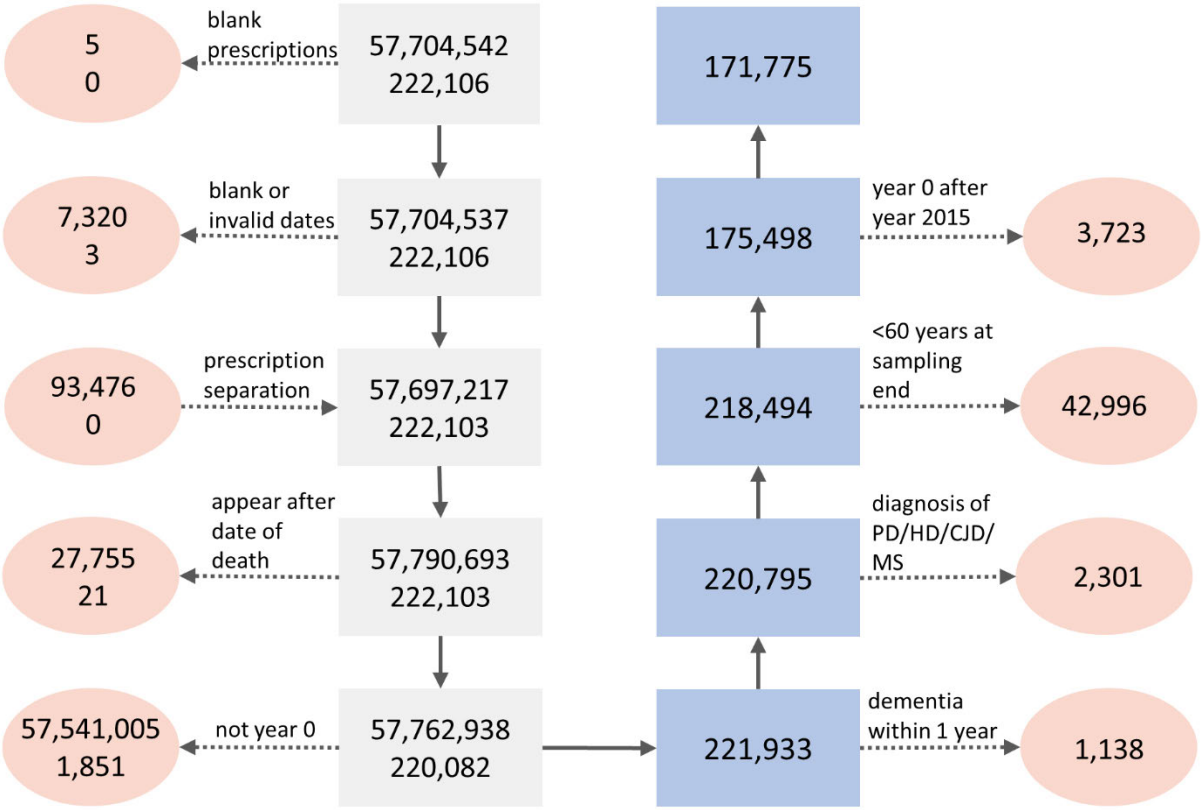
5.3 Conclusion

This chapter has presented the results of analyses exploring the associations between AChB and dementia. In contrast to most previous studies that probed anticholinergic use using a single anticholinergic scale, I explored the effects of several scales and differentiated between sources of AChB by studying individual drug classes. I found AChB according to most anticholinergic scales and the longitudinal slope of AChB to weakly associate with the risk of dementia. Like previous studies, only some drug classes, especially drugs to treat depression and epilepsy were associated with dementia. However, I did not find an effect for antipsychotics and urological drugs, despite the association consistently reported in the literature. Moreover, the effect sizes were generally smaller than most previously reported. These results highlight that anticholinergic use is a risk factor for dementia, but that differences in this effect exist between classes of drugs.

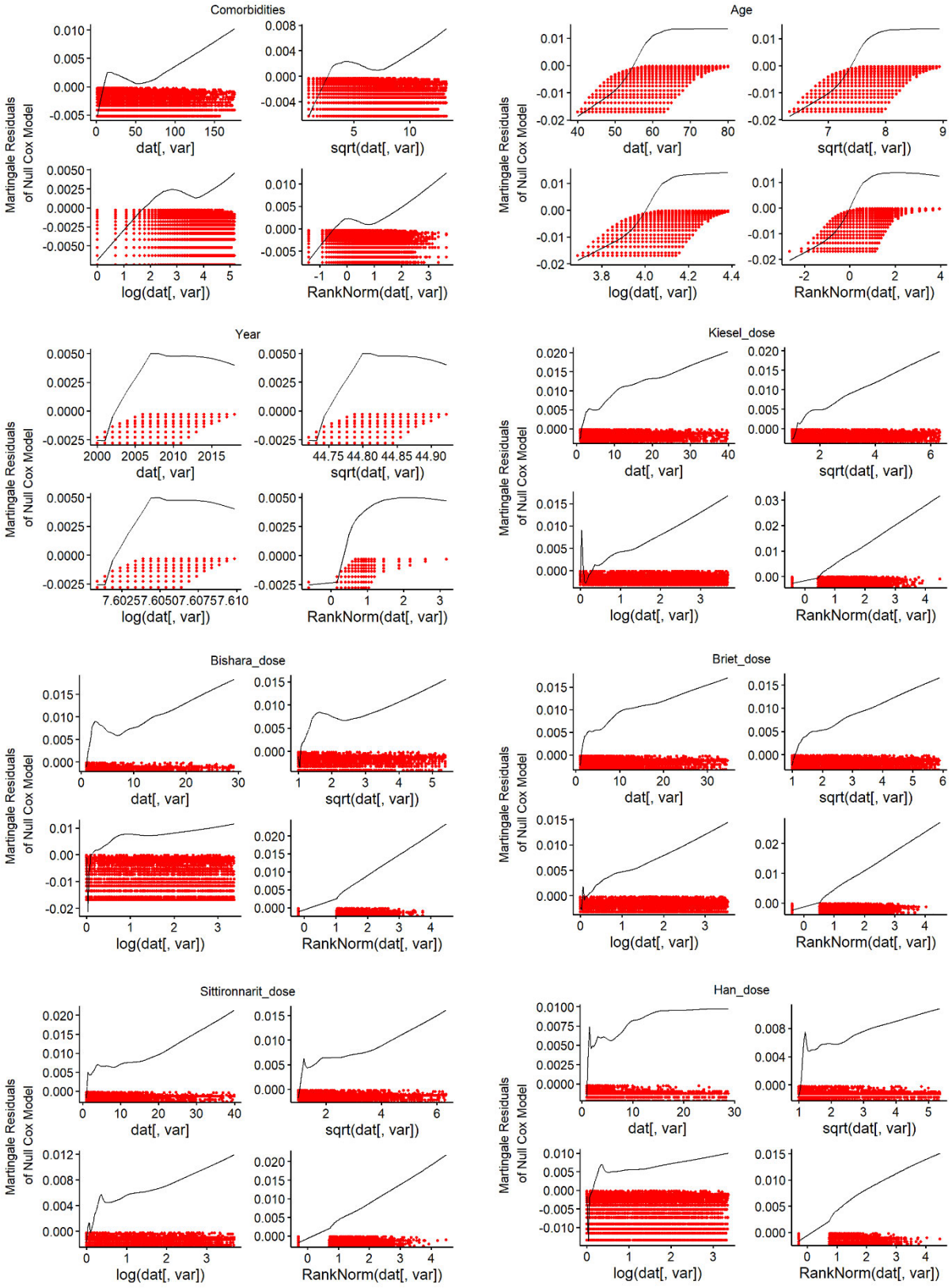
5.3.1 Supplementary material

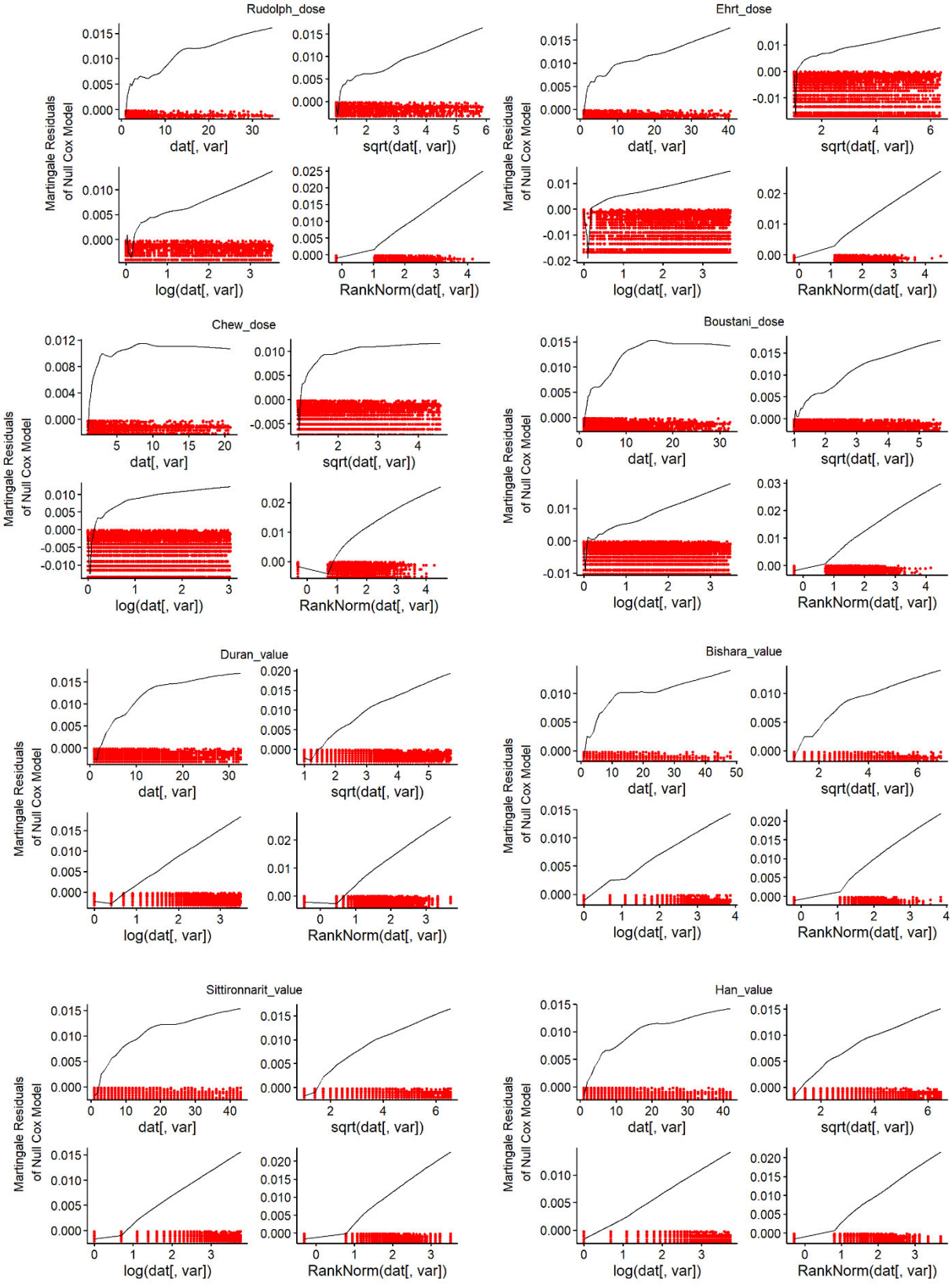
This section contains all Supplementary material that accompanies the manuscript presented in this chapter except Supplementary Table 3. In the published version, Supplementary Table 1 appears as a separate spreadsheet. Supplementary Table 3 was not included here, as it was originally made available as a spreadsheet, the width of which rendered it unpractical for publication in a word editor. It is available in full at https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9005668/bin/TRC2-8-e12290-s003.docx.

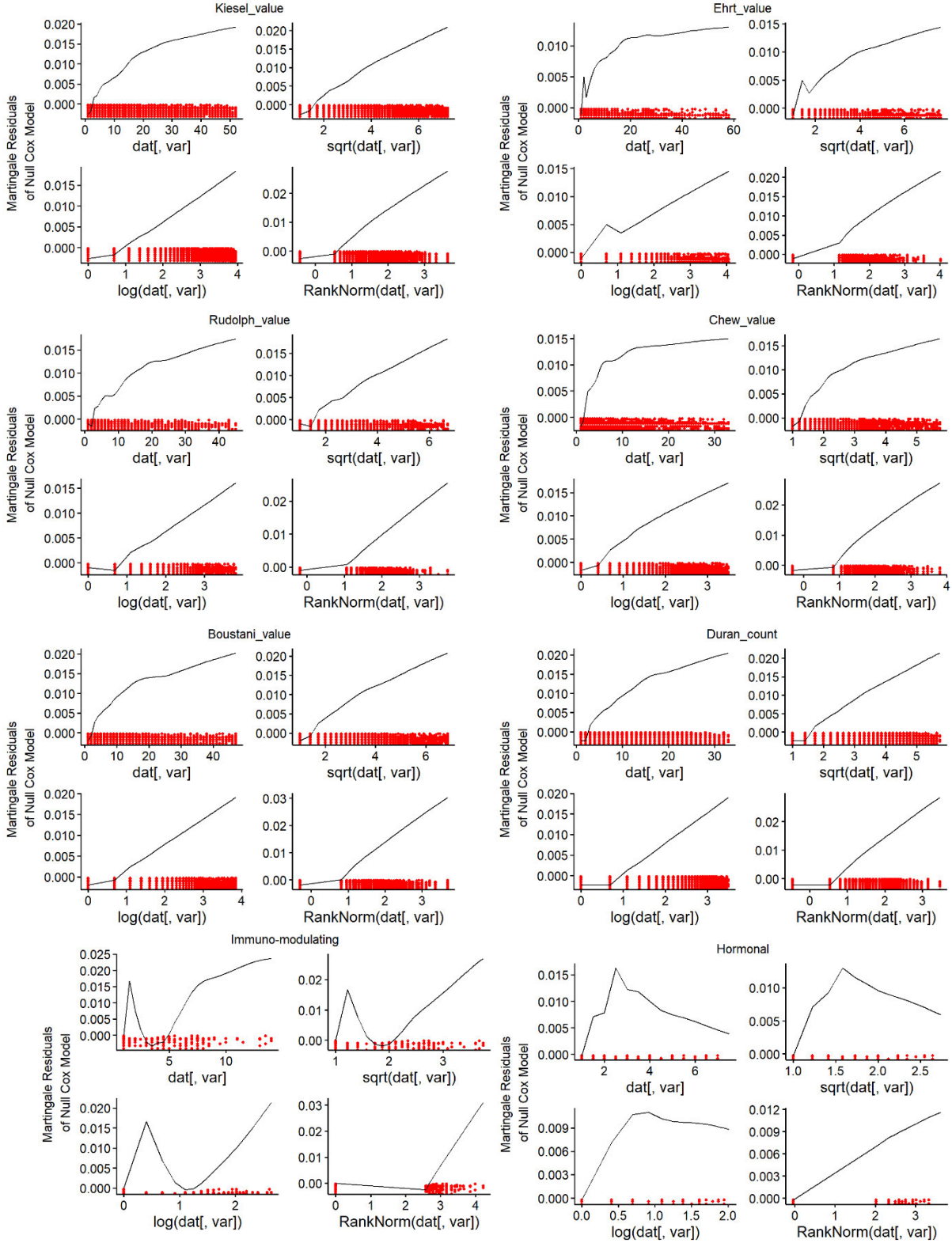
Supplementary Figure 1: The data cleaning procedure. The grey boxes contain the number of prescriptions (top row in the boxes) and participants (bottom row in the boxes) when a unit of observation was a single prescription; the blue boxes contain the number of prescriptions/participants (both take the same value) when the data was formatted so that the unit of observation was the yearly AChB for a participant in year 0. The orange ellipses contain numbers of prescriptions (top row in the ellipses) and participants (bottom row in the ellipses) that were removed at each data-cleaning step. The data cleaning steps include: (1) removal of prescription entries that were blank (i.e., did not list a drug), (2) removal of prescriptions without dates or with invalid dates, (3) the "separation" of prescriptions with multiple anticholinergic compounds into single entries, (4) removal of prescriptions occurring after the recorded dates of death, (5) removal of prescriptions in years other than year 0, (6) removal of participants diagnoses with dementia prior to year 0 or within one year of year 0, (7) removal of participants diagnosed with Parkinson's disease, Huntington's disease, Creutzfeldt-Jacob disease, or multiple sclerosis, (8) removal of participants younger than 60 at the end of sampling or when diagnosed with dementia, (9) removal of participants for whom year 0 was prior to 2015. Please note that in the third step, when prescriptions were "separated" so that prescriptions originally containing several anticholinergic compounds were divided into separate prescriptions (with a single anticholinergic compound each), the number of observations in the dataset effectively *increased*.

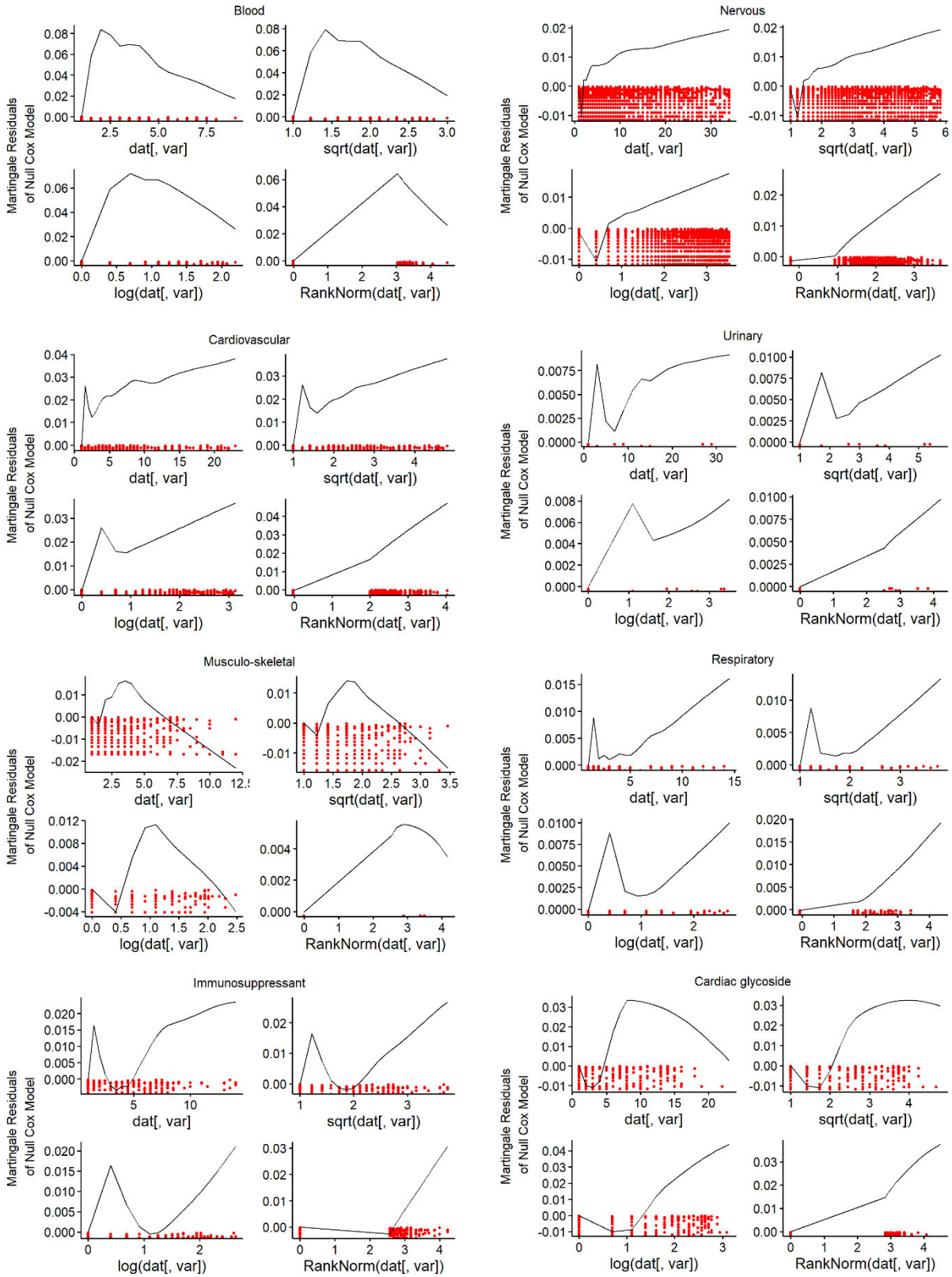


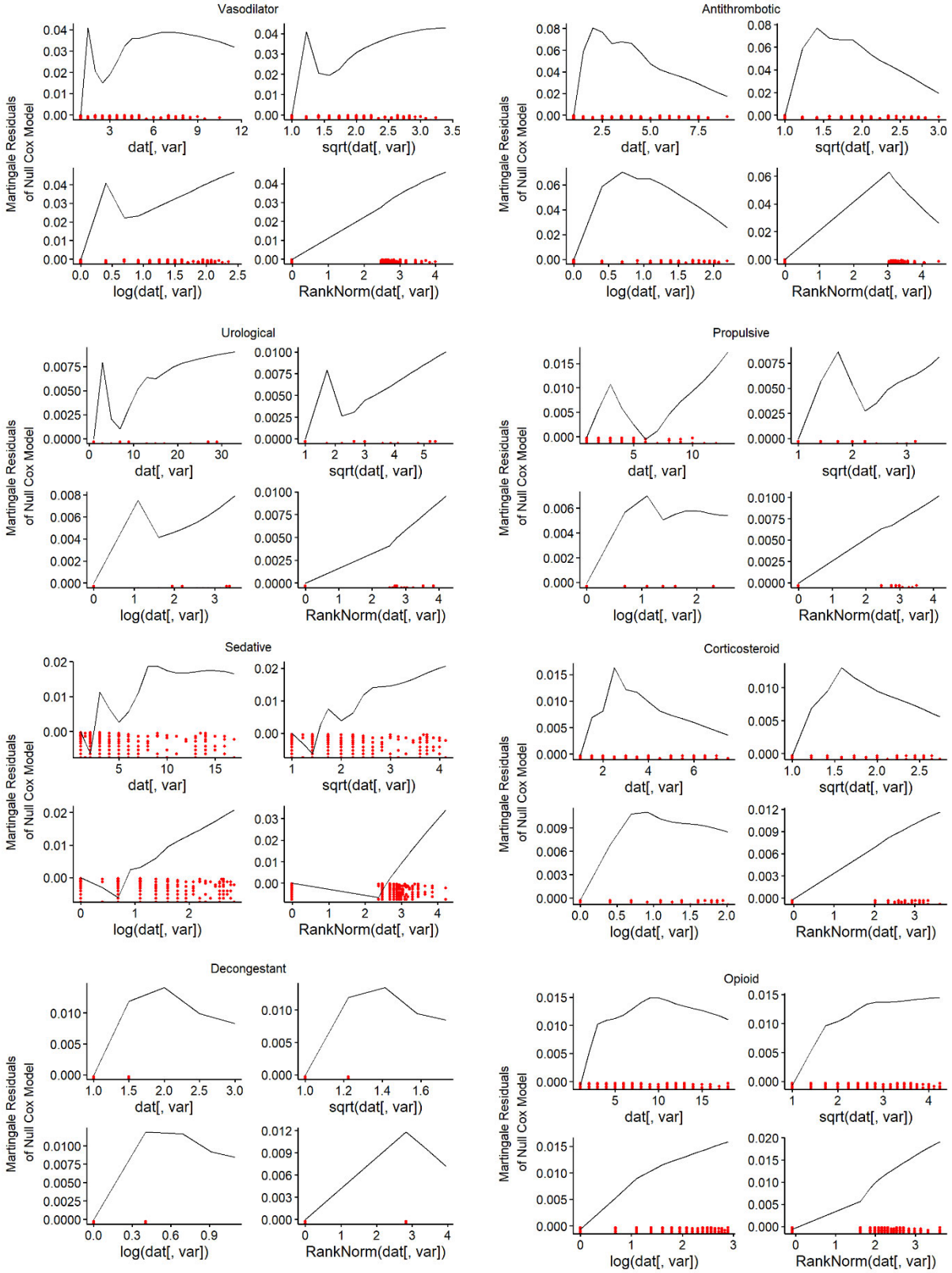
Supplementary Figure 2: Martingale residuals plotted against individual continuous covariates. Depicted are only those covariates for which this relationship was judged not to be linear before transforming. For each covariate, four plots are depicted, where the covariate is either untransformed (top left), or square-root-, log- , or rank-based-inverse-normal transformed.

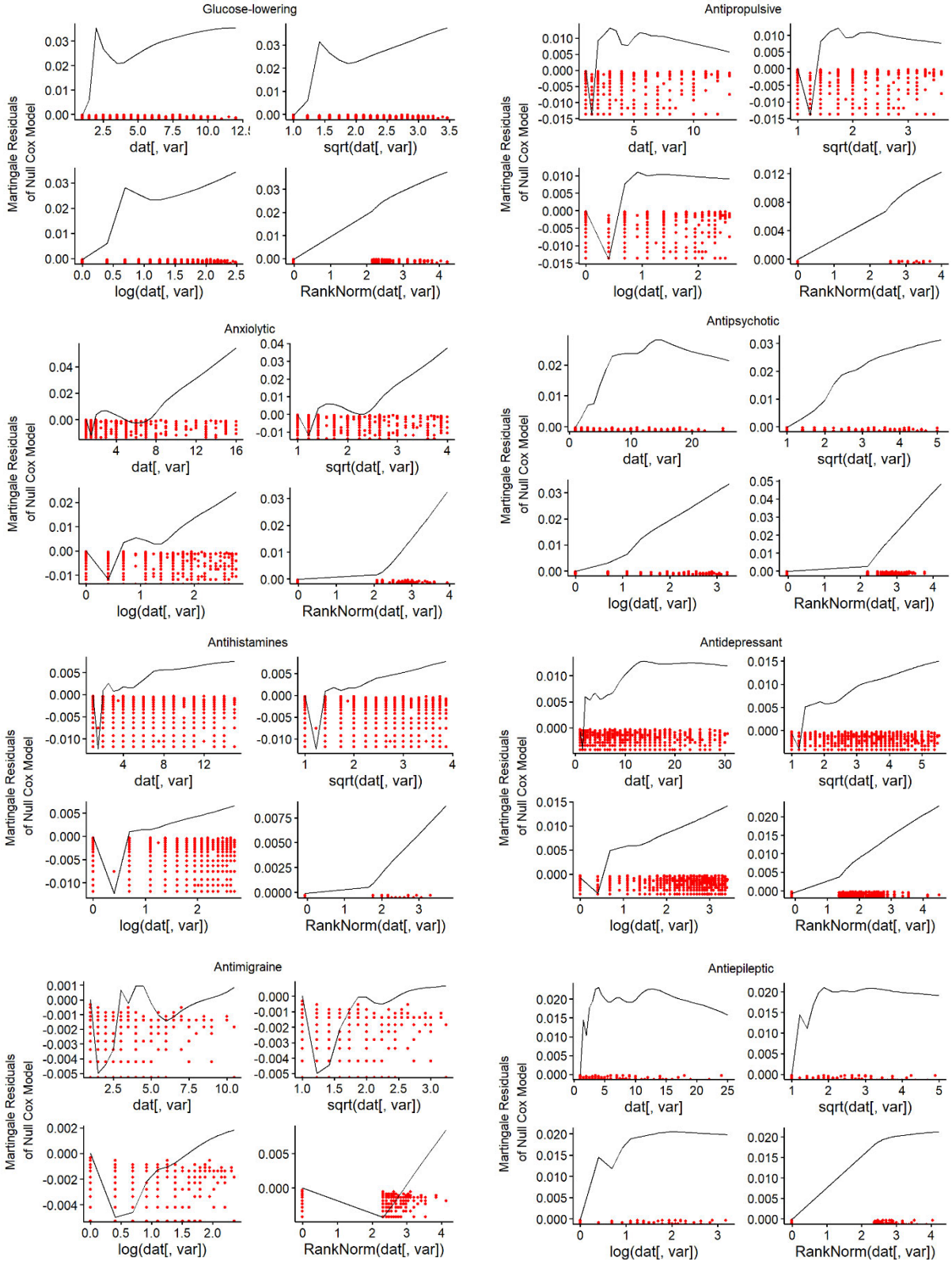



Rudolph_dose
Ehrt_dose
Chew_dose
Boustani_dose
Duran_value
Bishara_value
Sittironnarit_value
Han_value
Martingale Residuals of Null Cox Model
dat[, var]
sqrt(dat[, var])
log(dat[, var])
RankNorm(dat[, var])

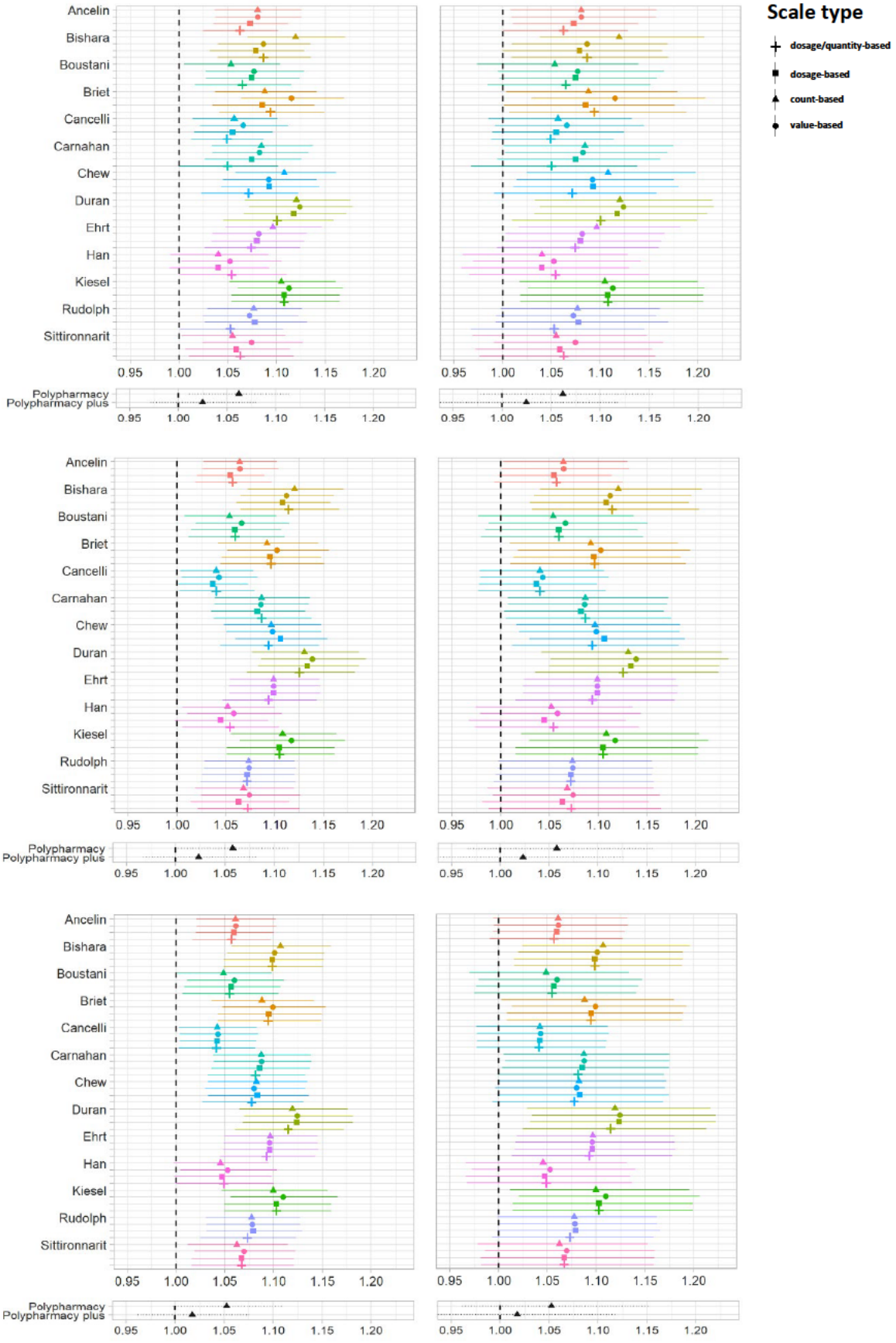

Kiesel_value
Ehrt_value
Rudolph_value
Chew_value
Boustani_value
Duran_count
Immuno-modulating
Hormonal
Martingale Residuals of Null Cox Model
dat[, var]
sqrt(dat[, var])
log(dat[, var])
RankNorm(dat[, var])


Blood
Nervous
Cardiovascular
Urinary
Musculo-skeletal
Respiratory
Immunosuppressant
Cardiac glycoside
Martingale Residuals of Null Cox Model
dat[, var]
sqrt(dat[, var])
log(dat[, var])
RankNorm(dat[, var])

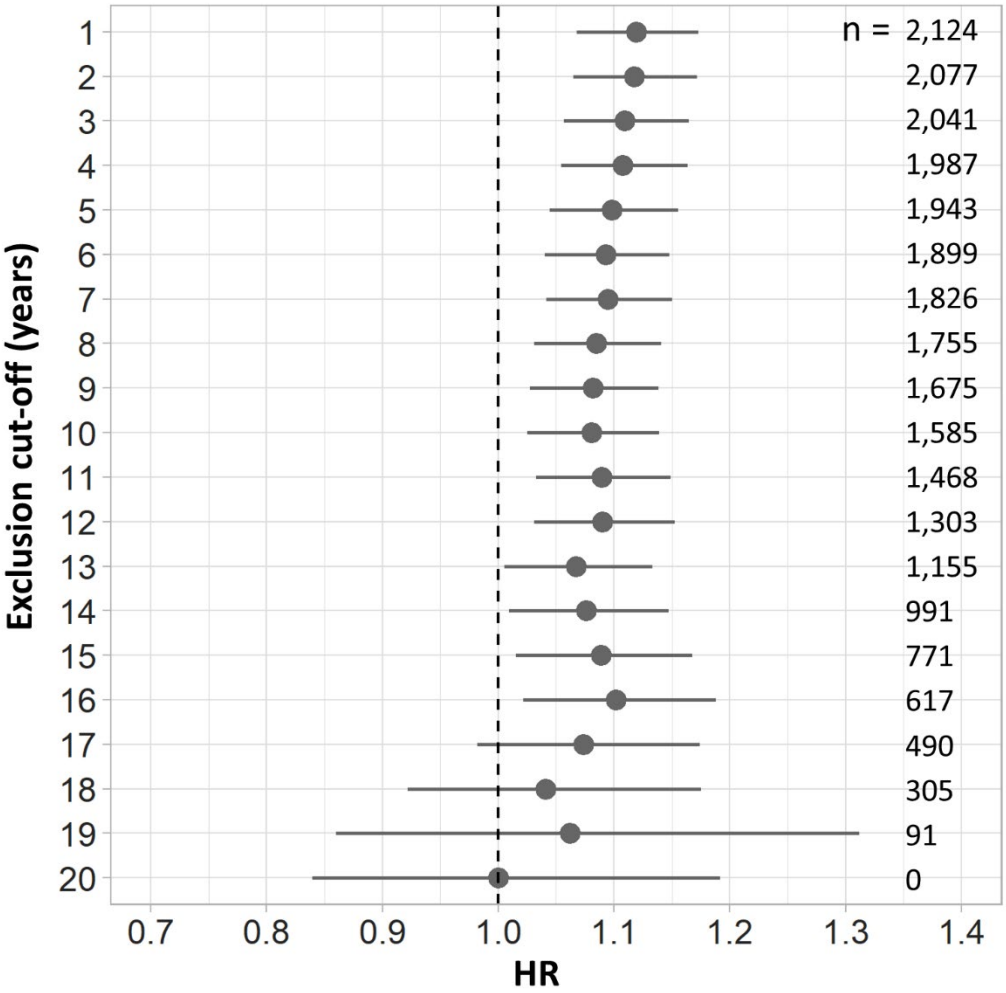

Vasodilator
Antithrombotic
Urological
Propulsive
Sedative
Corticosteroid
Decongestant
Opioid
Martingale Residuals of Null Cox Model
dat[, var]
sqrt(dat[, var])
log(dat[, var])
RankNorm(dat[, var])


Glucose-lowering
Antipropulsive
Anxiolytic
Antipsychotic
Antihistamines
Antidepressant
Antimigraine
Antiepileptic
Martingale Residuals of Null Cox Model
dat[, var]
sqrt(dat[, var])
log(dat[, var])
RankNorm(dat[, var])

Supplementary Figure 3: HRs for the association between AChB and dementia (top panels) and drug count and dementia (bottom panels) when AChB is scaled (**top row**), log-transformed and scaled (**middle row**), and transformed using the rank-inverse normal transformation and scaled (**bottom row**). The colour indicates the anticholinergic scales used for the calculation of the AChB; the symbols and line types indicate the type of scale computation used. The left and right columns show uncorrected results and results corrected for multiple testing, respectively.



Supplementary Figure 4: Association between AChB according to Dúran et al. (2013) and dementia, with different exclusion cut-offs for period of dementia diagnosis. Participants in the sample were diagnosed with dementia at different time points after year 0. If the diagnosis for a participant was established within a certain cut-off period after year 0, that participant was removed from the sample before analysis. The numbers on the y-axis specify this cut-off period (years after year 0); the x-axis represents the effect size (HR). The numbers on the right indicate the numbers of dementia cases for each model: as the exclusion period increases, more participant get excluded and the sample size decreases.



Supplementary Table 2: Anticholinergics scales identified in the present study. We considered anticholinergic scales that were available as complete lists of drugs, scored each drug for its anticholinergic potency, and did not utilize dosage. Grey shading indicates that the scale was not considered for further analysis. For two scales[1,2], updated versions were used (Aging Brain Care, 2012; Carnahan, 2014, personal communication on 21.10.2019). One scale[3] was modified to include newer drugs from the UK market as has been done before[4]. Some drugs from one scale[5] were categorised as "drugs with improbable or no anticholinergic action". For our analyses, the drugs in that category were scored with 0.5. Modified from Mur et al. (2022)[23].

Surname of first author	Scale name	Year of publication	Reason for exclusion
Summers[6]	Drug Risk Number (DRN)	1978	Outdated (based on the date of publication and on new scales developed on its basis).
Han[7,8]	Clinician-rated Anticholinergic Scale (CrAS)	2001	
Aizenberg[9]	Anticholinergic Burden Score (ABS)	2002	Publicly unavailable and no response from lead author to two email requests within a year.
Minzenberg[10]	n.a.	2004	Based on a reference compound.
Ancelin[11]	Anticholinergic Burden Classification (ABC) scale	2006	
Carnahan[1]	Anticholinergic Drug Scale (ADS)	2006	
Hilmer[12]	Drug Burden Index (DBI)	2007	Required information on drug dosage.
Chew[13]	Anticholinergic Activity Scale (AAS)	2008	
Cancelli[14]	n.a.	2008	
Rudolph[3]	Anticholinergic Risk Scale (ARS)	2008	
Ehrt[15]	Revised Anticholinergic Activity Scale (AAS-r)	2010	
Sittironnarit[16]	Anticholinergic Loading Scale (ALS)	2011	
Boustani[2]	Anticholinergic Cognitive Burden (ACB)	2008	
Whalley[17]	n.a.	2012	Unavailable in full.
Durán[5]	n.a.	2013	
Faure[18]	Drug Burden Index, International Version (DBI-WHO)	2014	Required information on drug dosage.
Klamer[19]	MARANTE	2017	Required information on drug dosage.
Bishara[20]	Anticholinergic effect on cognition (AEC) scale	2017	
Briet[21]	Anticholinergic impregnation scale	2017	
Kiesel[22]	n.a.	2018	

Supplementary Table 4: Numbers of participants diagnosed with dementia in different groups of the sample.

Group	N	N cases (% of group)
All	171,775	2,124 (1.2)
Sex		
Male	77,465	1,139 (1.5)
Female	94,310	985 (1.0)
Age group		
60-64	34,050	33 (0.10)
65-69	37,306	149 (0.40)
70-74	51,436	534 (1.04)
75-79	41,810	1,134 (2.7)
>= 80	7,173	274 (3.8)

Supplementary Table 5: Descriptive statistics of variables used in the models, presented separately for participants diagnosed with dementia and those not diagnosed with dementia. Data providers are suppliers of computer systems for general practitioners in the UK. The provide the IT framework for general practice surgeries to store information on patients, diagnoses, and prescriptions. The division of data providers in the UK is partially determined by the region in which a general practice surgery is located: England, Scotland, or Wales. *The total number of prescriptions was used along the number of anticholinergic drugs to calculate the scale-specific non-anticholinergic drug count.

		Dementia diagnosis	No dementia diagnosis
Participant count		2,124	169,631
Variable	**Level**	**Median (IQR) or n (%)**	
Age		59 (5)	54 (10)
Sex	Female	985 (46.4)	93,325 (55.0)
Education	No graduate degree	1,672 (80.5)	116,519 (69.6)
Deprivation		-1.96 (4.7)	-2.30 (3.8)
Alcohol consumption	Daily or almost daily	475 (22.5)	35,514 (21.0)
	Three or four times a week	382 (18.1)	39,365 (23.3)
	Once or twice a week	461 (21.8)	43,354 (25.6)
	Once to three times a month	190 (9.0)	17,959 (10.6)
	Only special occasions	317 (15.0)	19,356 (11.4)
	Never	289 (13.7)	13,735 (8.1)
Smoking	Current smoker	234 (11.1)	16,178 (9.6)
	Previous smoker	935 (44.4)	62,437 (37.0)
	Non-smoker	935 (44.4)	90,156 (53.4)
Physical activity	Strenuous	80 (4.3)	13,497 (8.6)
	Moderate	1105 (59.9)	102,016 (64.7)
	Light	661 (35.8)	42,116 (26.7)
BMI	<18.5	13 (0.62)	755 (0.45)
	18.5-25	606 (28.8)	51,043 (30.2)
	25-30	877 (41.7)	73,315 (43.4)
	30-35	431 (20.5)	31,376 (18.6)
	35-40	129 (6.1)	8,941 (5.3)
	>40	45 (2.1)	3,327 (2.0)
Data provider	England (Vision)	150 (7.1)	13,886 (8.2)
	Scotland	40 (1.9)	18,718 (11.0)
	England (TPP)	1,852 (87.2)	121,281 (71.5)
	Wales	82 (3.9)	15,766 (9.3)
Prior depression		257 (12.1)	12,879 (7.6)
Prior stroke		66 (3.1)	1,532 (0.90)
Prior diabetes		184 (8.7)	3,850 (2.3)
Prior hypercholesterolemia		145 (6.8)	4,756 (2.8)
Prior hypertension		415 (19.5)	15,737 (9.3)
Number of prior comorbidities		27 (44)	18 (40)
Total number of prescriptions*		7 (19)	3 (12)
APOE carrier	ε2	191 (7.8)	21,465 (13.0)
	ε3	920 (44.6)	101,820 (61.5)
	ε4	981 (47.6)	41,218 (25.5)

Supplementary Table 6: Frequency of anticholinergic prescribing in the sample from 2000 to 2015 and in year 0 according to each anticholinergic scale studied.

Scale	Number of distinct anticholinergic drugs in the sample	Number of anticholinergic drugs (% of prescriptions)	Number of distinct anticholinergic drugs in the sample in year 0	Number of anticholinergic drugs in year 0 (% of prescriptions)	Anticholinergic prescriptions per person in year 0
Ancelin	21	1,086,739 (2.5)	20	42,068 (2.6)	0.24
Bishara	58	2,876,150 (6.6)	56	126,195 (7.9)	0.72
Boustani	90	5,272,868 (12.2)	87	225,409 (14.1)	1.28
Briet	121	7,041,395 (16.3)	117	294,881 (18.4)	1.68
Cancelli	14	1,700,948 (3.9)	13	63,609 (4.0)	0.36
Carnahan	111	3,761,500 (8.7)	105	165,838 (10.4)	0.94
Chew	36	4,739,876 (11.0)	34	177,101 (11.1)	1.00
Durán	147	8,257,133 (19.1)	139	320,792 (20.0)	1.83
Ehrt	24	3,079,302 (7.1)	23	119,600 (7.5)	0.68
Han	54	4,378,190 (10.1)	52	193,108 (12.1)	1.10
Kiesel	141	9,495,193 (22.0)	136	371,757 (23.2)	2.12
Rudolph	61	2,201,774 (5.1)	59	102,540 (6.4)	0.58
Sittironnarit	47	5,129,912 (11.9)	46	207,085 (12.9)	1.18

Supplementary Table 7 (see also next page): HRs for scaled (mean=0; standard deviation=1) numerical variables in the Cox proportional risks model predicting the risk of dementia. Each row depicts the effect of anticholinergic burden according to a different anticholinergic scale. The different columns depict HRs for different transformations of the data.

Scale	Type	Untransformed		Log		Rank-based inverse-normal		n missing
		HR	99.9% CI	HR	99.9% CI	HR	99.9% CI	
Ancelin	count	1.08	1.01 - 1.16	1.06	1.00 - 1.13	1.07	0.99 - 1.13	23,367
	value	1.08	1.01 - 1.16	1.06	1.00 - 1.13	1.07	0.99 - 1.13	23,371
	dosage	1.07	1.01 - 1.14	1.05	1.00 - 1.11	1.07	0.99 - 1.13	23,934
	quantity	1.06	1.00 - 1.13	1.06	0.99 - 1.12	1.06	0.99 - 1.13	29,712
Bishara	count	1.12	1.04 - 1.21	1.12	1.04 - 1.21	1.07	1.02 - 1.20	23,472
	value	1.09	1.01 - 1.17	1.11	1.03 - 1.20	1.08	1.02 - 1.19	23,553
	dosage	1.08	1.00 - 1.16	1.11	1.03 - 1.19	1.08	1.02 - 1.19	24,130
	quantity	1.09	1.01 - 1.17	1.11	1.03 - 1.20	1.08	1.02 - 1.19	29,826
Boustani	count	1.05	0.97 - 1.14	1.05	0.98 - 1.14	1.10	0.97 - 1.13	23,525
	value	1.08	0.99 - 1.17	1.07	0.99 - 1.15	1.10	0.98 - 1.15	23,698
	dosage	1.07	1.00 - 1.16	1.06	0.98 - 1.14	1.11	0.98 - 1.14	24,424
	quantity	1.06	0.98 - 1.15	1.06	0.98 - 1.15	1.10	0.98 - 1.14	29,859
Briet	count	1.09	1.00 - 1.18	1.09	1.01 - 1.18	1.05	1.00 - 1.18	23,606
	value	1.12	1.03 - 1.21	1.10	1.02 - 1.19	1.05	1.01 - 1.19	23,784
	dosage	1.09	1.00 - 1.18	1.10	1.01 - 1.18	1.05	1.01 - 1.19	24,327
	quantity	1.09	1.01 - 1.19	1.10	1.01 - 1.19	1.05	1.01 - 1.19	29,935
Cancelli	count	1.06	0.99 - 1.13	1.04	0.98 - 1.11	1.09	0.98 - 1.11	23,387
	value	1.07	0.99 - 1.15	1.04	0.98 - 1.11	1.10	0.98 - 1.11	23,399
	dosage	1.05	0.99 - 1.12	1.04	0.98 - 1.10	1.10	0.98 - 1.11	23,966
	quantity	1.05	0.99 - 1.11	1.04	0.98 - 1.11	1.10	0.98 - 1.11	29,730
Carnahan	count	1.08	1.00 - 1.18	1.09	1.01 - 1.17	1.11	1.01 - 1.18	23,464
	value	1.08	1.00 - 1.17	1.09	1.01 - 1.17	1.12	1.01 - 1.18	23,571
	dosage	1.07	0.99 - 1.16	1.08	1.00 - 1.17	1.12	1.00 - 1.17	24,204
	quantity	1.05	0.97 - 1.14	1.09	1.01 - 1.17	1.12	1.00 - 1.17	29,947
Chew	count	1.11	1.02 - 1.20	1.10	1.02 - 1.18	1.08	1.00 - 1.17	23,497
	value	1.09	1.01 - 1.18	1.10	1.02 - 1.18	1.08	1.00 - 1.17	23,841
	dosage	1.09	1.01 - 1.18	1.11	1.03 - 1.19	1.08	1.00 - 1.17	24,447
	quantity	1.07	0.99 - 1.16	1.09	1.01 - 1.18	1.08	0.99 - 1.17	30,065
Durán	count	1.12	1.03 - 1.22	1.13	1.04 - 1.23	1.08	1.03 - 1.22	23,559
	value	1.12	1.04 - 1.22	1.14	1.05 - 1.23	1.09	1.03 - 1.22	23,768
	dosage	1.12	1.03 - 1.21	1.13	1.05 - 1.22	1.09	1.03 - 1.22	24,319
	quantity	1.10	1.01 - 1.20	1.13	1.04 - 1.22	1.09	1.02 - 1.21	29,896
Ehrt	count	1.10	1.02 - 1.18	1.10	1.02 - 1.18	1.04	1.02 - 1.18	23,412
	value	1.08	1.00 - 1.17	1.10	1.02 - 1.18	1.04	1.02 - 1.18	23,485
	dosage	1.08	1.00 - 1.16	1.10	1.02 - 1.18	1.04	1.02 - 1.18	24,091
	quantity	1.07	0.99 - 1.16	1.09	1.02 - 1.18	1.04	1.01 - 1.18	29,818
Han	count	1.04	0.96 - 1.13	1.05	0.97 - 1.14	1.09	0.97 - 1.13	23,577
	value	1.05	0.97 - 1.14	1.06	0.98 - 1.14	1.09	0.97 - 1.14	23,730
	dosage	1.04	0.96 - 1.13	1.04	0.97 - 1.13	1.10	0.97 - 1.13	24,270
	quantity	1.05	0.97 - 1.15	1.05	0.97 - 1.14	1.09	0.97 - 1.14	29,926
Kiesel	count	1.10	1.02 - 1.20	1.11	1.02 - 1.20	1.05	1.01 - 1.20	23,561
	value	1.11	1.03 - 1.21	1.12	1.03 - 1.21	1.06	1.02 - 1.21	23,750
	dosage	1.11	1.02 - 1.21	1.10	1.02 - 1.20	1.06	1.01 - 1.20	29,894
	quantity	1.11	1.02 - 1.21	1.10	1.02 - 1.20	1.05	1.01 - 1.20	29,894
Rudolph	count	1.08	1.00 - 1.16	1.07	1.00 - 1.15	1.10	1.00 - 1.16	23,487
	value	1.07	0.99 - 1.16	1.07	1.00 - 1.16	1.10	1.00 - 1.16	23,529
	dosage	1.08	0.99 - 1.17	1.07	0.99 - 1.15	1.10	1.00 - 1.17	24,060

	quantity	1.05	0.97 - 1.15	1.07	0.99 - 1.16	1.11	0.99 - 1.16	29,881
Sittironnarit	count	1.05	0.97 - 1.15	1.07	0.99 - 1.16	1.06	0.98 - 1.15	23,534
	value	1.07	0.99 - 1.17	1.07	0.99 - 1.16	1.06	0.99 - 1.16	23,678
	dosage	1.06	0.97 - 1.15	1.06	0.98 - 1.15	1.06	0.98 - 1.16	24,293
	quantity	1.06	0.98 - 1.16	1.07	0.99 - 1.16	1.06	0.98 - 1.16	30,097
Polypharmacy	count	1.06	0.94 - 1.12	1.06	0.97 - 1.16	1.05	0.95 - 1.15	24,103
Polypharmacy plus	count	1.02	0.98 - 1.15	1.02	0.93 - 1.13	1.02	0.92 - 1.12	24,103

Supplementary Table 8 (see also next page): HRs for scaled (mean=0; standard deviation=1) numerical variables in the Cox proportional risks model predicting the risk of dementia by value-based scales. Each row depicts the effect on dementia risk according to a different predictor. The estimates for the covariates in the different models (that used different anticholinergic scales) did not differ substantially from one another and are thus not depicted for each model separately. Instead, they are depicted as ranges: within the parentheses a range is given that was observed across all models. To fit model prerequisites, polypharmacy is transformed by taking the square root before scaling.

Variable	Level	HR	95% CI	99.9% CI
AChB		[1.02 , 1.12]	[1.00, 1.10] - [1.05, 1.15]	[0.94, 1.04] - [1.12, 1.21]
Polypharmacy		[1.01, 1.04]	[1.00, 1.03] - [1.03, 1.06]	[0.96, 1.00] - [1.04, 1.09]
Sex	Male	[1.44, 1.47]	[1.37, 1.39] - [1.52, 1.55]	[1.21, 1.23] - [1.72, 1.75]
Data provider	Scotland	[0.22, 0.24]	[0.18, 0.20] - [0.28, 0.30]	[0.11, 0.12] - [0.45, 0.48]
	England (TPP)	[1.40, 1.45]	[1.26, 1.30] - [1.56, 1.61]	[0.99, 1.03] - [1.98, 2.04]
	Wales	[0.52, 0.53]	[0.44, 0.45] - [0.61, 0.63]	[0.31, 0.32] - [0.87, 0.89]
Age		[1.22, 1.22]	[1.21, 1.21] - [1.22, 1.23]	[1.19, 1.20] - [1.24, 1.25]
Year		[0.98, 0.98]	[0.97, 0.97] - [0.99, 0.99]	[0.94, 0.95] - [1.01, 1.02]
Education	Graduate degree	[0.81, 0.82]	[0.76, 0.77] - [0.87, 0.88]	[0.66, 0.67] - [1.00, 1.01]
Deprivation		[1.03, 1.03]	[1.02, 1.02] - [1.04, 1.04]	[1.00, 1.00] - [1.06, 1.06]
BMI	18.5-25	[0.64, 0.71]	[0.47, 0.52] - [0.87, 0.98]	[0.24, 0.25] - [1.73, 2.01]
	25-30	[0.54, 0.59]	[0.40, 0.43] - [0.73, 0.82]	[0.20, 0.21] - [1.44, 1.67]
	30-35	[0.51, 0.57]	[0.37, 0.41] - [0.69, 0.78]	[0.19, 0.20] - [1.38, 1.61]
	35-40	[0.55, 0.61]	[0.40, 0.43] - [0.76, 0.85]	[0.19, 0.20] - [1.56, 1.81]
	>40	[0.49, 0.56]	[0.34, 0.38] - [0.71, 0.83]	[0.14, 0.16] - [1.66, 1.97]
Smoking	Previous smoker	[1.13, 1.14]	[1.07, 1.08] - [1.19, 1.20]	[0.94, 0.95] - [1.34, 1.36]
	Current smoker	[1.25, 1.28]	[1.14, 1.17] - [1.37, 1.41]	[0.93, 0.95] - [1.69, 1.73]
Alcohol consumption	Three or four times a week	[0.83, 0.85]	[0.77, 0.79] - [0.89, 0.92]	[0.65, 0.67] - [1.06, 1.09]
	Once or twice a week	[0.90, 0.91]	[0.83, 0.84] - [0.96, 0.98]	[0.71, 0.72] - [1.14, 1.15]
	Once to three times a month	[0.89, 0.91]	[0.81, 0.82] - [0.99, 1.00]	[0.65, 0.66] - [1.23, 1.25]
	Only special occasions	[1.12, 1.15]	[1.03, 1.05] - [1.22, 1.26]	[0.84, 0.87] - [1.49, 1.53]
	Never	[1.33, 1.38]	[1.21, 1.26] - [1.46, 1.51]	[0.99, 1.02] - [1.80, 1.86]

Physical activity	Moderate	[0.75, 0.76]	[0.71, 0.72] - [0.79, 0.81]	[0.63, 0.64] - [0.89, 0.91]
	Strenuous	[0.64, 0.66]	[0.56, 0.58] - [0.73, 0.75]	[0.42, 0.44] - [0.97, 0.99]
Number of prior comorbidities		[1.0, 1.0]	[1.00, 1.00] - [1.00, 1.00]	[1.00, 1.00] - [1.00, 1.00]
Prior depression		[1.15, 1.29]	[1.05, 1.18] - [1.25, 1.40]	[0.87, 0.98] - [1.52, 1.69]
Prior stroke		[1.46, 1.56]	[1.21, 1.30] - [1.75, 1.86]	[0.80, 0.88] - [2.64, 2.78]
Prior diabetes		[1.69, 1.86]	[1.49, 1.65] - [1.91, 2.11]	[1.13, 1.26] - [2.51, 2.78]
Prior hypercholesterolemia		[1.03, 1.08]	[0.91, 0.95] - [1.17, 1.22]	[0.69, 0.72] - [1.54, 1.61]
Prior hypertension		[1.20, 1.25]	[1.11, 1.16] - [1.30, 1.35]	[0.94, 0.98] - [1.53, 1.60]
APOE carrier	ε2	[0.82 ,0.84]	[0.74, 0.77] - [0.90, 0.93]	[0.60, 0.62] - [1.13, 1.16]
	ε4	[2.72, 2.76]	[2.58, 2.62] - [2.87, 2.90]	[2.30, 2.33] - [3.22, 3.26]

Supplementary Table 9: HRs for scaled (mean=0; standard deviation=1) numerical variables in the Cox proportional risks model predicting the risk of dementia. Anticholinergic burden was determined using the value-based scale by Durán et al. (2013)[5].

Variable	Level	HR	99% CI
AChB		1.12	1.04 - 1.22
Sex	Male	1.46	1.22 - 1.75
Year 0		0.98	0.95 - 1.02
Age 0		1.22	1.19 - 1.24
Education	Graduate degree	0.82	0.67 - 1.02
Deprivation		1.03	1.00 - 1.06
Alcohol consumption	Three or four times a week	0.83	0.65 - 1.07
	Once or twice a week	0.90	0.71 - 1.14
	Once to three times a month	0.90	0.65 - 1.25
	Only special occasions	1.13	0.85 - 1.51
	Never	1.34	0.99 - 1.83
Smoking	Current smoker	1.13	0.94 - 1.35
	Previous smoker	1.26	0.93 - 1.72
Physical activity	Strenuous	0.76	0.64 - 0.92
	Moderate	0.66	0.43 - 1.00
BMI	<18.5	0.65	0.23 - 1.78
	25-30	0.54	0.20 - 1.48
	30-35	0.51	0.18 - 1.42
	35-40	0.55	0.19 - 1.61
	>40	0.50	0.14 - 1.74
Data provider	England TPP	1.43	1.01 - 2.03
	Scotland	0.24	0.12 - 0.48
	Wales	0.53	0.31 - 0.90
Prior depression		1.18	0.89 - 1.57
Prior stroke		1.54	0.85 - 2.79
Prior diabetes		1.84	1.23 - 2.75
Prior hypercholesterolemia		1.06	0.70 - 1.59
Prior hypertension		1.25	0.97 - 1.61
Number of prior comorbidities		1	1.00 - 1.00
Non-anticholinergic drug count		1.02	0.97 - 1.07
APOE carrier	ε2	0.83	0.60 - 1.15
	ε4	2.73	2.30 - 3.25

Supplementary Table 10: HRs for scaled numerical variables in the Cox proportional risks model predicting the risk of dementia. Each row depicts the effect of anticholinergic burden due to a drug prescribed for a different anatomical group.

Anatomical group	HR	95% CI	n missing
nervous	1.12	1.07 - 1.18	19,700
cardiovascular	1.05	1.02 - 1.09	19,700
gastrointestinal	1.05	1.00 - 1.09	19,700
blood	1.02	1.00 - 1.05	19,700
hormonal	1.02	0.97 - 1.07	19,700
respiratory	1.01	0.96 - 1.06	19,700
antiinfective	1.01	0.97 - 1.06	19,700
urinary	0.99	0.95 - 1.04	19,700
immuno-modulating	0.99	0.94 - 1.04	19,700
musculo-skeletal	0.99	0.94 - 1.03	19,700

Supplementary Table 11: HRs for scaled numerical variables in the Cox proportional risks model predicting the risk of dementia. Each row depicts the effect of anticholinergic burden due to a drug prescribed for a different pharmacological group.

Pharmacological group	HR	95% CI	n missing
antidepressant	1.11	1.07 - 1.17	20,047
antiepileptic	1.07	1.04 - 1.11	20,047
high ceiling diuretic	1.06	1.02 - 1.10	20,047
acid reflux	1.04	0.99 - 1.08	20,047
propulsive	1.03	0.99 - 1.08	20,047
antipsychotic	1.02	0.98 - 1.07	20,047
corticosteroid	1.02	0.98 - 1.07	20,047
decongestant	1.02	0.98 - 1.06	20,047
cardiac Ca-blocker	1.02	0.98 - 1.05	20,047
antithrombotic	1.02	0.99 - 1.04	20,047
antihistamine	1.01	0.96 - 1.06	20,047
antipropulsive	1.01	0.97 - 1.05	20,047
penicillin	1.01	0.96 - 1.06	20,047
anxiolytic	1.01	0.96 - 1.05	20,047
glucose-lowering	1.01	0.96 - 1.06	20,047
vasodilator	1.00	0.96 - 1.04	20,047
cardiac glycoside	0.99	0.95 - 1.04	20,047
opioid	0.99	0.94 - 1.04	20,047
immunosuppressant	0.99	0.93 - 1.04	20,047
urological	0.99	0.94 - 1.04	20,047
sedative	0.98	0.93 - 1.03	20,047
antimigraine	0.98	0.92 - 1.03	20,047

Supplementary Table 12: HRs for scaled numerical variables in the Cox proportional risks model predicting the risk of dementia. Each row depicts the effect of anticholinergic burden due to a drug prescribed for a different category of anticholinergic potency. The last column indicates the number of participants that were issued at least one prescription from each potency group.

Potency score	HR	95% CI	n missing	n > 0
2	1.03	0.98 - 1.07	20,693	7,745
1	1.10	1.05 - 1.15	20,693	36,500
0.5	1.03	0.99 - 1.08	20,693	35,120
0	1.03	0.98 - 1.09	20,693	117,583

References

1. Carnahan RM, Lund BC, Perry PJ, Pollock BG, Gulp KR. The anticholinergic drug scale as a measure of drug-related anticholinergic burden: Associations with serum anticholinergic activity. J Clin Pharmacol. 2006;46(12):1481-1486. doi:10.1177/0091270006292126
2. Boustani M, Campbell N, Munger S, Maidment I, Fox C. Impact of anticholinergics on the aging brain: A review and practical application. Aging health. 2008;4(3):311-320. doi:10.2217/1745509X.4.3.311
3. Rudolph JL, Salow MJ, Angelini MC, McGlinchey RE. The anticholinergic risk scale and anticholinergic adverse effects in older persons. Arch Intern Med. 2008;168(5):508-513. doi:10.1001/archinternmed.2007.106
4. Sumukadas D, McMurdo MET, Mangoni AA, Guthrie B. Temporal trends in anticholinergic medication prescription in older people: Repeated crosssectional analysis of population prescribing data. Age Ageing. 2013;0:1-7. doi:10.1093/ageing/aft199
5. Durán CE, Azermai M, Stichele RHV. Systematic review of anticholinergic risk scales in older adults. Eur J Clin Pharmacol. 2013;69(7):1485-1496. doi:10.1007/s00228-013-1499-3
6. Summers WK. A clinical method of estimating risk of drug induced delirium. Life Sci. 1978;22:1511-1516.
7. Han L, McCusker J, Cole M, Abrahamowicz M, Primeau F, Élie M. Use of medications with anticholinergic effect predicts clinical severity of delirium symptoms in older medical inpatients. Arch Intern Med. 2001;161(8):1099-1105. doi:10.1001/archinte.161.8.1099
8. Han L, Agostini J V., Allore HG. Cumulative anticholinergic exposure is associated with poor memory and executive function in older men. J Am Geriatr Soc. 2008;56(12):2203-2210. doi:10.1111/j.1532-5415.2008.02009.x
9. Aizenberg D, Sigler M, Weizman A, Barak Y. Anticholinergic burden and the risk of falls among elderly psychiatric inpatients: A 4-year case-control study. Int Psychogeriatrics. 2002;14(3):307-310. doi:10.1017/S1041610202008505
10. Minzenberg MJ, Poole JH, Benton C, Vinogradov S. Assocation of Anticholinergic Load with Impairment of Complex Attention and Memory in Schizophrenia. Am J Psychiatry. 2004;161(1):116-124. doi:10.1176/appi.ajp.161.1.116
11. Ancelin ML, Artero S, Portet F, Dupuy AM, Touchon J, Ritchie K. Non-degenerative mild cognitive impairment in elderly people and use of anticholinergic drugs: Longitudinal cohort study. Br Med J. 2006;332(7539):455-458. doi:10.1136/bmj.38740.439664.DE
12. Hilmer SN, Mager DE, Simonsick EM, et al. A drug burden index to define the functional burden of medications in older people. Arch Intern Med. 2007;167(8):781-787. doi:10.1001/archinte.167.8.781
13. Chew ML, Mulsant BH, Pollock BG, et al. Anticholinergic activity of 107 medications commonly used by older adults. J Am Geriatr Soc. 2008;56(7):1333-1341. doi:10.1111/j.1532-5415.2008.01737.x
14. Cancelli I, Valentinis L, Merlino G, Valente M, Gigli GL. Drugs with anticholinergic properties as a risk factor for psychosis in patients affected by Alzheimer's disease. Clin Pharmacol Ther. 2008;84(1):63-68. doi:10.1038/sj.clpt.6100435
15. Ehrt U, Broich K, Larsen JP, Ballard C, Aarsland D. Use of drugs with anticholinergic effect and impact on cognition in Parkinson's disease: A cohort study. J Neurol Neurosurg Psychiatry. 2010;81(2):160-165. doi:10.1136/jnnp.2009.186239
16. Sittironnarit G, Ames D, Bush AI, et al. Effects of anticholinergic drugs on cognitive function in older Australians: Results from the AIBL study. Dement Geriatr Cogn Disord. 2011;31(3):173-178. doi:10.1159/000325171
17. Whalley LJ, Sharma S, Fox HC, et al. Anticholinergic drugs in late life: Adverse effects on cognition but not on progress to dementia. J Alzheimer's Dis. 2012;30(2):253-261. doi:10.3233/JAD-2012-110935
18. Faure R, Dauphinot V, Krolak-Simon, P., et al. A standard international version of the drug burden index for cross-national comparison of the functional burden of medications in older people. Journal of the American Geriatrics Society. 2013;61(7):1227-1228. doi: 10.1111/jgs.12343

19. Klamer TT, Wauters M, Azermai M, et al. A Novel Scale Linking Potency and Dosage to Estimate Anticholinergic Exposure in Older Adults: the Muscarinic Acetylcholinergic Receptor ANTagonist Exposure Scale. Basic Clin Pharmacol Toxicol. 2017;120(6):582-590. doi:10.1111/bcpt.12699
20. Bishara D, Harwood D, Sauer J, Taylor DM. Anticholinergic effect on cognition (AEC) of drugs commonly used in older people. Int J Geriatr Psychiatry. 2017;32(6):650-656. doi:10.1002/gps.4507
21. Briet J, Javelot H, Heitzman E, et al. The anticholinergic impregnation scale: towards the elaboration of a scale adapted to prescriptions in French psychiatric settings. Therapie. 2017;72:427-437. doi:10.1016/j.therap.2016.12.010
22. Kiesel EK, Hopf YM, Drey M. An anticholinergic burden score for German prescribers: Score development. BMC Geriatr. 2018;18(1):1-11. doi:10.1186/s12877-018-0929-6
23. Mur J, Cox SR, Marioni RE, Muniz-Terrera G, Russ TC. Increase in anticholinergic burden from 1990 to 2015: age-period-cohort analysis in UK Biobank. Br J Clin Pharmacol. 88(3), 983-993. doi:10.1111/bcp.15045

6 AChB, cognitive ability, and brain structure in UK Biobank

6.1 Introduction

The study presented in chapter 5.2 clarified the nature of the association between anticholinergic use and dementia. I found AChB according to most anticholinergic scales, but only some drug classes, to be predictive of dementia. Based on my analyses and previous work, anticholinergic use can be predictive of cognitive decline even when the exposure occurred years or decades prior to the diagnosis of dementia. Furthermore, it is possible that even in individuals that are never diagnosed with dementia, anticholinergic use causes a decreased ability in general cognitive functioning in middle age when compared to non-users. However, previous work on the topic has mostly been conducted using tests with low sensitivity for subtle cognitive changes or with a focus on narrow cognitive domains.

In the study presented in this chapter, I calculated a general factor of cognitive ability to study the associations with AChB. As before, I distinguished between anticholinergic scales and drug classes to address potential sources of heterogeneity. I also explored associations of AChB with brain structure. The previous studies on this topic had all found differences in brain structure between users and non-users, but were mostly conducted in older individuals, and assessed the risk for dementia (Chuang et al., 2017; Kilimann et al., 2021; Meng et al., 2022; Risacher et al., 2016). Only one study that found a negative association between AChB and the volume of the hippocampus, was conducted on relatively healthy individuals across the age range (Kilimann et al., 2021). That study did not explore differences between anticholinergic scales or drug classes, nor did they test for differences in cognitive ability.

This study was published in the British Journal of Clinical Pharmacology in February 2023. It was also presented at the 26th Nordic Congress of Gerontology as an oral presentation (June 8th – 10th 2022). The manuscript is available in full in section 6.2 and supplementary material for this work is available in section 6.3.1 and at https://bpspubs.onlinelibrary.wiley.com/doi/10.1111/bcp.15698.

6.2 Anticholinergic burden in middle and older age is associated with lower cognitive function, but not with brain atrophy

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ORIGINAL ARTICLE

Anticholinergic burden in middle and older age is associated with lower cognitive function, but not with brain atrophy

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Aims: The aim of this study is to estimate the association between anticholinergic burden, general cognitive ability and various measures of brain structural MRI in relatively healthy middle-aged and older individuals.

Methods: In the UK Biobank participants with linked health-care records (n = 163,043, aged 40–71 at baseline), of whom about 17 000 had MRI data available, we calculated the total anticholinergic drug burden according to 15 different anticholinergic scales and due to different classes of drugs. We then used linear regression to explore the associations between anticholinergic burden and various measures of cognition and structural MRI, including general cognitive ability, 9 separate cognitive domains, brain atrophy, volumes of 68 cortical and 14 subcortical areas and fractional anisotropy and median diffusivity of 25 white-matter tracts.

Results: Anticholinergic burden was modestly associated with poorer cognition across most anticholinergic scales and cognitive tests (7/9 FDR-adjusted significant associations, standardised betas (β) range: −0.039, −0.003). When using the anticholinergic scale exhibiting the strongest association with cognitive functions, anticholinergic burden due to only some classes of drugs exhibited negative associations with cognitive function, with β-lactam antibiotics ($\beta = -0.035$, $P_{FDR} < 0.001$) and opioids ($\beta = -0.026$, $P_{FDR} < 0.001$) exhibiting the strongest effects. Anticholinergic burden was not associated with any measure of brain macrostructure or microstructure ($P_{FDR} > 0.08$).

Conclusions: Anticholinergic burden is weakly associated with poorer cognition, but there is little evidence for associations with brain structure. Future studies might focus more broadly on polypharmacy or more narrowly on distinct drug classes, instead of using purported anticholinergic action to study the effects of drugs on cognitive ability.

This is a secondary investigation of an existing cohort study and therefore did not have a Principal Investigator.

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KEYWORDS
anticholinergic drugs, brain structural magnetic resonance imaging, cognitive ability, primary care

1 | INTRODUCTION

Anticholinergic drugs (anticholinergics) are medicines thought to block muscarinic receptors. Their anticholinergic action is ascertained by consulting anticholinergic scales that assign potency scores to individual drugs; the combined score for an individual patient is the anticholinergic burden (AChB). Anticholinergics are commonly prescribed for a variety of conditions,[1] and their transient side effects on cognition are well-known.[2–6] Moreover, their long-term use in old age[7] and middle age[8–11] has been associated with an increased risk of cognitive decline and dementia. It has been hypothesized[12] that this relationship is due to central anticholinergic effects, affecting areas of the brain crucial for cognition.[13–15] Therefore, a relationship might exist between AChB, cognitive ability and brain structure, even within the *normal* spectrum of cognitive functioning.

However, the existing evidence on this relationship is mixed. Most studies on anticholinergic prescribing in adults classify cognition as the absence *vs.* presence of a disorder or test separate cognitive modalities in isolation.[16,17] When measured this way, studies of AChB and cognitive ability often produce discordant results.[16] There are reports of positive associations between anticholinergic use and executive function,[12,18–21] associative learning,[22] visual,[23] episodic,[24,25] and short-term memory,[26] delayed and immediate recall,[27] language abilities,[28] visuospatial skills,[28] attention,[28] and reaction time.[28] However, some authors have found no evidence for delayed and immediate recall,[21,22,28,29] reaction time,[22] executive function,[23,27] language abilities,[27,29] working memory,[25,27] processing speed,[25] and implicit[28] and semantic[25] memory. Additionally, because anticholinergic scales sometimes include different drugs and score the same drugs differently,[30] they could represent another source of variation in reported findings.

It has been suggested that global composites of cognitive functioning might be more sensitive to subtle cognitive changes.[16] Individual test scores contain more random noise, and the results can limit generalisability and contribute to inconsistency among studies. By contrast, general cognitive ability (sometimes referred to as general intelligence or *g*) represents shared variation across cognitive domains, is predictive of various social outcomes,[31] health outcomes,[32,33] mortality,[34] and is referenced in widely utilized diagnostic manuals.[31] Analysing large samples on multiple anticholinergic scales can further strengthen the reliability of the results.

Past studies have demonstrated associations between cognitive ability and several measures of brain structural magnetic resonance imaging (MRI). While the effect sizes have varied depending on the sample characteristics and cognitive tests used, they have usually ranged from r 0.2 to 0.3.[35] One review found evidence for cross-sectional and longitudinal associations between global cognition and total brain size, global grey matter and hippocampal volume.[36] An analysis conducted on the UK Biobank sample found correlations between general cognitive ability and total brain volume, functional anisotropy (FA), mean diffusivity (MD) and several regional cortical volumes, especially those in the frontal lobe. Additionally, the authors found associations for multiple subcortical structures, especially the thalamus.[37] However, little is known about the neural correlates of potential anticholinergic-related cognitive decline.

To our knowledge, 4 studies[8,12,38,39] to date have assessed the relationship between these brain measures and regular anticholinergic use. While each study reported on associations between anticholinergic use and various metrics of brain structure and function, replication studies in larger samples are required. Furthermore, research is needed to probe potential differences between anticholinergic scales and between different classes of anticholinergics when exploring associations with cognitive function and cerebral correlates.

What is already known about this subject

- Long-term anticholinergic use is associated with a risk of dementia, but the evidence on the relationship with cognitive ability in healthy individuals is mixed. It is unclear if anticholinergic use is associated with measurable changes in brain structure before the onset of advanced age and dementia.
- The heterogeneity in previous studies may be due to differences in cognitive tests and anticholinergic scales used to measure the outcome and exposures, respectively, and in different effects of distinct classes of drugs.

What this study adds

- Our study suggests that while anticholinergic use according to most anticholinergic scales studied is associated with lower cognitive ability, the relationship holds only for some classes of drugs, especially β-lactam antibiotics and opioids.
- In contrast to previous studies linking anticholinergic use to changes in brain structure in individuals with dementia, we found no such relationship in healthy individuals.

In our study—conducted using the UK Biobank—we calculated a latent factor of general cognitive ability (*g*) and utilized MRI-imaging measures and prescriptions linked from primary care, to study the association between AChB, *g* and various brain structural MRI measures. Our goals were to assess: (i) whether there existed differences between anticholinergic scales and (ii) between drug classes in the association of AChB and cognitive ability; and (I ii) whether potential associations between AChB and cognitive ability were reflected in brain MRI measures, including brain atrophy, the volume of various cortical and subcortical brain structures, and measures of white matter microstructure. Based on previous findings, we hypothesised AChB to negatively associate with *g*, total brain volume, and the volumes of prefrontal cortical areas, the thalamus and hippocampus.

2 | METHODS

2.1 | Sample

UK Biobank[40] is a prospective study whose participants were recruited between 2006 and 2010 when they were aged 37–73 years. During the initial assessment, demographic and lifestyle questionnaires, physiological measurements and cognitive tests were administered. A subset of participants later underwent MRI structural imaging and additional cognitive testing. For ~230 000 participants, data on issued prescriptions and diagnoses are available. The diagnoses used were sourced from self-reported data, primary care and secondary hospital care. Self-reported data were provided at the time of the assessments, while data from primary care and secondary hospital care are available until August 2017 and March 2021, respectively. Prescriptions are complete until May 2016 and were sourced from primary care. The prescription entries contained names and dates of drugs prescribed by general practitioners and the (mostly region-specific) suppliers of the prescription data. For the variables described below, we provide specific Field IDs (and links to the descriptions page for each field) in Table S1.

2.2 | Cognitive ability

During the baseline assessment, most participants completed tests measuring visual declarative memory (Pairs Matching), processing speed (Reaction Time), with a subsample also completing tests of working memory (Numeric Memory), prospective memory (Prospective Memory), and verbal and numerical reasoning (Fluid Intelligence). During the imaging assessment, another subset of participants completed the above tests again, in addition to tests of executive function (Trail Making A and B, Tower Rearranging), verbal declarative memory (Paired Associate Learning), nonverbal reasoning (Matrix Pattern completion), crystallized ability (Picture Vocabulary) and another on processing speed (Symbol Digit Substitution; Table S2). Analyses of their psychometric properties in this sample have been reported previously.[41,42] We fitted a confirmatory factor analysis in a structural equation modelling (SEM) framework to calculate *g* from the cognitive tests (Figure S1 and Table S3), yielding 2 separate values, 1 for each assessment visit. SEM has been used to calculate *g* in UK Biobank before[37,43]; the proportional variance explained in our study is smaller (23% for the baseline assessment, 28% for the imaging assessment) than in prior work in UK Biobank that used fewer cognitive tests.[37] For participants for whom this was possible, *g* from the imaging assessment was used in our analyses.

2.3 | Brain imaging

Since 2014, UK Biobank has been enhancing the dataset with imaging data that includes brain MRI.[40,44] It consists of imaging-derived phenotypes, whose acquisition and quality control have been previously described.[45] Briefly, brain imaging data were obtained at 4 data collection sites (Cheadle, Newcastle, Reading and Bristol; all UK) using 3 identical scanners (3T Siemens Skyra), with a standard Siemens 32-channel receive head coil. Preprocessing and quality control were undertaken by the UK Biobank research team according to published procedures.[45] Our analyses included total brain volume, brain volumes of 68 cortical areas, 14 subcortical structures, FA and MD of 25 white matter tracts. The measures of brain volume were corrected for head size by multiplication with the T1-based scaling factor (UK Biobank field ID 25000). The brain regions and white matter tracts used in the study are depicted in Figure S2.

2.4 | Anticholinergic burden and drug classification

Anticholinergic scales typically score drugs on a 0–3 ordinal scale, with a higher score indicating greater anticholinergic potency. We considered 15 anticholinergic scales—13[28,46–57] were based on our previous analyses[1] while 2 scales[58,59] were identified through a recent review[7] (Table S4). Three scales[47,50,56] were modified to include newer drugs.[1,60] One scale[52] was modified so that drugs with *improbable anticholinergic action* were assigned an anticholinergic burden of 0.5 as was done before.[1] Using the British National Formulary (https://bnf.nice.org.uk/, last accessed on 11 March 2021), we replaced brand names with generic names. Combination prescriptions containing several anticholinergics were each separated to yield multiple prescriptions, each containing a single anticholinergic. Each prescription was then assigned a potency score from each anticholinergic scale. For analysis, the cumulative AChB was calculated by summing the AChB scores across all prescribed drugs in the sampling period. The sampling period excluded the year preceding the UK Biobank assessment to avoid acute effects of drugs. Prescriptions of drugs with ophthalmic, otic, nasal or topical routes of administration were all assigned an anticholinergic score of 0, as previously reported.[1,54–57] Each drug was assigned to a class in the WHO Anatomical Therapeutic Chemical (ATC) Classification system (https://www.whocc.no, last accessed on 11th March 2022)[61] that categorizes drugs in a 5-level hierarchy. In our analyses, the first (anatomical main group) and third (pharmacological subgroup) levels were used.

2.5 | Data preparation

Prescriptions issued before 2000 and after 2015 were removed due to low ascertainment and incomplete annual data, respectively.[1] Participants with a diagnosis of diseases that may affect brain structure or cognitive ability were removed. The data-cleaning process is depicted in Figure S3. Outliers for numerical variables were defined as values lying 4 or more standard deviations or interquartile ranges beyond the mean or median, whichever was most appropriate according to the distribution. The total number of prescribed drugs and the AChB scores were strongly right-skewed due to the high numbers of zero values. For these variables, zeroes were removed before identifying outliers. All outliers were removed before analysis. Model assumptions were mostly met, but some models exhibited non-normality in the distribution of residuals (Figure S4).

2.6 | Modelling

We applied principal component analysis to tract-specific FA and MD and used the first principal component to compute the *general* FA and MD (*g*FA and *g*MD), accounting for 44 and 50% of the variance, respectively. The standardized loadings and proportional variance for *g*FA and *g*FA are presented in Figure S5 and Table S5. We used linear regression models to estimate the association between AChB, cognitive ability and brain structure. To compare anticholinergic scales, we first modelled the association between *g* and AChB for each scale separately. This was later repeated for total brain volume as the outcome. The scale exhibiting the strongest association with *g* was selected for subsequent analyses. Second, we modelled the effects of AChB due to different drug classes on *g* and total brain volume. Finally, we computed the associations between AChB and the results from 9 cognitive tests, the volumes of 68 cortical areas, 14 subcortical areas, *g*FA and *g*MD, and FA and MD of 25 white matter tracts. We also conducted 2 sensitivity analyses. First, we repeated the analyses on the association between AChB and *g* including only the year preceding the UK Biobank assessment to calculate AChB. Second, we computed the association between AChB according to each scale and *g*, while including an interaction term between AChB and age at assessment.

Each model was corrected for potential confounders, which included age at assessment, number of years over which the cumulative AChB was calculated, number of prescribed nonanticholinergics (different for each anticholinergic scale), data supplier of prescriptions (region-specific—2 for England, and 1 each for Scotland and Wales), socioeconomic deprivation (higher values correspond to greater deprivation; range: −6.3–11.0),[62] smoking status (nonsmoker, previous smoker, current smoker), frequency of alcohol consumption (daily or almost daily; 3 or 4 times a week; once or twice a week; once to 3 times a month; only on special occasions; never), level of physical activity (strenuous; moderate; mild),[63] body mass index (kg/m^2), *APOE*-carrier status, comorbidities count before the first assessment date (total number of distinct diagnoses codes), history of mood disorders, anxiety disorders, schizophrenia, diabetes, hypercholesterolemia, hypertension and myocardial infarction before the assessment date. *APOE*-carrier status was defined through the *APOE* genotype, which is based on the nucleotides at SNP positions rs239358 and rs7412. Participants were denoted as ε2, ε3, or ε4 carriers, if they carried the ε2/ε2 or ε2/ε3 haplotype, ε3/ε3 or ε1/ε3 or ε2/ε4 haplotype, or ε3/ε4 or ε4/ε4 haplotype, respectively. Smoking status, alcohol consumption, physical activity, body mass index and genotype were ascertained at each of the 2 UK Biobank assessments; socioeconomic deprivation was ascertained during the baseline assessment.

When comparing anticholinergic scales, 2 additional models were run for which polypharmacy was the main predictor. The first of these models (*Polypharmacy* model) controlled for the same covariates as above, and the second (*Polypharmacy plus* model) further controlled for the total number of anticholinergics according to any scale. The models where a measure of brain imaging was the main outcome, were in addition to the covariates above controlled for age^2, age*sex, age^2*age, head position in the MRI-scanner (3 coordinates), ethnicity and assessment centre. The template for the linear models is described in Text S1. Results are presented for models before the adjustment for polypharmacy and after adjustment for polypharmacy. Unless explicitly stated otherwise, the results refer to the fully adjusted models.

In analyses where a single anticholinergic scale was used (as opposed to comparing several scales), AChB was calculated using the scale by Durán *et al.*,[52] as it exhibited the strongest association with *g* (see Section 3). All numerical variables were normalized to have a mean of 0 and a standard deviation of 1. When several independent models were run to predict the same outcome, *P*-values were corrected for multiple comparisons using the false discovery rate (FDR).[64] Otherwise, the *P*-threshold of 0.05 was used. Results are reported as standardized betas (β) and plotted with confidence intervals (CIs) adjusted for multiple comparisons (based on the Z-values of the quantile for the standard normal distribution for the FDR-adjusted *P*-values). All data cleaning and modelling were performed using R version 4.2.1 and Python version 3.9.7.

3 | RESULTS

3.1 | Sample

After removing outliers, among the 163 043 participants in our sample, ∼140 000 and ∼14 000 data points (exact value depended on the model) were available for analyses of cognitive ability and brain imaging, respectively. The demographic- and lifestyle variables are presented in Table 1. While the imaging sample was older, the distribution of other variables was similar to the rest of the sample (Table S6). In the period from 2000 to the year before the initial assessment, anticholinergics—depending on the anticholinergic scale—represented between 4.3 and 24.1% of prescriptions, with between 11.3 and 40.7% of participants prescribed an anticholinergic at least once (Table S7). We have previously characterized anticholinergic prescribing and its longitudinal trends in UK Biobank in detail.[1]

TABLE 1 Demographic and lifestyle characteristics of the sample at the baseline assessment after the removal of outliers.

Variable	Level	Median (IQR) or *n* (%)	N missing
Age (years)		58.3 (12.8)	
Sex	Male	72 184 (44.3)	
	Female	90 859 (55.7)	
Deprivation (z-score)		−2.3 (3.9)	174
Alcohol consumption	Daily or almost daily	32 244 (19.8)	326
	Three or 4 times a week	38 472 (23.6)	
	Once or twice a week	43 328 (26.6)	
	Once to 3 times a month	18 402 (11.3)	
	Only special occasions	18 045 (11.1)	
	Never	12 316 (7.6)	
Smoking	Current smoker	16 048 (9.9)	772
	Previous smoker	55 642 (34.3)	
	Non-smoker	90 581 (55.8)	
Physical activity	Strenuous	16 531 (10.8)	10 707
	Moderate	97 776 (64.2)	
	Light	38 029 (25.0)	
BMI (kg/m^2)		26.8 (5.8)	1029
Data provider	England (Vision)	14 393 (8.8)	
	Scotland	9571 (5.9)	
	England (TPP)	122 120 (74.9)	
	Wales	16 959 (10.4)	
Mood disorder		23.926 (14.7)	
Anxiety disorder		15.572 (9.6)	
Schizophrenia		590 (0.4)	
Myocardial infarction		7335 (4.5)	
Diabetes		14 477 (8.9)	
Hypercholesterolemia		29 994 (18.4)	
Hypertension		54 124 (33.2)	
Number of prior comorbidities		86 (98)	65
Polypharmacy		34 (96)	
APOE carrier	ε2	20 549 (12.9)	3871
	ε3	98 084 (61.6)	
	ε4	40 539 (25.5)	

Note: Polypharmacy is the total number of prescriptions issued over the sampling period (differs among participants; range: 1 16 years). Sex, deprivation, alcohol consumption, smoking, physical activity and BMI are self-reported or based on measurements during the baseline assessment. The variables are not scaled.

Abbreviations: BMI: body mass index; IQR: interquartile range; TPP: The Phoenix Partnership.

3.2 | AChB and cognition

When polypharmacy was not included as a control variable, all the tested anticholinergic scales exhibited significant negative associations with *g* (Table S8). The scales by Durán *et al.*[52] and by Cancelli *et al.*[49] showed the strongest (β −0.032, P_{FDR} < .001) and weakest (β −0.009, P_{FDR} < .001) effects, respectively. When the models were additionally corrected for polypharmacy, the median effect size of AChB across scales was reduced by 31%, but associations of all anticholinergic scales except the scale by Cancelli *et al.*[49] (β -4.4×10^{-5}, P_{FDR} .88) remained significant (Figure 1A, Table S8). The scale by Durán *et al.*[52] retained the strongest association (β −0.025, P_{FDR} < .001; Table S9). When the predictors were not standardized, this effect size corresponds to an at most 0.0017 decrease in *g* when AChB is increased by 1 standard deviation. The main predictors of each polypharmacy model also exhibited negative correlations with cognition, (*Polypharmacy*: β −0.034 P_{FDR} < .001; *Polypharmacy plus*: β −0.028, P_{FDR} < .001). The number of anticholinergics included in a scale was positively correlated with the strength of the observed effect when uncorrected for polypharmacy (*R* .70,

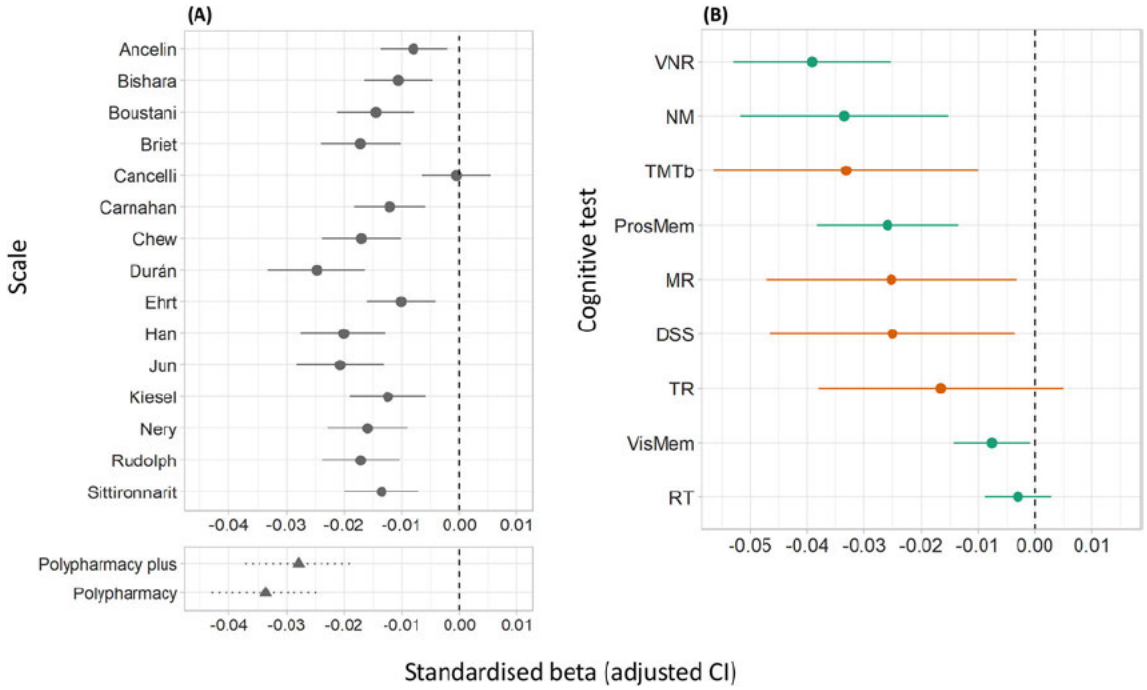

FIGURE 1 Associations between anticholinergic burden (AChB) and general cognitive ability for each anticholinergic scale (A) and associations between AChB according to the scale by Durán *et al.*[52] and each cognitive test included in the calculation of general cognitive ability (B). Results are displayed as standardized βs. (A) The y-axis indicates the main predictor for each model; in the upper panel, this was the AChB according to different anticholinergic scales; in the bottom panel, this was drug count (i.e., polypharmacy, controlled for in 2 different ways; see main text for details). (B) The y-axis indicates the cognitive test used as the outcome. The colours refer to when the test was taken, with green indicating assessment at baseline and orange indicating assessment during the imaging visit.

P .004) and when corrected for polypharmacy (*R* .60, *P* .02, Figure S6). I.e., the more drugs were identified as anticholinergic by an anticholinergic scale, the better predictor the scale was of lower *g*.

When a separate model was run for each cognitive test, AChB exhibited negative associations for each test. Among the cognitive tests, 7/9 were significant; Fluid Intelligence showed the strongest effect (β −0.039, P_{FDR} < .01) and Reaction Time (β −0.0030, P_{FDR} .33) exhibited the weakest effect (Figure 1B, Table S10).

When testing for the effects of drug classes, we found only limited instances in which higher AChB was associated with lower *g* (Figure 2, Table S11). Among the pharmacological classes, AChB due to drugs for migraine (β 0.015, P_{FDR} < .001) showed positive associations with *g*. AChB due to most other drugs exhibited negative associations with *g*, with β-lactam antibiotics (β −0.035, P_{FDR} < .001) and opioids (β −0.026, P_{FDR} < .001) showing the strongest effects, corresponding to respectively 0.033 and 0.010 decreases in the unstandardized *g* for each increase of AChB by 1 standard deviation.

3.3 | ACB and brain-imaging measures

AChB was not associated with brain atrophy irrespective of the anticholinergic scale used (range of β −0.004–0.017, P_{FDR} ≥ .21). While there were minor differences between the predictive power of different scales, the CIs overlapped across scale models and polypharmacy models (Figure 3, Tables S12 and S13).

AChB was also not associated with the volume of any cortical (range of β −0.018–0.028, P_{FDR} ≥ .26) or subcortical (β range −0.007–0.024, P_{FDR} ≥ .08) brain region, or the microstructure of white matter tracts (range of β −0.015–0.014, all P_{FDR} .98; Table S14). AChB due to no drug class was associated with brain atrophy (Table S15, Figure 4).

3.4 | Sensitivity analyses

When the analyses on the associations between AChB and cognitive function were repeated using only AChB in the year before the assessment as the predictor (Tables S16–S19), the results exhibited similar trends to those observed in the main analyses. Most anticholinergic scales tended to negatively associate with cognitive function, albeit the effect sizes were smaller. Additionally, AChB was associated with lower performance in 1/5 cognitive tests available for this analysis. Furthermore, AChB due to β-lactam antibiotics and opioids again exhibited the strongest negative associations with *g*.

When *g* was modelled with the inclusion of an interaction term between age at assessment and AChB, the interaction was not significant (β 3.0×10^{-4}, *P* .38), indicating that the observed effect

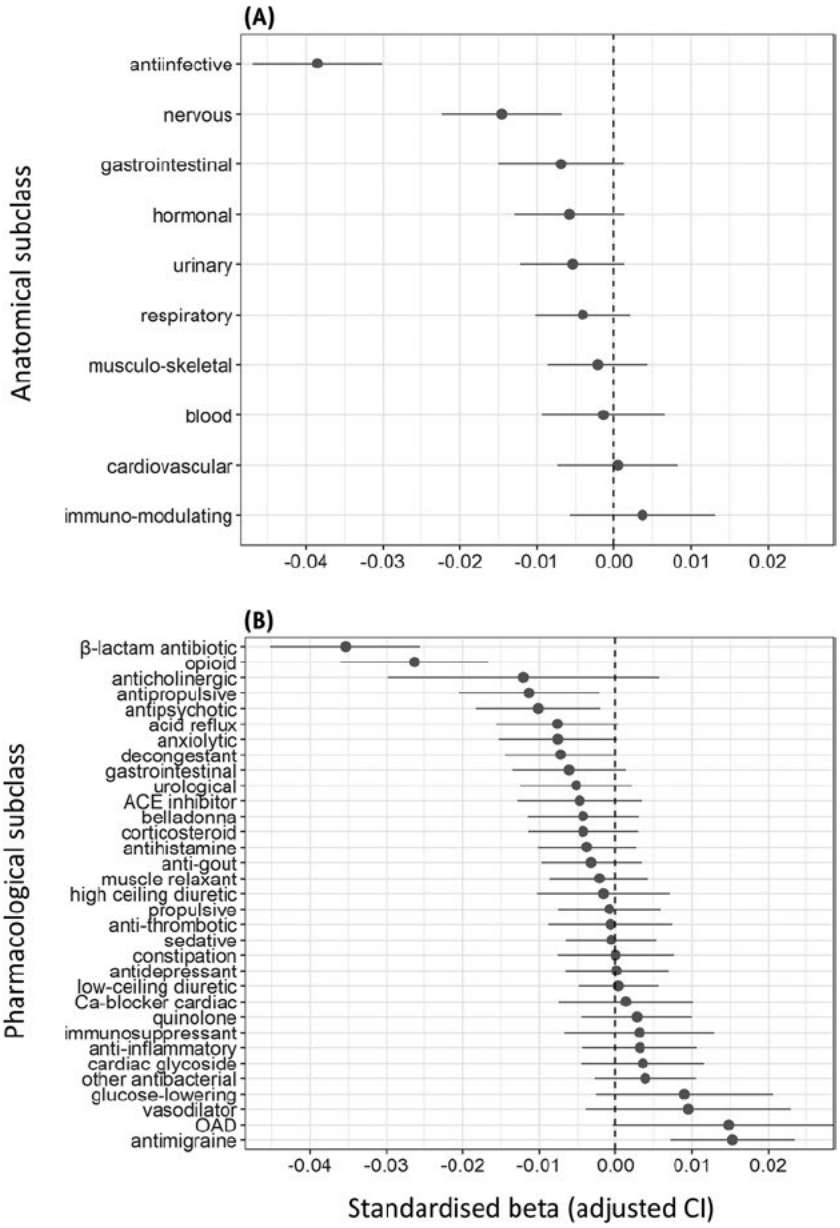

FIGURE 2 Associations between anticholinergic burden according to the scale by Durán *et al.*[52] due to different classes of drugs on the 1 hand and general cognitive ability on the other. Results are displayed as standardized βs. (A) Classification of drugs based on anatomical class; (B) Classification of drugs based on pharmacological subclass. Classes containing drugs that were together prescribed to too few participants (<100) were not included in the models.

sizes were not substantially larger or smaller in older compared to younger participants.

4 | DISCUSSION

In this study, we found that most of the 15 studied anticholinergic scales exhibited significant associations with cognitive ability. This remained the case after controlling for multiple potential confounds, including the history of certain disorders and polypharmacy. Interestingly, the size of the effect was not moderated by age—middle-aged and older adults showed similar AChB-cognitive associations. While the positive association between higher AChB and lower cognitive ability largely agrees with previous studies on the topic, past results have been mixed.[7,16] One potential source of heterogeneity between studies is different control for polypharmacy, which may alter the results considerably. In our study, the addition of polypharmacy substantially decreased the size of the observed effects and was a stronger predictor of lower cognitive ability than AChB according to any of the studied anticholinergic scales. Another source of heterogeneity may be the differential effect of distinct drug classes. We found large differences between drug classes when predicting cognitive ability, with β-lactam antibiotics exhibiting larger effects than other drug classes. Moreover, antimigraine drugs were associated with higher cognitive ability. The effect of a general anticholinergic score may thus strongly depend on the structure of the sample and the precise prescribing characteristics of the participants.

In our study, general AChB was not predictive of any measure of brain structural MRI studied, including the volumes of 68 cortical and 14 subcortical areas, and measures of brain microstructure for 25 white matter tracts. These findings are in contrast with previous research. To our knowledge, 4 studies have explored the association

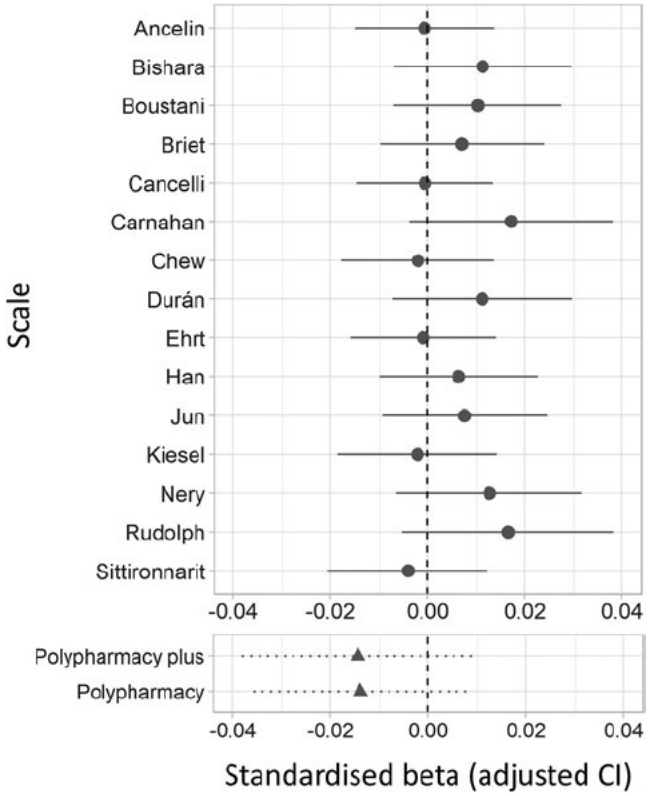

FIGURE 3 Associations between anticholinergic burden and brain atrophy for each anticholinergic scale. The y-axis indicates the main predictor for each model; in the upper panel, this was the anticholinergic burden according to different anticholinergic scales; in the bottom panel, this was drug count (i.e., polypharmacy, adjusted for covariates in different ways; see main text for details). Results are displayed as standardized βs.

between anticholinergic use and brain structure. They found anticholinergic use to associate with reduced cortical volume and reduced temporal lobe thickness,[12] increased rates of brain atrophy,[8] reduced grey matter density and functional connectivity in the nucleus basalis of Meynert,[38] and reduced volumes of both hippocampi.[39]

It is unclear why our results from MRI structural imaging diverge from previous findings, as the studies described above display a range of characteristics that overlap with our own, including longitudinal data,[8] control for polypharmacy,[12] and the inclusion of middle-aged participants.[8,39] One possibility is that the previous studies mostly classified the predictor (e.g., anticholinergic users *vs.* nonusers), while we used a continuous measure of AChB. The pitfalls of categorisation and the loss of power for true effects have been discussed before.[65] Furthermore, the size of our imaging sample (~16 000) was several times larger (~3000). As has been recently reported,[66] brain-wide association studies may require thousands of participants to minimize effect size inflation and increase replication rates. Finally, all but 1[39] of the above studies focused on cognitive disorders or decline later in life, with 1 reporting an effect for specifically those participants that later developed mild cognitive impairment.[38] It is possible that while brain atrophy occurs in ageing or dementia, subtle cellular changes in the cholinergic system occur before that but are not measurable by structural and diffusion MRI. This could include changes in the proportions or the integrity of muscarinic receptor subtypes or a shift in the balance of oscillation frequencies of neural networks.

Our study exhibits several advantages, including the use of a far larger sample than ever before in this area, use of linked prescriptions from primary care across a long period, exploration of several outcomes, the use of a latent factor of cognitive ability, and the comparison of different anticholinergic scales and classes of drugs.

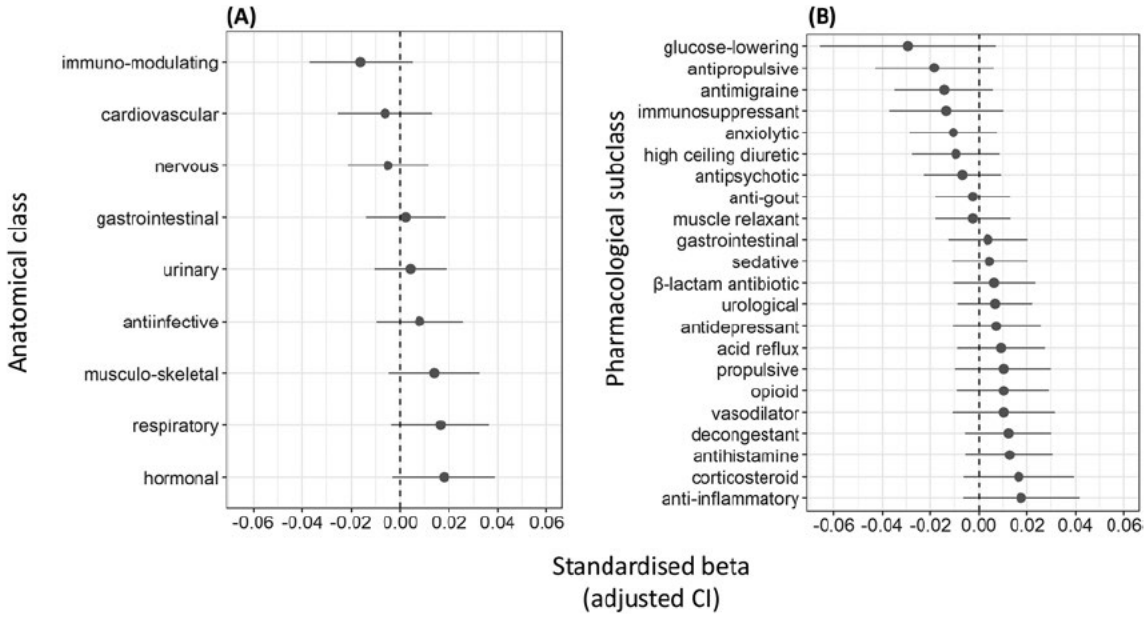

FIGURE 4 Associations between anticholinergic burden according to the scale by Durán *et al.*[52] due to different classes of drugs on the 1 hand and total brain volume on the other. Results are displayed as standardized βs. (A) Classification of drugs based on anatomical class; (B) Classification of drugs based on pharmacological subclass. Classes with too few prescriptions in the sample (<100) were not included in the models.

Furthermore, our models carefully incorporated several important control variables, including the history of relevant disorders, polypharmacy, and several lifestyle and demographic factors. Finally, we adopted a robust approach to measuring cognitive ability that can reduce variability common in the assessment of separate cognitive domains.

However, we recognize several limitations. First, the UK Biobank sample is on average less deprived and healthier than the UK population[67] and thus not representative. Participants in the imaging subsample exhibit even better indicators of psychological and physical health than the UK Biobank average.[68] Both factors are likely to result in an underestimate of the effects present in the population. Second, the prescriptions included in our study do not incorporate over-the-counter drugs and we also have no information on how many prescriptions were dispensed or taken by participants. Third, brain imaging was sometimes performed after the coverage for prescriptions had concluded and the drugs potentially prescribed in the intervening period were not accounted for. This probably decreased the accuracy of our AChB measure for those participants. Fourth, our study was cross-sectional and did not assess longitudinal changes in cognitive function and brain structure. This prevented us from establishing the sequence of events and from assessing associations between anticholinergic use and within-person changes. Finally, because AchB correlates with the number of anticholinergics, the effects of polypharmacy due to the use concurrently of several anticholinergic drugs and intrinsic anticholinergic activity of those drugs could not be completely separated.

Both the present study, as well as previous analyses have reported polypharmacy more broadly to be associated with poorer cognitive ability[69,70] and dementia.[71] A recent medication-wide association study[72] found that among 744 medicines, 30% were associated with dementia. Additionally, previous studies have reported on differences between drug classes in the association between AchB and dementia.[9–11] This finding was extended in the present study of general cognitive ability in a nonpathological sample. These results support a more nuanced approach that distinguishes between different classes of drugs beyond their assumed anticholinergic effects. For drug classes for which associations with lower cognitive ability or dementia can be demonstrated, more studies are needed to determine the effects of chronic use earlier in life, the impact of discontinuation and the potential neural correlates.

In summary, in this study, we found positive associations between long-term anticholinergic use and general cognitive ability across most studied anticholinergic scales. However, the associations held only for some drug classes and there was no evidence for differences in brain structure as a function of AChB. While the significant effect sizes observed in our study were modest, for complex, multicausal outcomes—especially in a large and relatively healthy sample—this is to be expected. For example, angiotensin converting enzyme inhibitors—one of the most common drugs to treat hypertension—have been shown to reduce systolic/diastolic pressure by merely −8/−5 mmHg.[73] When considered in the long-term and on the scale of entire populations, even tiny effects can accumulate to produce substantial health and economic consequences for society. Given sufficient confidence in a drug-outcome relationship and the availability of alternative treatments, changes in prescribing represent an intervention that is relatively simple to implement. This should serve as additional motivation for further research in the field.

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COMPETING INTERESTS

R.E.M. has received consulting fees from the Epigenetic Clock Development Foundation and speaker fees from Illumina. T.C.R. has received fees for medicolegal work from private solicitors. S.R.C. has received speaker fees from the Society of Biological Psychiatry. G.M.T. has received consulting fees for grants funded by the NIH. J.M. has nothing to disclose.

CONTRIBUTORS

J.M. conceived and planned the initial study, prepared the data, and conducted the statistical analysis. J.M. and S.R.C. contributed to the data analysis strategy. All authors contributed to the interpretation of findings, the revision of the manuscript, and the approval of the final version.

DATA AVAILABILITY STATEMENT

Data from UK Biobank is available to approved researchers directly from UK Biobank. The code used to clean and analyse the data is available at https://github.com/JuM24/UKB-AChB-cognition-MRI.

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REFERENCES

1. Mur J, Cox SR, Marioni RE, Muniz-Terrera G, Russ TC. Increase in anticholinergic burden from 1990 to 2015: age-period-cohort analysis in UK biobank. *Br J Clin Pharmacol*. 2022;88(3):983-993.
2. Green A, Ellis KA, Ellis J, et al. Muscarinic and nicotinic receptor modulation of object and spatial n-back working memory in humans. *Pharmacol Biochem Behav*. 2005;81(3):575-584. doi:10.1016/j.pbb.2005.04.010
3. Heller Brown J, Brandl K, Wess J. Muscarinic receptor agonists and antagonists. In: Brunton LL, Hilal-Dandan R, Knollmann BC, eds. *The Pharmacological Basis of Therapeutics*. 13th ed. McGraw-Hill; 2018:149-161.
4. Koller G, Satzger W, Adam M, et al. Effects of scopolamine on matching to sample paradigm and related tests in human subjects. *Neuropsychobiology*. 2003;48(2):87-94. doi:10.1159/000072883
5. Schon K, Atri A, Hasselmo ME, Tricarico MD, LoPresti ML, Stern CE. Scopolamine reduces persistent activity related to long-term encoding in the parahippocampal gyrus during delayed matching in humans. *J Neurosci*. 2005;25(40):9112-9123. doi:10.1523/JNEUROSCI.1982-05.2005
6. Sperling R, Greve D, Dale A, et al. Functional MRI detection of pharmacologically induced memory impairment. *Proc Natl Acad Sci U S A*. 2002;99(1):455-460. doi:10.1073/pnas.012467899
7. Taylor-Rowan M, Edwards S, Noel-Storr AH, et al. Anticholinergic burden (prognostic factor) for prediction of dementia or cognitive decline in older adults with no known cognitive syndrome. *Cochrane Database Syst Rev*. 2021;5(5):CD013540. doi:10.1002/14651858.CD013540.pub2
8. Chuang YF, Elango P, Gonzalez CE, Thambisetty M. Midlife anticholinergic drug use, risk of Alzheimer's disease, and brain atrophy in community-dwelling older adults. *Alzheimers Dement (N Y)*. 2017;3(3):471-479. doi:10.1016/j.trci.2017.06.004
9. Mur J, Russ TC, Cox SR, Marioni RE, Muniz-Terrera G. Association between anticholinergic burden and dementia in UK Biobank. *Alzheimers Dement (N Y)*. 2022;8(1):e12290. doi:10.1002/trc2.12290
10. Coupland CAC, Hill T, Dening T, Morriss R, Moore M, Hippisley-Cox J. Anticholinergic drug exposure and the risk of dementia: a nested case-control study. *JAMA Intern Med*. 2019;179(8):1084-1093. doi:10.1001/jamainternmed.2019.0677
11. Richardson K, Fox C, Maidment I, et al. Anticholinergic drugs and risk of dementia: case-control study. *BMJ*. 2018;361:k1315. doi:10.1136/bmj.k1315
12. Risacher SL, McDonald BC, Tallman EF, et al. Association between anticholinergic medication use and cognition, brain metabolism, and brain atrophy in cognitively normal older adults. *JAMA Neurol*. 2016;73(6):721-732. doi:10.1001/jamaneurol.2016.0580
13. Ballinger EC, Ananth M, Talmage DA, Role LW. Basal forebrain cholinergic circuits and signaling in cognition and cognitive decline. *Neuron*. 2016;91(6):1199-1218. doi:10.1016/j.neuron.2016.09.006
14. Knox D. The role of basal forebrain cholinergic neurons in fear and extinction memory. *Neurobiol Learn Mem*. 2016;133:39-52. doi:10.1016/j.nlm.2016.06.001
15. Solari N, Hangya B. Cholinergic modulation of spatial learning, memory and navigation. *Eur J Neurosci*. 2018;48(5):2199-2230. doi:10.1111/ejn.14089
16. Andre L, Gallini A, Montastruc F, et al. Association between anticholinergic (atropinic) drug exposure and cognitive function in longitudinal studies among individuals over 50 years old: a systematic review. *Eur J Clin Pharmacol*. 2019;75(12):1631-1644. doi:10.1007/s00228-019-02744-8
17. Ghezzi E, Chan M, Kalisch Ellett LM, et al. The effects of anticholinergic medications on cognition in children: a systematic review and meta-analysis. *Sci Rep*. 2021;11(1):219. doi:10.1038/s41598-020-80211-6
18. Attoh-Mensah E, Loggia G, Schumann-Bard P, et al. Adverse effects of anticholinergic drugs on cognition and mobility: cutoff for impairment in a cross-sectional study in young-old and old-old adults. *Drugs Aging*. 2020;37(4):301-310. doi:10.1007/s40266-019-00743-z
19. Bottiggi KA, Salazar JC, Yu L, et al. Lone-term cognitive impact of anticholinergic medications in older adults. *Am J Geriatr Psychiatry*. 2006;14(11):980-984. doi:10.1097/01.JGP.0000224619.87681.71
20. Kashyap M, Belleville S, Mulsant BH, et al. Methodological challenges in determining longitudinal associations between anticholinergic drug use and incident cognitive decline. *J Am Geriatr Soc*. 2014;62(2):336-341. doi:10.1111/jgs.12632
21. Neelamegam M, Zgibor J, Chen H, et al. The effect of cumulative anticholinergic use on the cognitive function of older adults: results from the personality and total health (PATH) through life study. *J Gerontol A Biol Sci Med Sci*. 2020;75(9):1706-1714. doi:10.1093/gerona/glaa145
22. Low LF, Anstey KJ, Sachdev P. Use of medications with anticholinergic properties and cognitive function in a young-old community sample. *Int J Geriatr Psychiatry*. 2009;24(6):578-584. doi:10.1002/gps.2157
23. Carrière I, Fourrier-Reglat A, Dartigues JF, et al. Drugs with anticholinergic properties, cognitive decline, and dementia in an elderly general population: the 3-city study. *Arch Intern Med*. 2009;169(14):1317-1324. doi:10.1001/archinternmed.2009.229
24. Moriarty F, Savva GM, Grossi CM, et al. Cognitive decline associated with anticholinergics, benzodiazepines and Z-drugs: findings from The Irish Longitudinal Study on Ageing (TILDA). *Br J Clin Pharmacol*. 2021;87(7):2818-2829. doi:10.1111/bcp.14687
25. Papenberg G, Bäckman L, Fratiglioni L, Laukka EJ, Fastbom J, Johnell K. Anticholinergic drug use is associated with episodic memory decline in older adults without dementia. *Neurobiol Aging*. 2017;55:27-32. doi:10.1016/j.neurobiolaging.2017.03.009
26. Han L, Agostini JV, Allore HG. Cumulative anticholinergic exposure is associated with poor memory and executive function in older men. *J Am Geriatr Soc*. 2008;56(12):2203-2210. doi:10.1111/j.1532-5415.2008.02009.x
27. Koyama A, Steinman M, Ensrud K, Hillier TA, Yaffe K. Long-term cognitive and functional effects of potentially inappropriate medications in older women. *J Gerontol A Biol Sci Med Sci*. 2014;69(4):423-429. doi:10.1093/gerona/glt192
28. Ancelin ML, Artero S, Portet F, Dupuy AM, Touchon J, Ritchie K. Non-degenerative mild cognitive impairment in elderly people and use of anticholinergic drugs: longitudinal cohort study. *BMJ*. 2006;332(7539):455-459. doi:10.1136/bmj.38740.439664.DE
29. Drag LL, Wright SL, Bieliauskas LA. Prescribing practices of anticholinergic medications and their association with cognition in an extended care setting. *J Appl Gerontol*. 2010;31(2):239-259. doi:10.1177/0733464810384592
30. Al Rihani SB, Deodhar M, Darakjian LI, et al. Quantifying anticholinergic burden and sedative load in older adults with polypharmacy: a systematic review of risk scales and models. *Drugs Aging*. 2021;38(11):977-994. doi:10.1007/s40266-021-00895-x
31. Farmer RL, Floyd RG, Reynolds MR, Berlin KS. How can general intelligence composites most accurately index psychometric g and what might be good enough? *Contemp Sch Psychol*. 2019;24(1):52-67.
32. Calvin CM, Batty GD, Der G, et al. Childhood intelligence in relation to major causes of death in 68 year follow-up: prospective population study. *BMJ*. 2017;357:j2708. doi:10.1136/bmj.j2708

33. Twig G, Tirosh A, Derazne E, et al. Cognitive function in adolescence and the risk for premature diabetes and cardiovascular mortality in adulthood. *Cardiovasc Diabetol*. 2018;17(1):154. doi:10.1186/s12933-018-0798-5
34. Batty GD, Deary IJ, Gottfredson LS. Premorbid (early life) IQ and later mortality risk: systematic review. *Ann Epidemiol*. 2007;17(4):278-288. doi:10.1016/j.annepidem.2006.07.010
35. Deary IJ, Cox SR, Hill WD. Genetic variation, brain, and intelligence differences. *Mol Psychiatry*. 2022;27(1):335-353. doi:10.1038/s41380-021-01027-y
36. Kaup AR, Mirzakhanian H, Jeste DV, Eyler LT. A review of the brain structure correlates of successful cognitive aging. *J Neuropsychiatry Clin Neurosci*. 2011;23(1):6-15. doi:10.1176/appi.neuropsych.23.1.6
37. Cox SR, Ritchie SJ, Fawns-Ritchie C, Tucker-Drob EM, Deary IJ. Structural brain imaging correlates of general intelligence in UK Biobank. *Dermatol Int*. 2019;76:101376. doi:10.1016/j.intell.2019.101376
38. Meng D, Mohammadi-Nejad AR, Sotiropoulos SN, Auer DP. Alzheimer's disease neuroimaging I. Anticholinergic drugs and forebrain magnetic resonance imaging changes in cognitively normal people and those with mild cognitive impairment. *Eur J Neurol*. 2022;29(5):1344-1353. doi:10.1111/ene.15251
39. Kilimann I, Wucherer D, Ittermann T, et al. Inverse association between the anticholinergic burden and hippocampus volume in a population-based cohort across the entire adult age range. *Geroscience*. 2022;44(3):1715-1726. doi:10.1007/s11357-021-00497-w
40. Sudlow C, Gallacher J, Allen N, et al. UK biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. *PLoS Med*. 2015;12(3):e1001779. doi:10.1371/journal.pmed.1001779
41. Fawns-Ritchie C, Deary IJ. Reliability and validity of the UK Biobank cognitive tests. *PLoS ONE*. 2020;15(4):e0231627. doi:10.1371/journal.pone.0231627
42. Lyall DM, Cullen B, Allerhand M, et al. Cognitive test scores in UK Biobank: data reduction in 480,416 participants and longitudinal stability in 20,346 participants. *PLoS ONE*. 2016;11(4):e0154222. doi:10.1371/journal.pone.0154222
43. MacPherson SE, Allerhand M, Cox SR, Deary IJ. Individual differences in cognitive processes underlying trail making test-B performance in old age: the Lothian birth cohort 1936. *Dermatol Int*. 2019;75:23-32. doi:10.1016/j.intell.2019.04.001
44. Littlejohns TJ, Holliday J, Gibson LM, et al. The UK Biobank imaging enhancement of 100,000 participants: rationale, data collection, management and future directions. *Nat Commun*. 2020;11(1):2624. doi:10.1038/s41467-020-15948-9
45. Alfaro-Almagro F, Jenkinson M, Bangerter NK, et al. Image processing and quality control for the first 10,000 brain imaging datasets from UK Biobank. *Neuroimage*. 2018;166:400-424. doi:10.1016/j.neuroimage.2017.10.034
46. Bishara D, Harwood D, Sauer J, Taylor DM. Anticholinergic effect on cognition (AEC) of drugs commonly used in older people. *Int J Geriatr Psychiatry*. 2017;32(6):650-656. doi:10.1002/gps.4507
47. Boustani M, Campbell N, Munger S, Maidment I, Fox C. Impact of anticholinergics on the aging brain: a review and practical application. *Aging Health*. 2008;4(3):311-320. doi:10.2217/1745509X.4.3.311
48. Briet J, Javelot H, Heitzman E, et al. The anticholinergic impregnation scale: towards the elaboration of a scale adapted to prescriptions in French psychiatric settings. *Therapie*. 2017;72(4):427-437. doi:10.1016/j.therap.2016.12.010
49. Cancelli I, Valentinis L, Merlino G, Valente M, Gigli GL. Drugs with anticholinergic properties as a risk factor for psychosis in patients affected by Alzheimer's disease. *Clin Pharmacol Ther*. 2008;84(1):63-68. doi:10.1038/sj.clpt.6100435
50. Carnahan RM, Lund BC, Perry PJ, Pollock BG, Culp KR. The anticholinergic drug scale as a measure of drug-related anticholinergic burden: associations with serum anticholinergic activity. *J Clin Pharmacol*. 2006;46(12):1481-1486. doi:10.1177/0091270006292126
51. Chew ML, Mulsant BH, Pollock BG, et al. Anticholinergic activity of 107 medications commonly used by older adults. *J Am Geriatr Soc*. 2008;56(7):1333-1341. doi:10.1111/j.1532-5415.2008.01737.x
52. Durán CE, Azermai M, Vander Stichele RH. Systematic review of anticholinergic risk scales in older adults. *Eur J Clin Pharmacol*. 2013;69(7):1485-1496. doi:10.1007/s00228-013-1499-3
53. Ehrt U, Broich K, Larsen JP, Ballard C, Aarsland D. Use of drugs with anticholinergic effect and impact on cognition in Parkinson's disease: a cohort study. *J Neurol Neurosurg Psychiatry*. 2010;81(2):160-165. doi:10.1136/jnnp.2009.186239
54. Han L, McCusker J, Cole M, Abrahamowicz M, Primeau F, Élie M. Use of medications with anticholinergic effect predicts clinical severity of delirium symptoms in older medical inpatients. *Arch Intern Med*. 2001;161(8):1099-1105. doi:10.1001/archinte.161.8.1099
55. Kiesel EK, Hopf YM, Drey M. An anticholinergic burden score for German prescribers: score development. *BMC Geriatr*. 2018;18(1):239. doi:10.1186/s12877-018-0929-6
56. Rudolph JL, Salow MJ, Angelini MC, McGlinchey RE. The anticholinergic risk scale and anticholinergic adverse effects in older persons. *Arch Intern Med*. 2008;168(5):508-513. doi:10.1001/archinternmed.2007.106
57. Sittironnarit G, Ames D, Bush AI, et al. Effects of anticholinergic drugs on cognitive function in older Australians: results from the AIBL study. *Dement Geriatr Cogn Disord*. 2011;31(3):173-178. doi:10.1159/000325171
58. Jun K, Hwang S, Ah YM, Suh Y, Lee JY. Development of an anticholinergic burden scale specific for Korean older adults. *Geriatr Gerontol Int*. 2019;19(7):628-634. doi:10.1111/ggi.13680
59. Nery RT, Reis AMM. Development of a Brazilian anticholinergic activity drug scale. *Einstein (Sao Paulo)*. 2019;17(2):eAO4435. doi:10.31744/einstein_journal/2019AO4435
60. Sumukadas D, McMurdo ME, Mangoni AA, Guthrie B. Temporal trends in anticholinergic medication prescription in older people: repeated cross-sectional analysis of population prescribing data. *Age Ageing*. 2014;43(4):515-521. doi:10.1093/ageing/aft199
61. ATC/DDD Index 2022. December 14th, 2021. https://www.whocc.no/atc_ddd_index/ (accessed February 9th, 2022 2022).
62. Townsend P. Deprivation. *J Soc Policy*. 1987;16(2):125-146. doi:10.1017/S0047279400020341
63. Hanlon P, Quinn TJ, Gallacher KI, et al. Assessing risks of polypharmacy involving medications with anticholinergic properties. *Ann Fam Med*. 2020;18(2):148-155. doi:10.1370/afm.2501
64. Benjamini Y, Hochberg Y. Controlling the false discovery rate a practical and powerful approach to multiple testing. *J R Stat Soc B*. 1995;57(1):289-300. doi:10.1111/j.2517-6161.1995.tb02031.x
65. Royston P, Altman DG, Sauerbrei W. Dichotomizing continuous predictors in multiple regression: a bad idea. *Stat Med*. 2006;25(1):127-141. doi:10.1002/sim.2331
66. Marek S, Tervo-Clemmens B, Calabro FJ, et al. Reproducible brain-wide association studies require thousands of individuals. *Nature*. 2022;603(7902):654-660. doi:10.1038/s41586-022-04492-9
67. Fry A, Littlejohns TJ, Sudlow C, et al. Comparison of sociodemographic and health-related characteristics of UK Biobank participants with those of the general population. *Am J Epidemiol*. 2017;186(9):1026-1034. doi:10.1093/aje/kwx246
68. Lyall DM, Quinn T, Lyall LM, et al. Quantifying bias in psychological and physical health in the UK Biobank imaging sub-sample. *Brain Commun*. 2022;4(3):fcac119. doi:10.1093/braincomms/fcac119

69. Jyrkka J, Enlund H, Lavikainen P, Sulkava R, Hartikainen S. Association of polypharmacy with nutritional status, functional ability and cognitive capacity over a three-year period in an elderly population. *Pharmacoepidemiol Drug Saf*. 2011;20(5):514-522. doi:10.1002/pds.2116
70. Rawle MJ, Cooper R, Kuh D, Richards M. Associations between polypharmacy and cognitive and physical capability: a British birth cohort study. *J Am Geriatr Soc*. 2018;66(5):916-923. doi:10.1111/jgs.15317
71. Leelakanok N, D'Cunha RR. Association between polypharmacy and dementia a systematic review and metaanalysis. *Aging Ment Health*. 2019;23(8):932-941. doi:10.1080/13607863.2018.1468411
72. Wilkinson T, Schnier C, Bush K, et al. Drug prescriptions and dementia incidence: a medication-wide association study of 17000 dementia cases among half a million participants. *J Epidemiol Community Health*. 2022;76(3):223-229. doi:10.1136/jech-2021-217090
73. Heran BS, Wong MM, Heran IK, Wright JM. Blood pressure lowering efficacy of angiotensin converting enzyme (ACE) inhibitors for primary hypertension. *Cochrane Database Syst Rev*. 2008;4:CD003823.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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6.3 Conclusion

This chapter has presented the results of analyses examining the association between AChB, general cognitive ability, and brain structure in relatively healthy people. In contrast to previous studies, I used a compound score of cognitive ability to minimise the noise of individual cognitive tests. Furthermore, when exploring brain structure, I used a much bigger sample than previous studies. I found effects for most anticholinergic scales, but only for some drug classes, especially β-lactam antibiotics and opioids. Moreover, there were no associations between AChB and any measure of brain structural MRI. These results suggest that while anticholinergic use is predictive of a slightly lower cognitive ability in healthy participants, there is no evidence for AChB-related brain changes before the onset of very old age and dementia.

6.3.1 Supplementary material

This section contains all Supplementary material that accompanies the manuscript presented in this chapter except Supplementary Tables 3, 5, and 8-19. Supplementary Tables 3, 5, and 8-19 were too numerous for inclusion in the thesis and are available in full at https://bpspubs.onlinelibrary.wiley.com/doi/10.1111/bcp.15698.

Supplementary Table 1: UK Biobank variables and their Field IDs used in the study.

Variable	Field ID	UK Biobank showcase link
Demographic and lifestyle		
Age	21003	https://biobank.ndph.ox.ac.uk/showcase/field.cgi?id=21003
Sex	31	https://biobank.ndph.ox.ac.uk/showcase/field.cgi?id=31
Deprivation	189	https://biobank.ndph.ox.ac.uk/showcase/field.cgi?id=189
Alcohol consumption	1558	https://biobank.ndph.ox.ac.uk/showcase/field.cgi?id=1558
Smoking	20116	https://biobank.ndph.ox.ac.uk/showcase/field.cgi?id=20116
Physical activity	6164	https://biobank.ndph.ox.ac.uk/showcase/field.cgi?id=6164
BMI	21001	https://biobank.ndph.ox.ac.uk/showcase/field.cgi?id=21001
Data provider	42039	https://biobank.ndph.ox.ac.uk/showcase/field.cgi?id=42039
APOE carrier	22418	https://biobank.ndph.ox.ac.uk/showcase/field.cgi?id=22418
Ethnicity	21000	https://biobank.ndph.ox.ac.uk/showcase/field.cgi?id=21000
Imaging assessment centre	54	https://biobank.ndph.ox.ac.uk/showcase/field.cgi?id=54
Date of attending assessment centre	53	https://biobank.ndph.ox.ac.uk/showcase/field.cgi?id=53
Prescriptions and diagnoses		
Inpatient diagnoses	41270 41271 41280 41281	https://biobank.ndph.ox.ac.uk/showcase/field.cgi?id=41270 https://biobank.ndph.ox.ac.uk/showcase/field.cgi?id=41271 https://biobank.ndph.ox.ac.uk/showcase/field.cgi?id=41280 https://biobank.ndph.ox.ac.uk/showcase/field.cgi?id=41281
Primary care diagnoses	42040	https://biobank.ndph.ox.ac.uk/showcase/field.cgi?id=42040
Primary care prescriptions	42039	https://biobank.ndph.ox.ac.uk/showcase/field.cgi?id=42039
Self-reported illness	20002	https://biobank.ndph.ox.ac.uk/showcase/field.cgi?id=20002
Cognitive tests		
DSS	23324	https://biobank.ndph.ox.ac.uk/showcase/field.cgi?id=23324
MR	6373	https://biobank.ndph.ox.ac.uk/showcase/field.cgi?id=6373
NM	4282	https://biobank.ndph.ox.ac.uk/showcase/field.cgi?id=4282
ProsMem	20018	https://biobank.ndph.ox.ac.uk/showcase/field.cgi?id=20018
RT	20023	https://biobank.ndph.ox.ac.uk/showcase/field.cgi?id=20023
TMTb	6350	https://biobank.ndph.ox.ac.uk/showcase/field.cgi?id=6350
TR	21004	https://biobank.ndph.ox.ac.uk/showcase/field.cgi?id=21004
VisMem	399	https://biobank.ndph.ox.ac.uk/showcase/field.cgi?id=399
VNR	20016	https://biobank.ndph.ox.ac.uk/showcase/field.cgi?id=20016

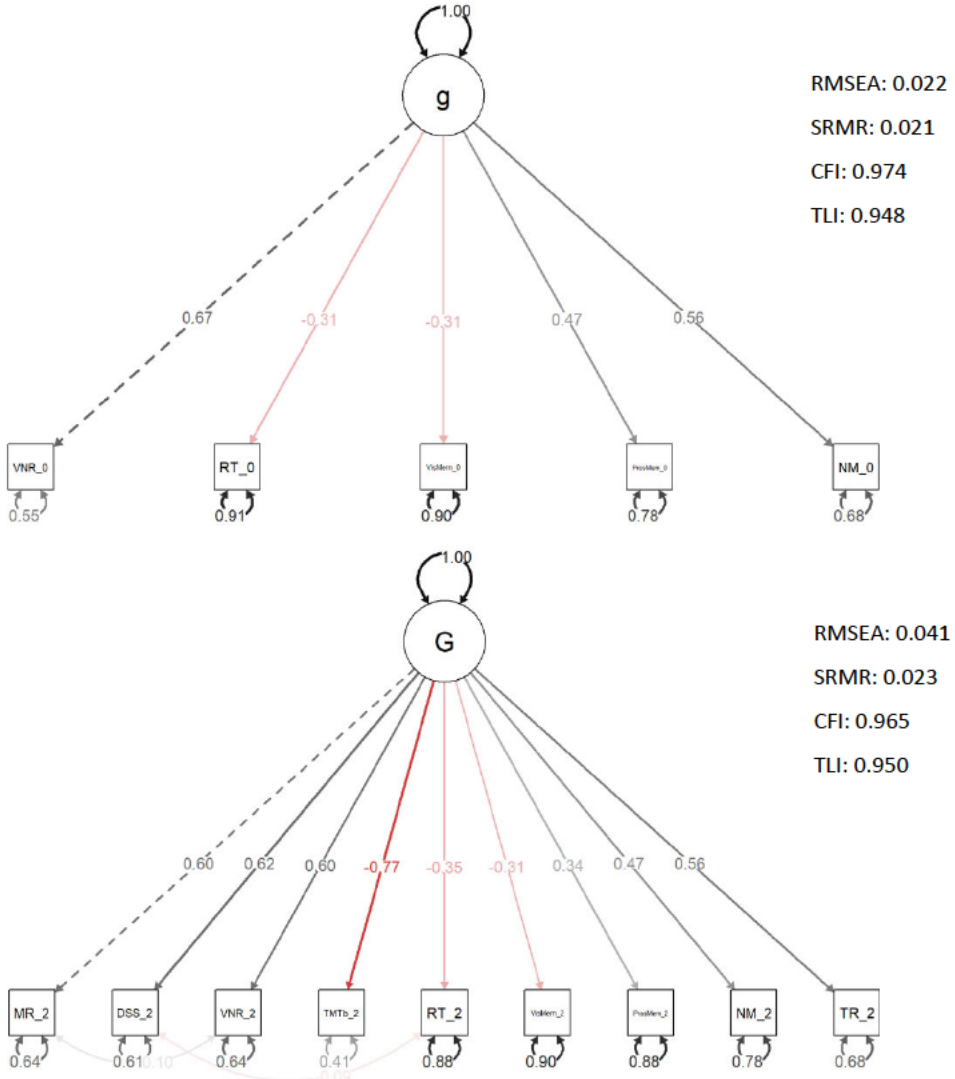
MRI imaging		
Total brain volume	25010	https://biobank.ndph.ox.ac.uk/showcase/field.cgi?id=25010
Cortical areas volume	27205-27235 27298-27328	https://biobank.ndph.ox.ac.uk/showcase/label.cgi?id=196
Subcortical areas volume	25011-25024	https://biobank.ndph.ox.ac.uk/showcase/label.cgi?id=1102
FA and MD of white matter	25488-25514 25515-25541	https://biobank.ndph.ox.ac.uk/showcase/label.cgi?id=135
Head position in scanner	25756-25758	https://biobank.ndph.ox.ac.uk/showcase/field.cgi?id=25756 https://biobank.ndph.ox.ac.uk/showcase/field.cgi?id=25757 https://biobank.ndph.ox.ac.uk/showcase/field.cgi?id=25758
T1 head size scaling factor	25000	https://biobank.ndph.ox.ac.uk/showcase/field.cgi?id=25000

Supplementary Table 2: All cognitive tests available in UK Biobank, with the mean and standard deviation for each test (before scaling), and the numbers of participants from our sample (and % of the sample) that underwent testing at either the baseline or the imaging assessment. The greyed-out tests were not used in our study, either due to measuring crystallised cognitive ability (Picture Vocabulary) or due to ceiling-effects (Paired Associative Learning). The Trail Making Tests, Tower Rearranging, Matrix Pattern Completion, and Symbol Digit Substitution were not administered during the baseline assessment. The data has been cleaned for outliers, defined as values four or more standard deviations below or above the mean.

Cognitive test	Baseline assessment			Imaging assessment		
	N (%)	Mean	Std.	N (%)	Mean	Std.
Pairs Matching	161,944 (99.3)	1.42	0.64	18,649 (11.4)	1.34	0.62
Reaction Time	160,571 (98.5)	6.30	0.18	18,546 (11.4)	6.37	0.17
Numeric Memory	25,565 (15.7)	6.71	1.32	13,856 (8.5)	6.78	1.27
Prospective Memory*	63,623 (39.0)	14,493 (22.7)		18,746 (11.5)	3,133 (16.7)	
Fluid Intelligence	61,599 (37.8)	6.0	2.13	18,403 (11.3)	6.60	2.06
Trail Making				13,082 (8.0)	6.29	0.34
Tower Rearranging				13,421 (8.2)	9.86	3.25
Paired Associative Learning						
Matrix Pattern Completion				13,539 (8.3)	7.93	2.13
Picture Vocabulary						
Symbol Digit Substitution				13,543 (8.3)	18.8	5.29

**Note:* Prospective memory was a binary variable; the values indicate the numbers (and %) of participants with correct recall.

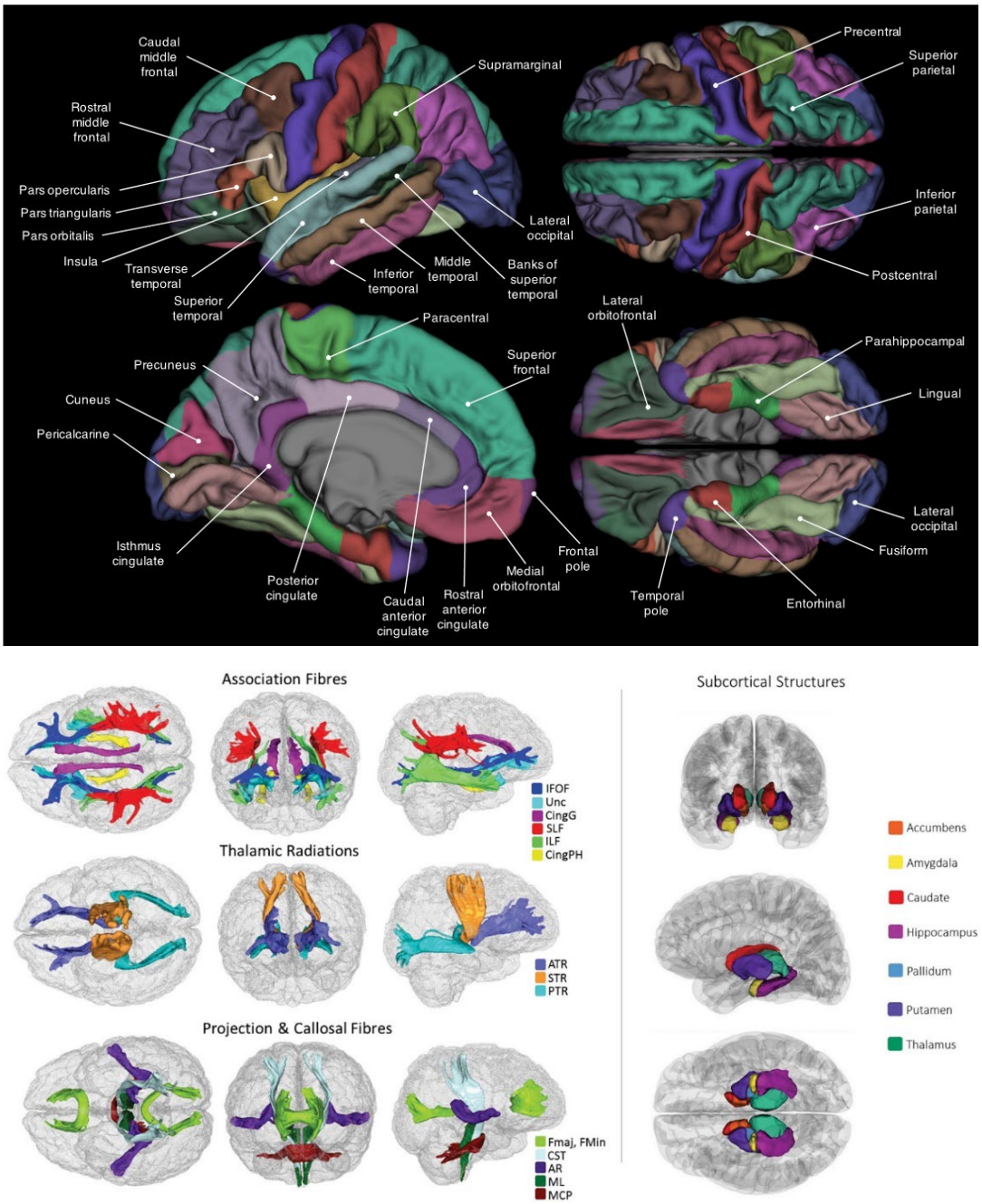
Supplementary Figure 1: Path diagram for SEM calculating the latent *g* from individual cognitive tests administered during the baseline assessment (**top**) or during the imaging assessment (**bottom**). The arrows depict standardised loadings (the latent variable and the observed variables have a variance of 1), with positive loadings depicted with grey one-way arrows and negative loadings depicted with red one-way arrows. The dotted line indicates that the (unstandardised) loading was fixed to 1. MR and VNR on the one hand, and DSS and RT one the other hand measure similar cognitive abilities and residual correlations between them were included in the model; this is depicted by two-way arrows between the cognitive



tests. Two-way arrows within individual cognitive tests represent residual variances. The total variance in cognitive test scores explained by the latent factor was 0.23 for the baseline assessment and 0.28 for the imaging assessment. The model-fit statistics are displayed on the right side of each diagram.

Note: MR: Matrix Pattern Completion; DSS: Digit Symbol Substitution; VNR: Fluid Intelligence; TMTb: Trail Making Test B; RT: Reaction Time; VisMem: Pairs Matching; ProsMem: Prospective Memory; NM: Numeric Memory; TR: Tower Rearranging.

Supplementary Figure 2: Cortical regions from the Desikan-Killiany neuroanatomical atlas (**top**), white matter tracts (**bottom left**) and subcortical structures (**bottom right**) measured



in the present study. Figures reused from previous studies[1,2].

Note: AR, acoustic radiation; ATR, anterior thalamic radiation; Cing, cingulum (gyrus and parahippocampal); CST, corticospinal tract; Fmaj and Fmin (forceps major and minor); IFOF, inferior fronto-occipital fasciculus; ILF, inferior longitudinal fasciculus; MCP, middle

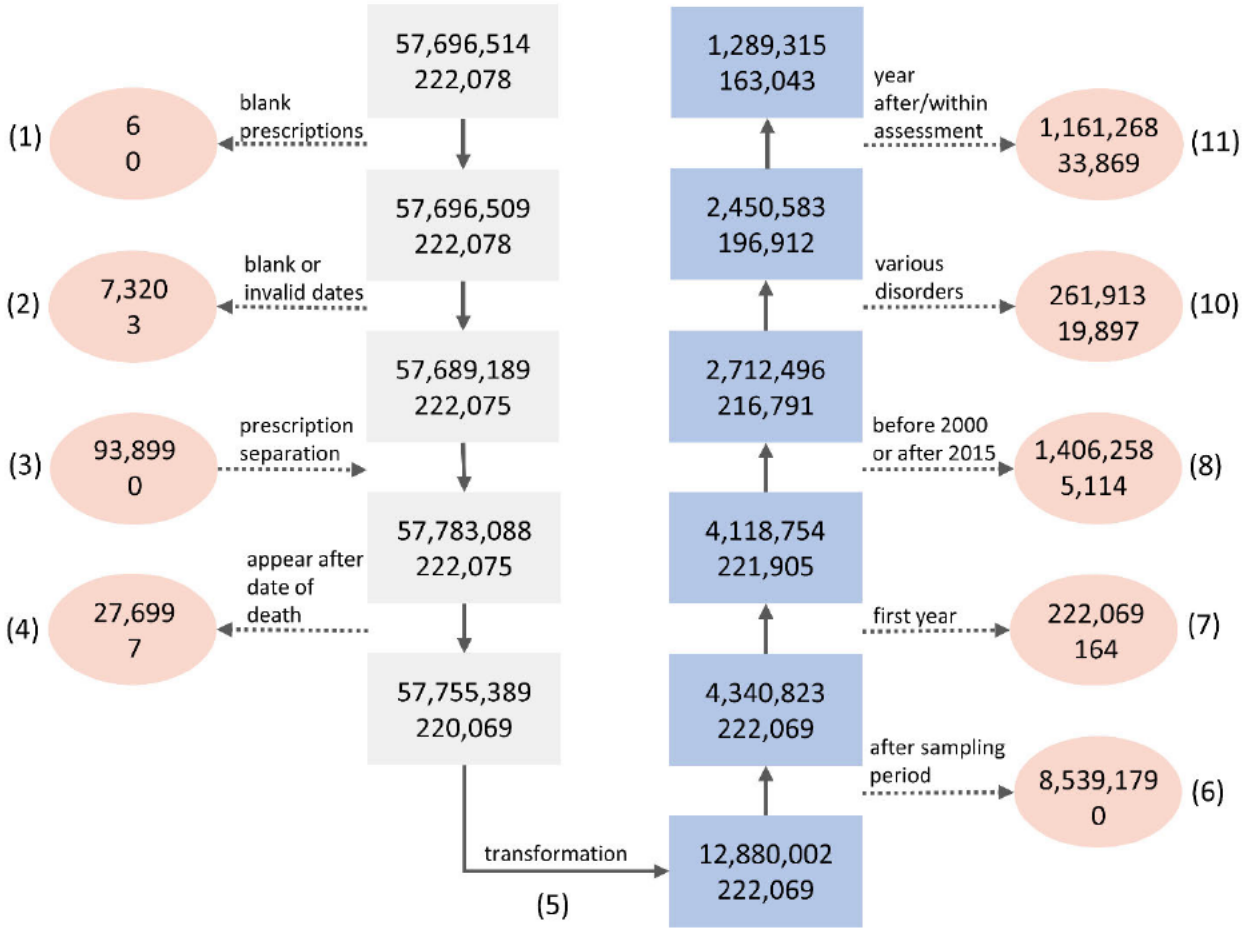
cerebellar peduncle; ML, medial lemniscus; PTR, posterior thalamic radiation; SLF, superior longitudinal fasciculus; STR, superior thalamic radiation; Unc, uncinate fasciculus.

Supplementary Table 4: Anticholinergics scales identified in the present study. We considered anticholinergic scales that were available as complete lists of drugs, scored each drug for its anticholinergic potency, and did not require information on dosage. Grey shading indicates that the scale was not considered for further analysis. For two scales[3,4], updated versions (Aging Brain Care, 2012; Carnahan, 2014, personal communication on 21.10.2019) were used. One scale[5] was updated to include newer drugs from the UK market as has been done before[6]. The table was modified from a previous study[7].

Surname of first author	Scale name	Year of publication	Reason for exclusion
Summers[8]	Drug Risk Number (DRN)	1978	Outdated (based on the date of publication and on new scales developed on its basis).
Han[9]	Clinician-rated Anticholinergic Scale (CrAS)	2001	
Aizenberg[10]	Anticholinergic Burden Score (ABS)	2002	Publicly unavailable and no response from lead author to two email requests within a year.
Minzenberg[11]	n.a.	2004	Based on a reference compound.
Ancelin[12]	Anticholinergic Burden Classification (ABC) scale	2006	
Carnahan[4]	Anticholinergic Drug Scale (ADS)	2006 (2014)	
Hilmer[13]	Drug Burden Index (DBI)	2007	Required information on drug dosage.
Chew[14]	Anticholinergic Activity Scale (AAS)	2008	
Cancelli[15]	n.a.	2008	
Rudolph[5]	Anticholinergic Risk Scale (ARS)	2008 (2013)	
Ehrt[16]	Revised Anticholinergic Activity Scale (AAS-r)	2010	
Sittironnarit[17]	Anticholinergic Loading Scale (ALS)	2011	
Boustani[3]	Anticholinergic Cognitive Burden (ACB)	2008 (2012)	
Whalley[18]	n.a.	2012	Unavailable in full.
Durán[19]	n.a.	2013	
Faure[20]	Drug Burden Index, International Version (DBI-WHO)	2014	Required information on drug dosage.

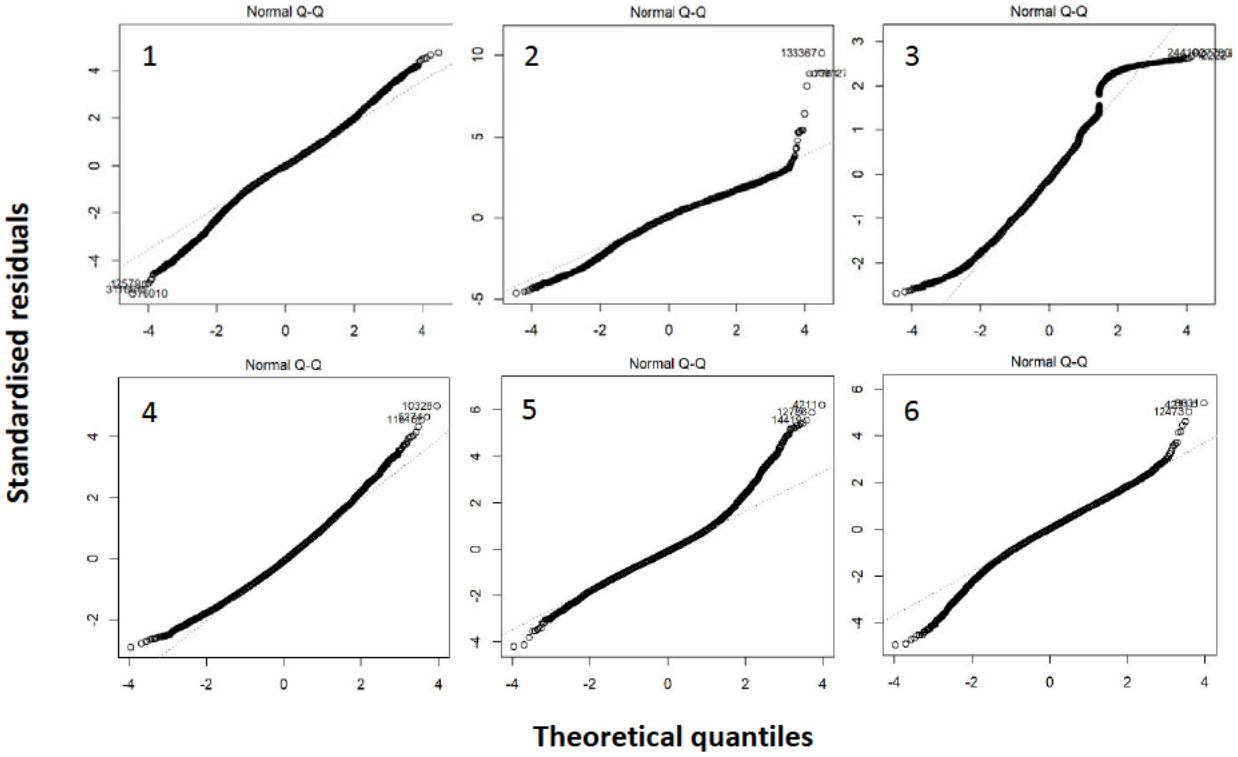
Klamer[21]	MARANTE	2017	Required information on drug dosage.
Bishara[22]	Anticholinergic effect on cognition (AEC) scale	2017	
Briet[23]	Anticholinergic impregnation scale	2017	
Kiesel[24]	n.a.	2018	
Nery[25]	Brazilian anticholinergic activity drug scale	2019	
Jun[26]	Korean Anticholinergic Burden Scale (KABS)	2019	

Supplementary Figure 3: The data-cleaning pipeline. The upper and lower rows of numbers represent the numbers of observation and participants, respectively. The rectangular boxes display the numbers of observations/participants at each point of the data-cleaning process, while the ovals display the numbers of observations/participants removed. The colour of the rectangles indicates the type of observation: in the grey boxes, the basic observation was a

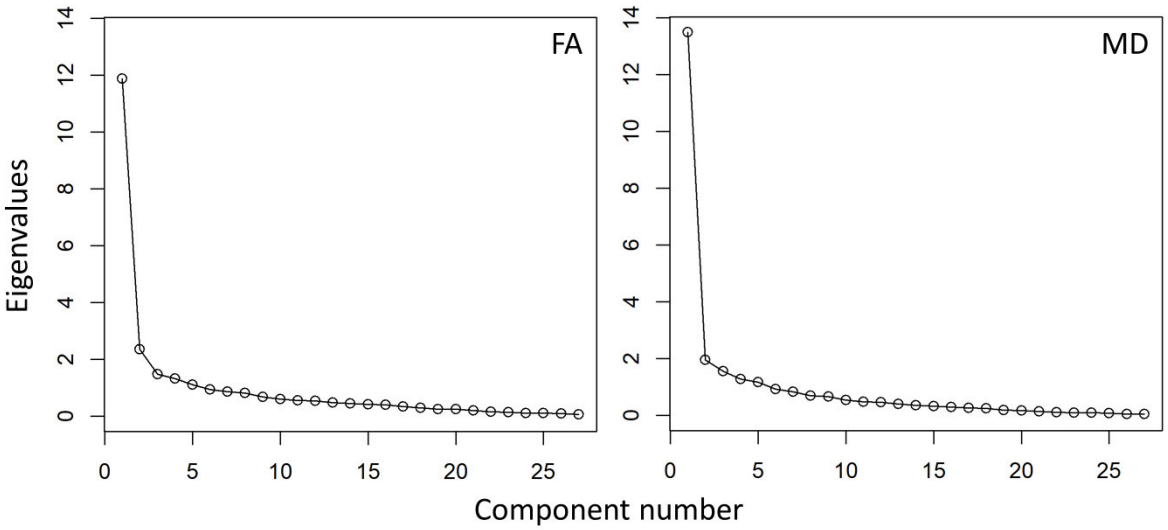


single prescription; in the blue boxes, it was a year-participant pair, with each pair representing the AChB of a single participant in a given year. The actions performed at each step are written above the arrows and signify the (1) removal of prescriptions without any content (i.e., no drug indicated), (2) removal of prescriptions without dates or impossible dates (e.g., in the future or far in the past), (3) separation of combination drugs into individual compounds, (4) removal of prescriptions that appear after the death of the participant, (5) transformation into the year-participant format, (6) removal of observations (generated in step (5)) occurring after the end of the prescription-sampling period for any participant, (7) removal of observations for the first year in the dataset for each participant (as it is unlikely to be complete), (8) removal of observations prior to the year 2000 and after the year 2015, (9) removal of observations for participants diagnosed with a disorder that may affect cognitive or brain function, (10) removal of observations after or within the year of the UK Biobank assessment.

Supplementary Figure 4: Q-Q plots of theoretical quantiles (x-axis) vs. standardised residuals (y-axis) for some models used in our study. Only the examples of models exhibiting most extreme kurtosis are shown. The examples here are for the association between AChB according to Durán et al. (2013) [19] on the one hand and either *g* (1), reaction time (2), visual memory (3), volume of the right pastriangularis (4), volume of the left pallidum (5), or volume of the right hippocampus (6), on the other.



Supplementary Figure 5: Scree plots of eigenvalues for FA (**left**) and MD (**right**). The principal component for FA explained 43.9% of the total variance and the principal component for MD



explained 49.9% of the total variance.

Supplementary Text 1: The models used in our study. (1.0) was used to compare anticholinergic scales (with each model using g as the outcome) and to compare cognitive tests (with each model using the scale by Durán et al. (2013) [19] as the predictor and a different cognitive test as the outcome). (1.1) is the basic polypharmacy model; it differs from (1.0) in that the number of non-anticholinergic drugs is the main predictor. (1.2) is the Polypharmacy Plus model that differs from (1.1) in that the number of anticholinergic drugs (according to any anticholinergic scale) is included as a covariate. (2.0) comprises of models that predict any measure of brain-MRI by AChB (according to the scale by Durán et al. (2013) [19] when not comparing scales) and include additional covariates. Polypharmacy models (analogous to 1.1 and 1.2, but with additional covariates as in 2.0) were also run for total brain volume when comparing anticholinergic scales.

1.0 $Cognition_{scale}$

$$= lm(cognition \sim AChB_{scale} + non.anticholinergic.drug.number + age + number.prescription.years + data.provider + deprivation + smoking + alcohol + physical.activity + BMI + APOE + comorbidities + mood.disorder + anxiety.disorder + schizophrenia + diabetes + hypercholesterolemia + hypertension + myocardial.infarction)$$

1.1 $Cognition_{polypharmacy}$

$$= lm(g \sim drug.count + age + number.prescription.years + data.provider + deprivation + smoking + alcohol + physical.activity + BMI + APOE + comorbidities + mood.disorder + anxiety.disorder + schizophrenia + diabetes + hypercholesterolemia + hypertension + myocardial.infarction)$$

1.2 $Cognition_{polypharmacy.plus}$

$$= lm(g \sim drug.count + anticholinergic.drug.number + +age + number.prescription.years + data.provider + deprivation + smoking + alcohol + physical.activity + BMI + APOE + comorbidities + mood.disorder + anxiety.disorder + schizophrenia + diabetes + hypercholesterolemia + hypertension + myocardial.infarction)$$

$$2.\ MRI_{scale} = lm(MRI \sim AChB_{scale} + non.anticholinergic.drug.number + +age + number.prescription.years + data.provider + deprivation + smoking + alcohol + physical.activity + BMI + APOE + comorbidities + mood.disorder + anxiety.disorder + schizophrenia + diabetes + hypercholesterolemia + hypertension + myocardial.infarction + age^2 + age * sex + age^2 * sex + head.position + ethnicity + asessment.centre)$$

Supplementary Table 6: Demographic and lifestyle characteristics of separately the imaging subsample and the rest of the sample after the removal of outliers. The columns indicate the median and IQR (or n and % for categorical variables) and the number of missing observations. The variables are not scaled. Note that the counts may not always add up to those depicted in **Table 1**, as the imaging subsample underwent separate data-cleaning before running the analyses.

Variable	Level	Imaging subsample		Rest of sample	
		Median (IQR) or n (%)	N missing	Median (IQR) or n (%)	N missing
Age (years)		64.8 (11.9)		58.6 (12.9)	
Sex	Male	8,072 (46.6)		62,640 (43.8)	
		9,265 (53.4)		80,412 (56.2)	
Deprivation (z-score)		-2.7 (3.2)	21	-2.2 (3.9)	149
Alcohol consumption	Daily or almost daily	2,926 (17.0)	117	27,778 (19.5)	316
	Three or four times a week	4,927 (28.6)		32,812 (23.0)	
	Once or twice a week	4,536 (26.3)		38,103 (26.7)	
	Once to three times a month	1,994 (11.6)		16,225 (11.4)	
	Only special occasions	1,713 (9.9)		16,430 (11.5)	
	Never	1,124 (6.5)		11,388 (8.0)	
Smoking	Current smoker	561 (3.3)	165	14,819 (10.4)	740
	Previous smoker	5,744 (33.4)		49,029 (34.5)	
	Non-smoker	10,867 (63.3)		78,464 (55.1)	
Physical activity	Strenuous	2,232 (13.3)	513	13,583 (10.2)	10,030
	Moderate	11,346 (67.4)		84,918 (63.8)	
	Light	3,246 (19.3)		34,521 (26.0)	
BMI (kg/m²)		25.8 (5.3)	590	26.9 (5.8)	987
Region	England (Vision)	1,524 (8.8)		12,592 (8.8)	
	Scotland	1,500 (8.7)		7,862 (5.5)	
	England (TPP)	14,133 (81.5)		105,849 (74.0)	
	Wales	180 (1.0)		16,749 (11.7)	
Mood disorder		2,126 (12.3)		21,431 (15.0)	
Anxiety disorder		1,250 (7.2)		14,037 (9.8)	
Schizophrenia		20 (0.12)		555 (0.4)	
Myocardial infarction		370 (2.1)		6,762 (4.7)	
Diabetes		766 (4.4)		13,425 (9.4)	

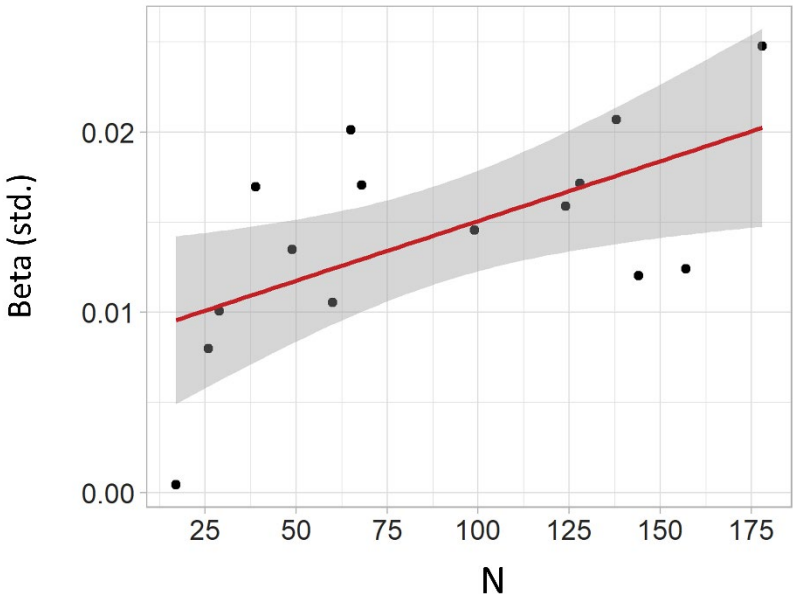
Hypercholesterolemia		2,041 (11.8)		27,259 (19.1)	
Hypertension		3,729 (21.5)		49,191 (34.4)	
Number of prior comorbidities		135 (103)	2	81 (92)	47
Polypharmacy		49 (132)		33 (92)	
APOE carrier	ε2	2,224 (13.1)	329	18,010 (12.9)	3,490
	ε3	10,498 (61.7)		85,949 (61.6)	
	ε4	4,286 (25.2)		35,603 (25.5)	
Ethnicity	British	15,942 (93.0)	189	128,246 (91.1)	2,215
	Irish	376 (2.2)		3,307 (2.3)	
	Any other white background	481 (2.8)		3,906 (2.8)	
	White and black Caribbean	16 (0.09)		169 (0.12)	
	White and black African	7 (0.04)		94 (0.07)	
	White and Asian	29 (0.17)		204 (0.15)	
	Any other mixed background	22 (0.13)		250 (0.18)	
	Indian	131 (0.76)		2,006 (1.4)	
	Pakistani	33 (0.19)		606 (0.43)	
	Bangladeshi	2 (0.01)		47 (0.03)	
	Any other Asian background	27 (0.16)		434 (0.31)	
	Caribbean	46 (0.27)		1,005 (0.71)	
	African	35 (0.20)		538 (0.38)	
	Any other black background	1 (0.006)		25 (0.02)	
Imaging assessment centre	Cheadle	11,534 (60.7)			
	Reading	1,208 (7.0)			
	Newcastle	5,581 (32.2)			
	Bristol	14 (0.08)			

Supplementary Table 7: Numbers (and % of total prescriptions) of anticholinergic prescriptions per anticholinergic scale and numbers (and % of total sample) of participants prescribed at least one anticholinergic prescription in the sampling period. The data include the period between the year 2000 and the year prior to attending the assessment visit (differs between participants).

Anticholinergic scale	**N (%)**	**>1 n (%)**
Ancelin et al. (2006)	632,410 (4.3)	19,656 (12.1)
Bishara et al. (2017)	1,328,952 (8.9)	41,871 (25.7)
Boustani et al. (2008*)	2,158,487 (14.5)	54,480 (33.4)
Briet et al. (2017)	2,814,146 (18.9)	60,975 (37.4)
Cancelli et al. (2008)	778,080 (5.2)	18,445 (11.3)
Carnahan et al. (2006*)	1,642,406 (11.1)	48,411 (29.7)
Chew et al. (2008)	1,912,596 (12.9)	58,773 (36.0)
Durán et al. (2013)	3,118,524 (21.0)	66,289 (40.7)
Ehrt et al. (2010)	1,336,023 (9.0)	37,931 (23.3)
Han et al. (2001)	1,952,053 (13.1)	54,311 (33.3)
Jun et al. (2019)	2,075,559 (14.0)	55,657 (34.1)
Kiesel et al. (2018)	3,579,841 (24.1)	62,889 (38.6)
Nery et al. (2019)	2,745,039 (18.5)	59,915 (36.7)
Rudolph et al. (2008*)	1,108,629 (7.5)	41,556 (25.5)
Sittironnarit et al. (2011)	2,169,407 (14.6)	55,948 (34.3)

Note*: some scales were updated after their initial date of publication, as noted in **Supplementary Table 3.

Supplementary Figure 6: Scatterplot for the association between the number of drugs identified as having an anticholinergic effect and the association with lower cognitive ability. The x-axis represents the number of drugs identified as possessing anticholinergic effects, the y-axis represents the absolute value of the standardised β for the association between AChB and *g*. Each dot in the scatterplot represents an anticholinergic scale. The red line represents the line of best fit, with grey shading indicating the 95% CI.



Supplementary references

1. Cox SR, Lyall DM, Ritchie SJ, et al. Associations between vascular risk factors and brain MRI indices in UK Biobank. *Eur Heart J* 2019; **40**(28): 2290-300.
2. Ritchie SJ, Cox SR, Shen X, et al. Sex Differences in the Adult Human Brain: Evidence from 5216 UK Biobank Participants. *Cereb Cortex* 2018; **28**(8): 2959-75.
3. Boustani M, Campbell N, Munger S, Maidment I, Fox C. Impact of anticholinergics on the aging brain: A review and practical application. *Aging Health* 2008; **4**(3): 311-20.
4. Carnahan RM, Lund BC, Perry PJ, Pollock BG, Culp KR. The Anticholinergic Drug Scale as a measure of drug-related anticholinergic burden: associations with serum anticholinergic activity. *J Clin Pharmacol* 2006; **46**(12): 1481-6.
5. Rudolph JL, Salow MJ, Angelini MC, McGlinchey RE. The anticholinergic risk scale and anticholinergic adverse effects in older persons. *Archives of Internal Medicine* 2008; **168**(5): 508-13.
6. Sumukadas D, McMurdo ME, Mangoni AA, Guthrie B. Temporal trends in anticholinergic medication prescription in older people: repeated cross-sectional analysis of population prescribing data. *Age Ageing* 2014; **43**(4): 515-21.
7. Mur J, Russ TC, Cox SR, Marioni RE, Muniz-Terrera G. Association between anticholinergic burden and dementia in UK Biobank. *Alzheimers Dement (N Y)* 2022; **8**(1): e12290.
8. Summers WK. A clinical method of estimating risk of drug induced delirium. *Life Sci* 1978; **22**(17): 1511-6.
9. Han L, McCusker J, Cole M, Abrahamowicz M, Primeau F, Élie M. Use of medications with anticholinergic effect predicts clinical severity of delirium symptoms in older medical inpatients. *Archives of Internal Medicine* 2001; **161**(8): 1099-105.
10. Aizenberg D, Sigler M, Weizman A, Barak Y. Anticholinergic burden and the risk of falls among elderly psychiatric inpatients: a 4-year case-control study. *Int Psychogeriatr* 2002; **14**(3): 307-10.
11. Minzenberg MJ, Poole JH, Benton C, Vinogradov S. Association of anticholinergic load with impairment of complex attention and memory in schizophrenia. *Am J Psychiatry* 2004; **161**(1): 116-24.
12. Ancelin ML, Artero S, Portet F, Dupuy AM, Touchon J, Ritchie K. Non-degenerative mild cognitive impairment in elderly people and use of anticholinergic drugs: longitudinal cohort study. *BMJ* 2006; **332**(7539): 455-9.
13. Hilmer SN, Mager DE, Simonsick EM, et al. A drug burden index to define the functional burden of medications in older people. *Arch Intern Med* 2007; **167**(8): 781-7.
14. Chew ML, Mulsant BH, Pollock BG, et al. Anticholinergic activity of 107 medications commonly used by older adults. *J Am Geriatr Soc* 2008; **56**(7): 1333-41.
15. Cancelli I, Valentinis L, Merlino G, Valente M, Gigli GL. Drugs with anticholinergic properties as a risk factor for psychosis in patients affected by Alzheimer's disease. *Clinical Pharmacology and Therapeutics* 2008; **84**(1): 63-8.
16. Ehrt U, Broich K, Larsen JP, Ballard C, Aarsland D. Use of drugs with anticholinergic effect and impact on cognition in Parkinson's disease: a cohort study. *J Neurol Neurosurg Psychiatry* 2010; **81**(2): 160-5.
17. Sittironnarit G, Ames D, Bush AI, et al. Effects of anticholinergic drugs on cognitive function in older Australians: results from the AIBL study. *Dement Geriatr Cogn Disord* 2011; **31**(3): 173-8.
18. Whalley LJ, Sharma S, Fox HC, et al. Anticholinergic drugs in late life: adverse effects on cognition but not on progress to dementia. *J Alzheimers Dis* 2012; **30**(2): 253-61.
19. Durán CE, Azermai M, Vander Stichele RH. Systematic review of anticholinergic risk scales in older adults. *Eur J Clin Pharmacol* 2013; **69**(7): 1485-96.
20. Faure R, Dauphinot V, Krolak-Salmon P, Mouchoux C. A standard international version of the drug burden index for cross-national comparison of the funcional burden of medications in older people. *Journal of the American Geriatrics Society* 2013; **61**(7): 1227-8.
21. Klamer TT, Wauters M, Azermai M, et al. A Novel Scale Linking Potency and Dosage to Estimate Anticholinergic Exposure in Older Adults: the Muscarinic Acetylcholinergic Receptor ANTagonist Exposure Scale. *Basic Clin Pharmacol Toxicol* 2017; **120**(6): 582-90.
22. Bishara D, Harwood D, Sauer J, Taylor DM. Anticholinergic effect on cognition (AEC) of drugs commonly used in older people. *Int J Geriatr Psychiatry* 2017; **32**(6): 650-6.
23. Briet J, Javelot H, Heitzman E, et al. The anticholinergic impregnation scale: towards the elaboration of a scale adapted to prescriptions in French psychiatric settings. *Thérapie* 2017; **72**: 427-37.
24. Kiesel EK, Hopf YM, Drey M. An anticholinergic burden score for German prescribers: score development. *BMC Geriatr* 2018; **18**(1): 239.

25. Nery RT, Reis AMM. Development of a Brazilian anticholinergic activity drug scale. *Einstein (Sao Paulo)* 2019; **17**(2): eAO4435.
26. Jun K, Hwang S, Ah YM, Suh Y, Lee JY. Development of an Anticholinergic Burden Scale specific for Korean older adults. *Geriatr Gerontol Int* 2019; **19**(7): 628-34.

7 Discussion

7.1 General findings

The work described in this thesis examined the prescribing trends of anticholinergic drugs in UK Biobank and the associations between AChB and various cognitive outcomes. The following chapter reviews the empirical findings and the strengths and limitations of the sample and methodological approach. Finally, I discuss the potential implications for future studies on this topic.

7.1.1 Anticholinergic trends

The results in section 4.2 showed that the proportion of anticholinergic drugs identified in the sample strongly depended on the anticholinergic scale used, with proportions varying from 2.5% to 23.1%. Most anticholinergic prescriptions were antidepressants, constituting 32.5% of all prescribed anticholinergic drugs. Higher AChB was associated with female sex, lower education, greater deprivation, higher body mass index, less frequent alcohol consumption, and lower physical activity. Previous studies that typically used cross-sectional designs reported similar findings for antidepressants (Byrne et al., 2018; Rémillard, 1996; Rhee et al., 2018), female sex, lower education and greater deprivation (Kachru et al., 2015a; Reinold et al., 2021; Sumukadas et al., 2014).

Less work had previously been done on trends of anticholinergic prescribing in the UK. Data from other countries had been conflicting, with recent studies from the US and Europe reporting decreasing (Campbell et al., 2021; Malagaris et al., 2020; Rhee et al., 2018) and increasing (Aalto et al., 2020; Hovstadius et al., 2014) temporal trends, respectively. It is unclear whether this represents true differences between countries or different approaches to measure AChB. To my knowledge, the only published study on anticholinergic trends in the UK dated back to 2014 and compared AChB between the years 1995 and 2010 for individuals living in Tayside, Scotland (Sumukadas et al., 2014). While the authors found AChB to be higher in 2010 (n=73,465) compared to 1995 (n=67,608), they did not have longitudinal data

and relied on a sample restricted both in terms of the age of participants and location of sampling.

My results expanded on the study by Sumukadas et al. (2014) to include a wider age range, longitudinal prescribing up to a more recent period, and an exploration of age-period-cohort (APC) effects. I also depicted the longitudinal trend of multiple commonly used scales and used them to compute an averaged "Meta-scale". The results demonstrated that AChB has increased in UK Biobank in the period between 1991 and 2015 and that this increase was due to age- and period/cohort-related changes in prescribing. In other words, anticholinergic prescribing has increased due to both the ageing of the sample, as well as changes in prescribing trends over this period. This increase was observed across birth cohorts in the sample. A later study using data from England reported similar findings, showing that prescribing in individuals aged 65 years or older nearly doubled over the last 20 years (Grossi et al., 2020). When polypharmacy was included as a covariate in the APC models, the trend was reversed, with age and period/cohort (later period or earlier cohort) negatively associated with AChB. However, the effect was small and decreased by almost an order of magnitude when compared to effects before correcting for polypharmacy.

As described in chapter 4, the Age-Period-Cohort question is mathematically unresolvable. Because the three terms are colinear (age = period - cohort), they cannot all be included in a regression analysis. While there have been attempts to separate the unique linear contributions of the individual terms (Yang et al., 2008), I agree with those (Bell & Jones, 2013, 2018) who have argued that a mathematical problem cannot be solved by model structuring. In my analyses, I assumed that age effects would be present in the data. Higher age has consistently been associated with polypharmacy (Guthrie et al., 2015; Hajjar et al., 2005) and anticholinergic use (Reinold et al., 2021; Rémillard, 1996), and there are convincing mechanistic reasons for this association: ageing is associated with multimorbidity and greater severity of disease. These factors lead to a greater need for pharmacological intervention, which increases the number of prescribed drugs and the AChB.

I was interested in whether anticholinergic trends can be explained by factors other than age-related increases in morbidity of the participants. If age does associate with an increase in AChB, my results suggest that either later cohorts or later time periods are associated with higher AChB in addition to the age-related effect. While clear biological arguments can be made to support the notion of age-related rise in AChB, this is not the case for potential cohort- or period effects. Several explanations are possible, including a rise in the preparedness of medical practitioners to prescribe drugs and a higher willingness of later-born individuals to request prescriptions. While these are important considerations, they share the implication that AChB has been rising in the UK.

7.1.2 Anticholinergic drugs and dementia

The results in section 5.2 reported the association between higher AChB and elevated risk of dementia, and the distinct effects of different drug classes for this association. Previous systematic reviews on the topic suggested a link between anticholinergic use and cognitive decline but demonstrated considerable heterogeneity in the results of original studies (Taylor-Rowan et al., 2021; Zheng et al., 2021). Recent studies found evidence for associations between anticholinergic use and dementia risk, even when the exposure occurred decades before diagnosis (Coupland et al., 2019; Richardson et al., 2018). In my analyses, I attempted to replicate previous findings in a large sample and explore potential causes of the discrepancies in the literature by comparing different anticholinergic scales and exploring the effects of different classes of anticholinergic drugs in greater detail.

I showed that AChB according to most anticholinergic scales was associated with the risk of dementia. I also compared different ways of calculating AChB and found that counting drugs (as opposed to assigning potency scores) and correcting for dosage did not substantially alter the associations. For the anticholinergic scale that exhibited the largest effect size for the association between AChB and dementia (Durán et al., 2013), there was a negligible attenuation after accounting for the competing risk of death. Additionally, AChB was associated with the risk of all-cause mortality when using the above anticholinergic scale. The effect size for the association between AChB and dementia did not depend on the elapsed

time between the baseline measurement of AChB and the diagnosis of dementia. Some authors (Richardson et al., 2018) have interpreted an existence of such a relationship to strengthen the case for a causal link. However, even in the absence of such a relationship, causality may still exist. Anticholinergic drugs might decrease baseline cognitive functioning (the intercept of the longitudinal trajectory) as opposed to affecting the longitudinal trajectory/slope itself. In addition to an effect from AChB measured at baseline, the evolution of AChB throughout the course of the study was associated with the risk for dementia. This suggests that longitudinal changes in anticholinergic prescribing relate to the risk of cognitive decline.

When I analysed AChB due to different classes of medicines, drugs for treating the nervous, gastrointestinal, and cardiovascular systems were associated with an increased risk of dementia. Specifically, the strongest effects were exhibited by antiepileptic drugs, antidepressants, and the diuretic furosemide. Interestingly, when I analysed separately the effects of drugs ascribed different anticholinergic potencies (from 0 – no anticholinergic effect – to 3 – strongly anticholinergic), I found only the number of drugs that were assigned a potency rating of 1 to associate significantly with the risk of dementia.

Our result overlapped well with previous findings and extended the knowledge of different classes of drugs and differences between anticholinergic scales. However, some hypotheses were not supported by the evidence and some results exhibited discrepancies with previous studies. First, in contrast to most previous studies (Taylor-Rowan et al., 2021), I did not find a dose-response relationship between AChB and dementia. One explanation for this is that the potential adverse effect of AChB on cognitive status may plateau at moderate levels of anticholinergic use. Second, the findings on class-based effects only partially overlapped with previous studies on this topic (Coupland et al., 2019; Richardson et al., 2018). For example, I did not find an effect of AChB due to antipsychotics or urinary drugs, despite the link with dementia previously reported in the literature. This could be due to the relatively low use of this these drugs in the sample. As reported in the publication described in section 4.2, urinary drugs contributed only ~2.3% of all prescribed anticholinergic drugs. An older sample may be

needed to detect this effect. Third, the number of drugs with an anticholinergic rating of 1, but not those with an anticholinergic rating of 2, was associated with dementia risk. The reason for this might be that those anticholinergic drugs with a rating of 1 are most strongly represented in anticholinergic scales and were most common in the sample. It also conforms to the finding of simple drug counts being equally good predictors of dementia as more complicated algorithms of AChB. However, especially given the link between non-anticholinergic polypharmacy and dementia risk, anticholinergic use may merely represent an alternative – and more complicated – way of quantifying/weighing polypharmacy (see section 7.3.2). The findings may raise doubts about (1) the validity of AChB as a measure of anticholinergic potency or (2) the reliability of AChB *per se* to predict dementia and cognitive decline. Finally, even if the distinct effects of polypharmacy and AChB could be completely separated, the field still faces the issue of causality (see section 7.3.3): how can we determine if the increased risk of dementia is due to the drugs themselves or due to the underlying disease for which the drugs are prescribed? This is especially the case since the strongest effects in this study and others were observed for drug classes that treat disorders that themselves increase the probability of dementia or that are associated with significant changes in brain structure or function, such as cardiovascular disorders, schizophrenia, depression, epilepsy, and lower urinary tract symptoms (Chiang et al., 2015; Diniz et al., 2013; Fischer & Agüera-Ortiz, 2018; Haijma et al., 2013; Kempton et al., 2011; Livingston et al., 2020; Sen et al., 2018).

7.1.3 Anticholinergic drugs and cognitive ability

The results in section 6.2 cover the association between AChB, general cognitive ability and various measures of brain structural MRI. Many studies had previously been conducted on the association between anticholinergic use and cognitive ability. However, most either used specialised cognitive tests or the MMSE to explore dementia and cognitive decline. The former only probe individual cognitive domains and are susceptible to domain-specific noise and confounding. The MMSE, on the other hand, has well-known ceiling effects, which can affect the validity of effect estimation (Franco-Marina et al., 2010; Tombaugh & McIntyre,

1992; Wang et al., 2009). Most studies using these approaches found a negative association between anticholinergic use and cognitive ability, but the literature is very heterogenous and many counterexamples can be found for claims of purported effects (Andre et al., 2019; Ghezzi et al., 2021). In my analyses, I aimed to address the potential sources of heterogeneity by (1) adopting a robust measure of general cognitive ability, comparing (2) different anticholinergic scales and (3) the effects of different classes of drugs. I found AChB across most anticholinergic scales to associate with lower general cognitive ability. Additionally, non-anticholinergic polypharmacy was mostly just as strong a predictor of lower cognitive ability as AChB. When applying the scale (Durán et al., 2013) that exhibited the strongest effect for general cognitive ability, AChB was also associated with lower performance on most cognitive tests. There were differences between different classes of drugs, with β-lactam antibiotics and opioids exhibiting the strongest negative associations with general cognitive ability. To my knowledge, this work was the first class-based analysis of the association between anticholinergic use and cognitive ability in a sample without dementia. Interestingly, for the class-based associations, results for cognitive ability correspond poorly to prior work in dementia (Coupland et al., 2019; Richardson et al., 2018) (see sections 2.4.4.1 and 5.2). Drug classes most strongly or consistently linked with dementia across these studies, such as antiepileptics and antidepressants namely did not exhibit a significant effect when related to general cognitive ability.

To my knowledge, three previous observational studies had explored the association between anticholinergic drugs and measures of brain structure or function. One cross-sectional study (Risacher et al., 2016) found reduced glucose metabolism in the hippocampus and globally, reduced total cortical volume, larger lateral- and inferior lateral ventricles, and reduced temporal cortical lobe thickness in users of anticholinergic drugs when compared to non-users. Another study that used a longitudinal design compared definite and possible anticholinergic users (Chuang et al., 2017). The former were defined as those taking drugs with clinically known anticholinergic effects, while the latter were defined as taking drugs without clinically known anticholinergic effects. Surprisingly, only possible anticholinergic users had an increased risk of dementia and greater rates of atrophy when compared to non-

users. Finally, a recent cross-sectional study of older participants found that cognitively normal anticholinergic users exhibited increased loss of functional integrity in the NBM, while users of anticholinergic drugs with MCI also exhibited decreased NBM grey matter density (Meng et al., 2022).

We aimed to expand these findings in a larger sample by exploring associations with a high number of cortical and subcortical measures, and measures of white matter microstructure. Interestingly, I did not find AChB according to any anticholinergic scale to associate with brain atrophy. Moreover, when using the scale that exhibited the strongest negative effect for its relationship with cognitive ability, no associations were found between AChB and any of the 135 measures of brain structural MRI.

The disagreement of my results with those from previous studies could be due to differences in the exact cognitive outcomes of interest. The three cited studies all probed dementia or cognitive decline, while I was interested in variations in general cognitive ability within the "normal" spectrum of cognition.

The absence of evidence for an effect does not necessarily provide evidence for the absence of an effect. However, the sample in my analysis included ~16,000 individuals and thus provided with the necessary power to detect very small changes. If the potential effects of AChB on brain structure are so small that even greater sample sizes are required to detect them, this raises questions about the real-world importance of such associations. These results thus provide convincing evidence for the lack of meaningful changes in brain structure following anticholinergic use in middle-aged and older individuals without symptoms of dementia.

As before, this study is also vulnerable to confounding by indication: some disorders for which anticholinergics are prescribed (e.g., schizophrenia and depression) are themselves associated with altered cholinergic processing (Higley & Picciotto, 2014; Wallace et al., 2011).

7.1.4 Clinical- and policy implications

The role of research in the social- and health sciences can be classified into description, prediction, and causal inference. Description provides a "quantitative summary of certain features of the world" (Hernán et al., 2019, p. 43), prediction aims to map input features to output features, and causal inference uses counterfactual prediction to predict the outcome, had certain alternative events occurred (Hernán et al., 2019). While not explicitly stated, the objectives of the present work initially included the first two above goals. My analyses provided a thorough description of anticholinergic use in the UK and contributed to a better understanding of the predictive utility of AChB for dementia and cognitive ability. However, the results have limited utility for practical decision making in the real world.

The analysis of anticholinergic trends (chapter 4) described the state of anticholinergic prescribing in the UK across a period of 25 years. This included a comparison of AChB and its evolution according to multiple anticholinergic scales, a presentation of the relative contributions of different classes of drugs to anticholinergic prescribing, and a detailed description of long-term changes in prescribing trends. These observations contribute to our understanding of the prescribing landscape in the UK and may inform future hypotheses about potential drivers of longitudinal change. However, the results should not be viewed as definitive answer and cannot by themselves directly influence the decision-making of policymakers or health practitioners. For example, the smooth linear trend in the rise of AChB over time might suggest an inefficacy of any potential deprescribing initiatives that may have been attempted during this period. However, the occurrence of other events during the same period could have cancelled out the effects of any such an initiative. We cannot know what trend anticholinergic prescribing might have exhibited, had those initiatives not occurred. More generally, without the knowledge of the underlying causal drivers of the observed longitudinal changes, these results cannot be used for practical action. They can only be used to inform future research.

The analyses of associations between AChB and dementia (chapter 5), cognitive ability and brain structural MRI (chapter 6) provided an examination of the predictive performance of

different anticholinergic scales for the diagnosis of dementia or the detection of different cognitive abilities or brain volumes. The prediction of health risks by using tools that account for facets of drug use has enormous practical utility. It could enable clinicians to assess patients that are at risk and prioritise those patients for targeted interventions to reduce that risk. The analyses in chapters 5 and 6 represent comprehensive accounts of the influences of the choice of anticholinergic scale, drug classification, and confounder inclusion on the predictive value of AChB models. Given the large size of the sample used in the analyses and the relatively small effects for the studied associations, the results may also provide information on the appropriate balance between the medicinal value of these drugs and their purported long-term risks. However, as above, the results of my analyses cannot be drawn upon for the development of practical guidelines, because they do not probe causal mechanisms. Moreover, given the risk of residual confounding, even the potential predictive capacity of these tools in the real world is doubtful.

In summary, while the analyses presented in this thesis provided additional details and nuance to the field and may help spur additional research, they likely cannot directly assist practical decision-making in the clinical- or policy settings.

7.2 Limitations

In the following section, I critically appraise the work conducted as part of this thesis. First, I evaluate the appropriateness of UK Biobank and describe its limitations. Next, I critique my methodological approach. Many limitations that fall in the latter category follow to a certain extent from limitations inherent to the sample. However, not every dataset allows for the exploration of any hypothesis and the application of any statistical procedure. It is the role of the researcher to appropriately adapt these to the data.

7.2.1 The sample

The initial aim of UK Biobank to recruit a sample representative of the UK population was not entirely successful. The response rate of 5.47% among individuals invited to participate (Allen

et al., 2012) was already an early indicator of non-representativeness. Subsequent analyses (Fry et al., 2017) indeed demonstrated this with respect to several domains. First, participants in UK Biobank exhibit relatively low socioeconomic deprivation: they are more likely to own property, less likely to have a mortgage or a loan, to share ownership, or to live in rental accommodation than the general UK population in the same age range. Second, UK Biobank participants are healthier: both men and women are leaner than the national average and have lower rates of obesity. Across all age groups, they are also less likely to be current smokers and less likely to never drink alcohol. Additionally, UK Biobank participants have a lower prevalence of self-reported health conditions, including cardiovascular disease, stroke, hypertension, diabetes, chronic kidney disease, and respiratory disease. Furthermore, all-cause mortality and total cancer rates at 6-7 years of follow-up are lower than in the general population (Fry et al., 2017).

The lack of representativeness has also been demonstrated in the brain-imaging subsample. In a study of various characteristics of psychological and physical health, Lyall et al. (2022) found the imaging subsample exhibited even better health than the participants that were not scanned. This included lower socioeconomic deprivation, a lower proportion of smokers, less depression and unhappiness, lower neuroticism, lower prevalence of several health conditions, and better performance on multiple cognitive tests. Moreover, associations of established cognitive risk factors were smaller in the imaging subsample compared with the full sample (Jiang et al., 2022).

While the lack of representativeness is acknowledged and authors have advised against using UK Biobank to estimate prevalence- or incidence rates of disease, it has been argued that this issue does not represent a limitation to the primary purpose of the resource – the investigation of associations between exposures and outcomes (Fry et al., 2017). However, the effect that an exposure has on the outcome depends on the prevalence of other variables that interact with the exposure (Keyes & Westreich, 2019). A sample that exhibits non-representativeness in such variables will lead to estimates of effect sizes that are incorrect for the population. For example, comparing the UK Biobank sample and a subsample post-

stratified to be nationally representative, Stamatakis et al. (2021) found that while alcohol use acted as a protective factor for cardiovascular disease in the total sample, that association disappeared when using the representative sample. Thus, researchers using UK Biobank must remain cognisant of this issue and take care when generalising findings of exposure-outcome associations to the entire population.

Second, as described in section 3.4, minimal data curation is performed on the prescriptions by the UK Biobank team (UK Biobank, 2019b). While this allows more flexibility for researchers, it also increases the likelihood of a multitude of errors. First, the released dataset may include (false or true) duplicates and/or missing information. Second, medical records often contain more than one clinical code, with contradictions likely to occur. Third, due to local variation in code use, some codes may not match official code lists. While UK Biobank has compiled such lists for their use in research, the lists – along with definitions and mappings – have often not been verified by specialists before utilisation in my analyses. Thus, incorrect mappings may have occurred. Finally, the analyses described in this thesis assume that the accuracy of the diagnostic codes would remain stable across the value of the exposure (AChB); this assumption cannot be validated.

Possibly due to the recent availability of primary care records for research in UK Biobank, little work has been done on quality assessment and the development of guidelines and suggestions for researchers for best-practice use of the available data. To my knowledge, only one study has explored the accuracy of UK Biobank prescription data. While the authors found relatively high concordance with self-reported prescriptions, this varied between different classes of drugs (Darke et al., 2022).

7.2.2 My approach

7.2.2.1 Confounding by indication

An important issue in any observational study concerns the risk of confounding, i.e., when the factors that determine the exposure also affect the probability of the outcome. When the factors that determine the exposure are unmeasured or insufficiently accounted for, it can be

impossible to determine to what extent the association between the exposure and the outcome may have been confounded by such common factors. In analyses that measure the associations between prescription drugs and health outcomes, a major type of confounding is *confounding by indication*. It denotes a process by which the common factor that affects both the exposure and the outcome is the indication for which the drugs are prescribed. Users and non-users of anticholinergic drugs are not assigned to their respective exposure groups (use vs. non-use of anticholinergic drugs) by a random process. The indication for treatment with those drugs is usually difficult to characterise, as many factors may account for a physician's decision to prescribe a medicine. These factors may also differ from patient to patient – populations that are prescribed a drug can differ from non-users in a variety of ways, including age, sex, and underlying medical conditions. The indication for treatment is usually not available, as is the case in my study: the available information included drug name, date, and quantity, but not the reason for which the drug was prescribed. Furthermore, not only the presence of the disease itself but also its severity may pose a confounder. For example, different stages of an illness may necessitate different forms of treatment or varying concentrations of the active substance. Again, such information is rarely directly available in observational data. Finally, drug treatment may be indicated for prodromal symptoms of a disease, such as antidepressants to treat prodromal symptoms of dementia. In such a case, even the complete information on the indication for the prescribing would not capture its true cause and prevent confounding. While confounding by indication is often used to include the types of biases described above, some have argued for the use of the more appropriate terms of *confounding by severity* and *protopathic bias*, respectively (Salas et al., 1999).

Regardless of terminology, the lack of the direct measurement of these confounders and their danger to the interpretation of results in observational studies entails that the indication – if we wish to control for it – must be inferred from other data. I attempted this by controlling for several possible indications for the most prescribed anticholinergics, including the diagnoses of mood disorders, hypertension, and cardiovascular disorders. I also included the total number of diagnosed health conditions as a stand-in for potentially unaccounted-for indications. However, the list of control variables was surely neither exhaustive nor were the

indirect methods for ascertaining them perfectly appropriate for this aim. One proposed solution to confounding by indication in pharmacoepidemiology is active comparators: treatment alternatives for the "control group" that are indicated for the same disorder and severity as drugs in the "treatment group" (Lund et al., 2015) (see section 7.3.3). However, given the sheer number of included anticholinergics and their associated indications, this was not feasible for the present study. Thus, while efforts were made to prevent it, I cannot discount the possibility of substantial confounding in the reported results.

7.2.2.2 Associations without causality

The methodological approach adopted in my analyses and the results that these engendered cannot be used to infer causality. Predicting the future is a valid scientific endeavour. The discovery of associations between variables can help formulate causal hypotheses, assist in diagnosis, and prognosis (van Diepen et al., 2017). However, they often do not provide concrete suggestions for action for policymakers or physicians. The prescribing and deprescribing of drugs are relatively mutable practices that physicians can flexibly adapt to current science-based guidelines. Thus, it would be of great value if the results of my analyses could guide practical decision-making and public policy. Observational studies can be used to infer causality by emulating clinical trials (Didelez, 2016; Hernán & Robins, 2016). However, this was not the case for analyses conducted as part of this thesis. The data I used was neither appropriate to gauge truly incident anticholinergic use nor was the broad category of anticholinergics appropriate for an active comparator design.

7.2.2.3 A dearth of longitudinal trends

Another limitation of my work is that the longitudinal dynamics of the relevant variables were explored only to a limited extent. The analyses of anticholinergic trends (see section 4.2) provide a descriptive account of anticholinergic use in the UK over 15 years, while the study of the association between AChB and dementia (see section 5.2) includes evidence on the effect of the slope of change in AChB on the risk of dementia. However, I did not explore differences in anticholinergic trajectories between individuals or groups, nor how these differences relate to health outcomes. Moreover, all other analyses conducted as part of this

thesis did not account for temporal trends. Anticholinergic use can occur over the entire life course, but for most participants ascertainment only began in middle age. Demographic- and lifestyle variables were mostly only ascertained at a single point at study entry but may have also varied over time. More importantly, this variation might have been affected by prior levels of AChB. In other words, the pattern of association between confounders and AChB might have changed during the exposure period. Methods do exist to address these problems within a causal framework (Glass et al., 2013), including the parametric g-formula (Robins, 1986; Taubman et al., 2009), inverse probability of marginal structural models (Hernán et al., 2001; Robins et al., 2000), and g-estimation of nested structural models (Hernán et al., 2005; Robins, 1993). However, the application of these methods to address my questions was not possible using UK Biobank.

7.2.2.4 Use of clinical codes

The use of appropriate and accurate clinical codes to identify a diagnosis of interest is of vital importance for studies using linked health data. The quality assessment of linked data to identify disorders (see section 3.4.2) is valid only under the assumption of the utilisation of correct diagnostic codes for the disorders of interest. The accuracy of some diagnostic codes, along with recommendations for their use, are often available in the scientific literature. For example, Wilkinson et al. (2019) validated and suggested a list of codes for ascertainment of dementia in UK Biobank. While I eventually utilised these resources to improve diagnostic accuracy for the analyses described in this thesis, I originally did not sufficiently appreciate the importance of coding accuracy. During the early stages of the PhD project, I chose diagnostic codes by manually browsing term dictionaries that link disorders with codes used in the UK Biobank coding systems. This was laborious and error-prone work. As the project matured, I was made aware of two important resources in UK Biobank, first occurrences (UK Biobank, 2019a) and algorithmically-defined outcomes (UK Biobank, 2022). First occurrences are dates for when a diagnosis was first made. Algorithmically-defined outcomes are additionally curated to maximise the PPV based on the existing literature on diagnostic codes. While first occurrences and algorithmically-defined outcomes are not available for all

disorders, they cover the most prevalent ones. Moreover, they provide mappings across ICD-10, ICD-9, Read2, and Read3 coding systems, thus mostly obviating the need for manually searching term dictionaries. For example, if codes provided by UK Biobank were considered as true diagnoses, the sensitivity to detect the disorder by using my manually-retrieved codes was over 0.9 for all-cause dementia, hypertension, both types of stroke, and hypercholesterolemia, but only 0.59 and 0.75 for diabetes, and the combined diagnosis of either Parkinson's disease, multiple sclerosis, Huntington's disease, or Creutzfeldt-Jakob disease, respectively. Although the work described as part of this thesis mostly uses codes recommended by UK Biobank, the failure to familiarise myself with these resources during the early stages of the project substantially increased my workload and likely decreased the accuracy of my initial analyses. It serves as a lesson and a reminder that one must always understand the data, including its advantages and shortcomings, before attempting to analyse it.

7.2.2.5 Assumption of completeness

Two final issues pertain to the implicit assumption of data completeness inherent in my analyses and interpretations of the results. First, all prescribed medications may not have been dispensed and/or used by the participants. Second, although my analyses assumed that the measured AChB represented the total burden of all compounds used by the participants, they do not include over-the-counter-medications or prescriptions from secondary hospital care. Considering that previous analyses found prescriptions by GPs to contribute to ~50% of all anticholinergic use (Reinold et al., 2021; Rhee et al., 2018), my analyses likely underestimated the total AChB in the sample. This likely additionally decreases the generalisability and validity of the results.

7.2.2.6 Alternative approaches

The relationship between anticholinergic prescribing and cognitive outcomes is a complex topic that includes many stakeholders – including policymakers, clinicians, and patients – and can incorporate all previously mentioned goals of the health sciences: description, prediction, and causal inference (section 7.1.4). My analyses represent some possible lines of inquiry

within this field. There are many methodological alternatives to techniques using observational data as presented in this thesis. For example, causal questions could be explored if data were analysed like randomised experiments (see section 7.3.3). Furthermore, many other approaches exist that do not use observational data. This section aims to provide a brief overview of some of those methods.

RCTs are often viewed as an alternative to observational studies and are widely viewed as the gold standard for the ascertainment of cause and effect. They differ from observational studies in many ways (Caparrotta et al., 2019; Sørensen et al., 2006). Observational studies often exhibit selection bias, lack information about potentially important confounder variables, or rely on incomplete or inaccurate data. In RCTs, the assignment to the treatment and control groups is randomised, thus preventing baseline confounding. Furthermore, treatments and outcomes are defined and often registered in advance of the study, which increases internal validity and decreases the risk of selection bias. They also have strict inclusion and exclusion criteria and are often blinded to avoid observer bias. However, RCTs do have substantial disadvantages. The standard treatment in RCTs may differ from the regular treatment in standard clinical practice, and the strict exclusion criteria reduce the generalisability of the results to an often very narrow segment of the population. Moreover, RCTs normally do not account for therapies given outside of the study protocol, can exhibit substantial loss to follow-up, and problems with adherence. Additionally, because they are performed prospectively, RCTs must meet ethical standards of human experimentation. Due to the presumed harm of prolonged anticholinergic use, this renders RCTs of anticholinergic prescribing unfeasible for ethical reasons. Furthermore, RCTs usually incur high monetary costs, because data are gathered solely for the purpose of the study (as opposed to administrative data often used in observational research, for example). Thus, even in the absence of ethical constraints, a well-designed RCT of chronic anticholinergic use that provides sufficiently long follow-up and includes enough participants to detect cognitive decline, would be prohibitively expensive.

In the wider research field of the health sciences, good agreement has been demonstrated between RCTs and observational studies. A 2001 analysis found the correlation of summary ORs to be r=0.75 between studies using either design (Ioannidis et al., 2001). A more recent Cochrane systematic review reported the ratio of ORs between RCTs and observational studies to be 1.08 (95% CI=0.96-1.22), indicating little evidence for differences in effect estimates between RCTs and observational studies (Anglemyer et al., 2014). However, due to the relatively low number of RCTs about the effects of anticholinergic prescribing, it is not known whether these results extend to the area of anticholinergic research.

A viable approach to adopt RCTs in anticholinergic research are deprescribing trials that probe the effects of reducing AChB in patients already using anticholinergic drugs. However, too few such trials exist for a coherent synthesis (Salahudeen et al., 2014; Taylor-Rowan et al., 2021), and the results from the existing trials have been disappointing. One study in 87 nursing home residents demonstrated no effect of AChB reduction on immediate recall, mouth dryness, or SAA (Kersten et al., 2013). Interventions in some other studies did not even succeed in decreasing anticholinergic use. Van der Meer (2018) found that a pharmacist-led medication review in 157 community-dwelling participants failed in reducing AChB within three months. Another study of a similar type of intervention found no effect for the reduction of AChB in 363 community-dwelling participants within six months (Jamieson et al., 2023).

The failure of these trials indicates a lack of understanding of the factors that promote or hinder the successful application od anticholinergic deprescribing. These could include concerns about negative consequences of stopping medications, or miscommunication between clinicians and patients. A systematic review of qualitative studies on the barriers and facilitators to reduce AChB identified several barriers and facilitators for successful deprescribing. However, the authors did not find any studies that involved patients or carers (Stewart, Gallacher, et al., 2021). In recent years, the idea that research that directly concerns patients is to be conducted “with” or “by” members of the public has become more prominent. It involves including patients and the public in various aspects of the research process, including planning and design, implementation, and presentation and dissemination

of results. For example, a patient advisory group could collaborate with researchers to better inform the latter about the wishes and needs of the patient population (Jackson et al., 2020; Staley, 2015).

A more recent study by Cunningham et al. (2021) adopted such an approach on the topic of anticholinergic deprescribing. The authors performed qualitative interviews and focus groups with 22 members of the public and patients prescribed anticholinergic drugs and 25 health care professionals involved in anticholinergic prescribing. All stakeholders were supportive of a trial to reduce AChB. The study also identified important points to consider when designing such a trial, including patient engagement, the ability to continuously address patient concerns, ensure clear communication and allow patients to understand the reasons for AChB reduction, and the provision of points of contact throughout the duration of the trial. While not explicitly probed in this study, patient participation could also provide support in balancing the various effects of prescribing choices; for example, the relative importance of treating symptoms of underlying disease and the potential risks of acute side effects and long-term deleterious health outcomes. However, the health implications of chronic anticholinergic use are currently possibly too unclear to foster such engagement.

Finally, animal experiments can be used in anticholinergic research as well. First, this includes basic research on the chemistry and bioavailability of anticholinergic drugs. For example, one group used rodents to study in vivo muscarinic receptor binding of drugs to treat overactive bladder. A comparison of this binding to the selectivity for the urinary bladder over the brain then demonstrated the differential binding affinities for the CNS versus the periphery (Maruyama et al., 2008). Second, animal experiments can be used to directly probe mechanistic questions. For example, anticholinergic administration has been demonstrated to enhance tau pathology, synaptic loss, and inflammatory cytokine expression in a tauopathy mouse model. This neurodegeneration was mild for propiverine, but more severe for trihexyphenidyl, which corresponds to the relative affinities for CNS binding exhibited by these two drugs (Yoshiyama et al., 2015; Yoshiyama et al., 2012). However, the analyses presented in this thesis probed the chronic use of anticholinergic drugs in humans. It is unclear

if relatively short-term use of these compounds in organisms with a different metabolism and with cognitive abilities adapted to a different environment can adequately answer such questions. Animal models may be more suitable in cases where the causal relationship between the exposure and outcome is recognised, and the biological correlates of this relationship are sought.

7.3 Future research

Prescribing drugs should be highly malleable to new recommendations. Thus, the topic of potentially inappropriate use of anticholinergics could be of great relevance to both policymakers and medical practitioners. However, in my view, the field is struggling to suggest practical guidance to enact changes that could benefit patients. The reasons for this are manifold and multiple areas of anticholinergic research would benefit from adjustments and additions to current practice. Based on previous work, results conducted as part of this thesis, and recent developments in the field, I suggest some possible improvements and suggestions for future work in the following section. I focus mostly on the potential role of anticholinergics on cognition, but many of these observations could be applied to other health outcomes. I conclude the section by describing some tentative biological explanations for the potential effect of anticholinergics on cognition.

7.3.1 Anticholinergic activity

Research of various outcomes related to anticholinergic prescribing, including dementia (Hsu et al., 2017; Taylor-Rowan et al., 2021; Tristancho-Perez et al., 2022), all-cause mortality (Ali et al., 2020), and falls and fractures (Akgün et al., 2022; Ogawa et al., 2021), has demonstrated that the choice of anticholinergic scale may substantially affect the size of the observed effects. In my analyses (see sections 5.2, 6.2), the different scales generally exhibited similar trends but differed in the strength of their associations with both dementia and cognitive ability. These results are unsurprising, because scales differ in various aspects of the construction procedure, including country-specific prescribing landscape, scoring criteria, and

outcome validation (see section 2.3). Moreover, while studies that utilise different scales are available, they are too different to allow for comparisons. A recent systematic review concluded that the study designs, populations, and methods to measure various clinical outcomes are so heterogeneous that a meta-analysis on the topic was impossible to conduct (Lisibach et al., 2021). Thus, more individual studies are needed that compare anticholinergic scales when evaluating health outcomes. This would not only enable the development of an understanding of the relative merits of individual scales but also increase the reliability of studies assessing associations with outcomes. Care should also be taken to consider scales in their appropriate clinical contexts, accounting for their intended use.

There is a difference between (a) validity – the ability of a tool to measure what it purports to measure – and (b) its predictive value. The previous paragraph only addresses (b), but not (a). Comparisons of scales can inform us of their relative utility to act as markers for outcome measures, but such research does not necessarily demonstrate the validity of an anticholinergic scale to assess anticholinergic potency. The most widely used methods to score anticholinergic activity at present are either SAA, an assay based on serum levels of anticholinergic activity, or expert opinion. Both approaches are problematic and there is currently no gold standard for measuring anticholinergic activity (see section 2.3.1). Recently, some groups have attempted to address this issue by automatising the assignment of anticholinergic scores. For example, Xu et al. (2017) used *DrugBank* (Knox et al., 2011) to retrieve the molecular structures of 25 drugs of interest and queried a database (Gaulton et al., 2012) for information on bioactivity between those drugs and mAChRs. Similarities in chemical structure between the drugs of interest and the queried bioactive molecules were then used to infer antimuscarinic activity for receptor subtypes for the drugs of interest. Using multiple linear regression, the authors used the newly calculated AChB and several pharmacokinetic parameters, including BBB permeability, as predictors for ataxia incidence. They reported their model to exhibit high predictive performance when using leave-one-out cross-validation (R2=0.64). Moreover, when compared with two previously published anticholinergic scales, they found the AChB according to the new scale to better correspond to the cumulative incidence rate of ataxia. This research demonstrates the potential of large

molecular databases of medicines in combination with bioavailability parameters to inform pharmacological risk factors. However, the study used a relatively small number of drugs and compared the performance of the new tool to just two existing anticholinergic scales. Another study (Secchi et al., 2022) used natural language processing on a website portal to calculate AChB based on reported adverse effects and drug chemical structure. They then made the tool available to members of research groups with expertise in prescribing, pharmacy, geriatric medicine, mental health, and health service (n=110). A subsequent survey found that 74% of respondents rated the already available scales as very helpful but rated the new scale as least as helpful in their clinical decision-making. The survey confirmed that there is a need among practitioners for anticholinergic scales and that new advances in this field may be of great interest. However, the group did not report the methodology for the development of their scale. Novel tools to compute anticholinergic activity must be thoroughly documented so that they can be scrutinised, applied, and improved by the scientific community. Studies that utilise automatised approaches in this way, along with improvements in lab assays to measure anticholinergic activity (Chandramouleeshwaran et al., 2021; Chandramouleeshwaran et al., 2022), could help to create an anticholinergic scale with acceptable validity.

More research is also needed on the basic biology of molecular compounds to inform databases such as those used in the above research. Studies in animals can inform us of the BBB permeability of a drug (Callegari et al., 2011; Chancellor et al., 2012; Pahlman et al., 2001), which affects the concentration that the drug attains in the brain. This property is affected by both passive and active transport (Chancellor et al., 2012; Pagoria et al., 2011; Welk et al., 2021). Passive passage of molecules across the BBB is affected by molecular properties of the compounds, including polar surface area, molecular weight, lipophilicity, etc. (Callegari et al., 2011; Geldenhuys et al., 2015; van de Waterbeemd et al., 1998). Active transport refers to the activity of transporter proteins that actively transport compounds against their concentration gradients (Geyer et al., 2009; Roberts et al., 2008). Anticholinergics seem to exert most of their cognitive effects through M_1 and, to a lesser extent, through M_2 and M_4 receptors (see section 1.4.3). Thus, even in the case of substantial

BBB permeability, not all antimuscarinic drugs will exhibit the same effects in the CNS. Imaging techniques can measure the binding of anticholinergic compounds to different mAChR subtypes (Salahudeen et al., 2014; Tsukada et al., 2016; Yoshida et al., 2010). The knowledge of these molecular characteristics can help explain the differential effects of drugs. For example, darifenacin and trospium are actively transported out of the CNS and preferentially bind to the M_2 and M_3 receptors (Callegari et al., 2011; Pak et al., 2003; Scheife & Takeda, 2005; Staskin et al., 2010). On the other hand, oxybutynin exhibits relatively high BBB penetration (Callegari et al., 2011; Jakobsen et al., 2011), is not selective for receptor subtypes (Callegari et al., 2011; Welk et al., 2021), and exhibits the highest mAChR antagonism in the CNS among medications to treat overactive bladder (Maruyama et al., 2008; Welk et al., 2021; Zinner, 2007). Unsurprisingly, within this drug class, oxybutynin is the drug most often associated with dementia (Dantas et al., 2022; Duong et al., 2021; Malcher et al., 2022). Of course, there is a multitude of other genetic and environmental factors, including age, sex, and the concomitant use of other medications that can affect drug properties (Abdel-Rahman et al., 2004; Chancellor et al., 2012; Kay et al., 2005; Pakulski et al., 2000; Starr et al., 2003), and thus complicate the calculation of a score that would apply equally to all individuals.

7.3.2 Polypharmacy and drug classes

The absence of robust and well-validated measures of anticholinergic activity for medications raises issues about the value of current anticholinergic scales. Moreover, even in the presence of a valid measure of AChB and its biological plausibility as a risk factor for cognitive decline, the utility of such a measure would be questionable. This is true for AChB both as a predictive tool to assess risk, as well as a testable cause of cognitive decline.

All drugs taken by the individual will contribute to their score of polypharmacy, and the latter will always be due to both anticholinergic and non-anticholinergic drugs (i.e., general polypharmacy will consist of both anticholinergic and non-anticholinergic polypharmacy). The anticholinergic component of polypharmacy cannot be completely distinguished from AChB. In fact, anticholinergic polypharmacy could itself be considered an unweighted AChB (i.e., a

score where each drug is assigned the same potency). It this sense, the conundrum is reminiscent of the APC problem discussed in chapter 4. There, the change in either one of the components of age-period-cohort inevitably altered the other two. Here, the increase in polypharmacy will on average inevitably lead to an increase in the AChB. In UK Biobank, the correlation between total AChB and anticholinergic polypharmacy between the years 2000 and 2015 ranges from 0.88 to 0.99 (depending on the scale), with a mean of r=0.94. When the same association is calculated using general polypharmacy (anticholinergic + non-anticholinergic polypharmacy) instead of only anticholinergic polypharmacy, the coefficient is only moderately high, with values between 0.25 and 0.65, and a mean of r=0.44. That is, whereas AChB is not especially well represented by the overall level of polypharmacy, it is intimately related to anticholinergic polypharmacy. In other words, both index something beyond overall prescriptions, but weighted or unweighted measures of AChB are virtually statistically indistinguishable.

Polypharmacy has previously been associated with dementia. For example, a 2019 meta-analysis of seven original studies on the association between polypharmacy and dementia found an adjusted RR of 1.30 (95% CI: 1.16-1.46) (Leelakanok & D'Cunha, 2019). Another systematic review found that out of 50 identified studies, most that sufficiently adjusted for comorbidity reported a positive association between polypharmacy and dementia (Fried et al., 2014). Finally, a recent medication-wide study of 17,000 dementia cases among half a million people found that almost a third of all the prescribed medications were associated with dementia (Wilkinson et al., 2022).

The problem of AChB as a predictive tool for cognitive outcomes is due to its close relationship with polypharmacy. In the creation of such a tool, it may very well be useful to isolate from polypharmacy an assumed anticholinergic component, and then to mark the individual drugs constituting that component to yield an AChB score. However, in my analyses, a weighted AChB did not exhibit greater or more precise estimates in its associations with dementia than did the unweighted AChB (i.e., anticholinergic polypharmacy; see section 5.2). Moreover, as shown in my work on the association between AChB and general cognitive ability (see section

6.2), the number of drugs included as anticholinergics in an anticholinergic scale associated with the effect size of the AChB according to that scale and general cognitive ability. Finally, not only did the introduction of weighted scores not improve the association between AChB and dementia, neither the weighted, nor the unweighted AChB showed greater associations than did general polypharmacy (see section 5.2). A valuable test of the utility of adding complexity to any predictive tool is that it should increase its predictive performance. While it is possible that this is realised for some health outcomes, the evidence for this in the field of dementia and cognitive ability – as shown in this thesis – is limited.

The second issue with AChB relates to its role as an element in the causal pathway to cognitive outcomes. Any association between anticholinergic use and dementia may be confounded by non-anticholinergic polypharmacy. In the sample, the correlation between AChB and non-anticholinergic polypharmacy ranged from 0.21 to 0.48, with a mean of r=0.34. While this is a modest association, it does demonstrate the possibility of non-anticholinergic polypharmacy confounding the causal relationship between AChB and cognitive outcomes. Surprisingly, this issue is rarely addressed in the literature. In my analyses, I attempted to account for both predictors by controlling for the number of non-anticholinergic drugs. The addition of non-anticholinergic polypharmacy to the models as a covariate decreased the observed effect size for AChB on average by 30%. Additionally, non-anticholinergic polypharmacy was consistently – across anticholinergic scales – just as strongly associated with cognitive outcomes as AChB.

Another problem about using AChB for mechanistic questions relates to the nature of drugs involved in computing the score. Given their ubiquity, many presumed anticholinergics will also be grouped in drug classes that themselves – possibly independently of anticholinergic action – have been linked to reduced cognitive function. For example, benzodiazepines (Gray et al., 2016), antiepileptics (Taipale et al., 2018), and proton-pump inhibitors (Haenisch et al., 2015) have been linked to dementia, and anticholinergic drugs can be found in all three groups. Moreover, drugs that exhibit anticholinergic action may also display secondary effects at other receptor types. For example, many anticholinergic antidepressants also exhibit

antiadrenergic action (Richelson, 1996). It is difficult if not impossible to discriminate between anticholinergic effects and other, class-based mechanisms that may contribute to adverse effects on cognition by these drugs. In other words, we cannot determine to what extent the adverse cognitive effects of anticholinergics – given their presumed ubiquity – are due to a different mechanism entirely, or a combination of several mechanisms.

As presented in section 2.4.4, drug class can be an important variable to consider in the association between anticholinergic use and several health outcomes, including dementia. As opposed to studying the entire group of drugs that is defined based on a putative, poorly measured biological property, it may be more informative to investigate different classes of drugs within that group. I.e., instead of studying all anticholinergic drugs as a single group, one could focus on antidepressants, anxiolytics, urological drugs, etc. Several recent reviews (Ghezzi et al., 2021; Neal et al., 2020; Taylor-Rowan et al., 2021; Welk et al., 2021; Zheng et al., 2021) and original investigations by us (sections 5.2 and 6.2) and others (Coupland et al., 2019; Joung et al., 2019; McMichael et al., 2021; Richardson et al., 2018; Welk et al., 2022) have adopted the approach of reporting results at the level of individual drugs or drug classes. As described in the next section, such approaches may offer a better way to test causal hypotheses.

7.3.3 Causality

To my knowledge, there have been no explicit attempts to explore whether the relationship between anticholinergic use and health outcomes is causal. Interestingly, some researchers (Attoh-Mensah et al., 2020; Cardwell et al., 2020; Coupland et al., 2019) nonetheless frequently conclude their articles with practical suggestions for policymakers and practitioners, despite professing to merely report *associations*, and often explicitly warning against causal interpretations from their work. Furthermore, more studies seem to exist that explore the effects of interventions on reductions of anticholinergic prescribing than those attempting to evaluate the effects of such interventions on health outcomes. In a recent systematic review, Salahudeen et al. (2022) found seven RCTs that explored the efficacy of interventions to improve practices of anticholinergic prescribing in older people. They

reported that single-component interventions, most frequently medication reviews and provision of education to healthcare practitioners, were very common. In most studies, such interventions successfully reduced the incidence of "errors" in anticholinergic prescribing. Another systematic review found four RCTs and reported mixed results of intervention efficacy in reducing AChB (Nakham et al., 2020). Yet another group that explored "*barriers and facilitators to reducing anticholinergic burden*" (Stewart, Gallacher, et al., 2021) provided readers with several research questions to tackle anticholinergic prescribing.

However, it is yet unclear whether alternative non-anticholinergic prescribing or the deprescribing of anticholinergics benefits patients. Few clinical trials on anticholinergic prescribing exist and all are deprescribing trials. Thus, they explore the effects of reducing or discontinuing existing anticholinergic use. A systematic review on the effects of anticholinergic discontinuation on cognitive outcomes in older people found only four studies between the years 1946 and 2013 that fit the inclusion criteria, two of which were RCTs (Salahudeen et al., 2014). Whereas the two prospective cohort studies included in the review found a positive effect of deprescribing, no significant effects were reported by the RCTs that explicitly tested causal questions. Additionally, none of the studies was performed entirely on cognitively normal, healthy participants, most used mainly the MMSE to detect cognitive change, and all had relatively small sample sizes (n<50). Furthermore, due to differences in the heterogeneity of study designs, interventions, and definitions of outcome measures, a meta-analysis was not possible. Two more recent systematic reviews on the risk of dementia and cognitive decline following the use of anticholinergics reported a similar shortage of RCTs on this topic (Taylor-Rowan et al., 2021; Wang et al., 2021).

While deprescribing trials are a worthwhile pursuit with clear implications for the work of physicians, a lack of positive results does not necessarily indicate a lack of any causal relationship between anticholinergic use and health outcomes. The effects of long-term anticholinergic use could be permanent or highly resistant and may not respond to attempts at reducing AChB. Because RCTs to explore the effects of initiating anticholinergic therapy are unethical, such questions can only be answered by observational studies. However, most

observational studies ask vague, poorly defined questions that provide little if any information that can be directly acted upon by policymakers (Glass et al., 2013). This gap between research and decision-making in public health can be bridged by target trial emulation that uses observational data to mimic idealised RCTs (Hernán & Robins, 2016). This approach is based on the framework first suggested by the Polish mathematician Jerzy Neyman in the 1920s (Splawa-Neyman et al., 1990), with further advances (Rubin, 1974, 1978) and a generalisation to include time-varying exposures (Robins, 1986) proposed in the following decades. There are many examples of successful applications of this approach in epidemiology (Cain et al., 2016; Hernán et al., 2008), with some authors (Didelez, 2016; Glass et al., 2013) arguing for its wider implementation.

In the specific case of anticholinergic effects on cognition, a clinical trial could be emulated using the active comparator, new user (ACNU) design. Here, participants are divided into those that have never been prescribed a drug of interest and those that have never been prescribed a comparator drug. The groups are then followed over time and the relevant health outcomes are compared. Two requirements of this design are notable. First, it does not include prevalent users of the drug of interest, but only participants that were not exposed to the drug before ("new users"). Some previous studies on the associations between anticholinergic use and health outcomes have indeed distinguished between incident and prevalent users and sometimes reported differences in effects (Moriarty et al., 2021; Richardson et al., 2018; Shah et al., 2013). Second, the ACNU does not compare users to non-users; it includes only individuals with the indication for the drug of interest and compares those taking the drug of interest to another drug ("active comparator"). Thus, the research question changes from "*Should I treat patients of indication X with the treatment of interest or not?*" to "*Given that a patient with indication X needs treatment, should I initiate treatment with the treatment of interest or the active comparator?*" (Sendor & Stürmer, 2022). The difference is subtle but important: while the former question can only be answered with precise measurement of underlying indications, the latter does not have that requirement. Active comparators require clinical equipoise with the study treatment. This means that a physician could prescribe either the treatment of interest or the comparator treatment for

any given patient. While this assumption leaves room for residual confounding by age, sex, and other demographic- and lifestyle factors, these can be more easily controlled for. In ACNU, all study participants have an above zero chance of being selected for either exposure group (a.k.a. “positivity”, a prerequisite for causal inference). Since individuals for which the treatment is not indicated do not receive that treatment, positivity is not given when we compare users vs. non-users of drugs (Sendor & Stürmer, 2022).

This approach does pose a substantial problem for anticholinergic research as it is traditionally conducted. If we measure the effect of a large group of drugs – as is commonly done when consulting anticholinergic scales – the drugs of interest will have been prescribed for a multitude of indications. This again could be resolved by focusing on individual drugs and classes of drugs. Assessing causality within each drug class might then even provide a clue for the plausibility of the proposed mechanism of a general anticholinergic effect on the studied health outcome.

It is important to emphasise that several factors, including the potential long-term harm of medications, must be accounted for when formulating science-based guidelines for practitioners. This includes knowledge of the characteristics of the individuals to whom drugs are prescribed, as effects can vary between different populations. For example, children might be less susceptible to the cognitive risks associated with anticholinergic use than middle-aged or older adults (Ghezzi et al., 2021). Furthermore, the effects of drugs on several outcomes must be considered. For example, while the cognitive effects of anticholinergic deprescribing are mixed (Salahudeen et al., 2014; Taylor-Rowan et al., 2021), the reduction of AChB might lead to a decrease in reported behavioural and psychological symptoms in individuals with dementia (Jaïdi et al., 2019; Jaïdi et al., 2018). Finally, the likelihood of adverse effects must be always evaluated in consideration of the drugs’ efficacy to treat underlying diseases. For example, anticholinergics to treat overactive bladder lead to only very minor reductions in urinary symptoms (Reynolds et al., 2015; Samuelsson et al., 2015) and alternative treatments are available (Welk et al., 2021), thus increasing the attractiveness of anticholinergic deprescribing.

7.3.4 Biological correlates

If it can be demonstrated that a valid and reliable measure of anticholinergic activity associates with relevant cognitive outcomes and that the relationship is likely causal, biological correlates of such a relationship should be explored. Given the uncertainty of a causal effect of anticholinergic use on cognitive outcomes, it may be premature to extensively hypothesise on the potential biological correlates of such a relationship at this time. However, perhaps due to the ubiquity of anticholinergic processing in the human body, combined with the wealth of research on potential associations with cognition, several suggestions have been advanced in the literature. In this section, I briefly describe three hypotheses that may warrant further study.

7.3.4.1 AD-related neuropathology

One potential mechanism for the adverse effect of long-term anticholinergic use on cognition is the direct action of cholinergic processing on neuropathological features associated with AD. As presented in section 1.4.4, Aβ plaques – a suggested cause of AD – exhibit an intimate relationship with cholinergic processing. Inhibition of ACh signalling increases the levels of Aβ in mice (Liskowsky & Schliebs, 2006), while increases in ACh signalling in guinea pigs (Beach, Kuo, et al., 2001) and targeted activation of M_1 receptors in rabbits (Beach, Walker, et al., 2001) or humans with AD (Hock et al., 2003; Nitsch et al., 2000), decrease the quantity of Aβ in the cortex and cerebrospinal fluid, respectively. A study in 24 individuals suggested that carriers of the APOE ε4 allele – which affects Aβ aggregation and clearance and substantially increases the risk of developing AD (Farrer, 1997) – may be more vulnerable to anticholinergic effects of drugs (Pomara et al., 2003). However, the study only tested cognitive performance a few hours after anticholinergic administration and did not test chronic use. Moreover, only one drug, trihexyphenidyl was tested. Another study of 688 individuals and a 10-year follow-up similarly showed an interaction between APOE ε4 carrier status and anticholinergic use in the risk of developing mild cognitive impairment (Weigand et al., 2020). Consequently, some authors (Welk et al., 2021) have suggested that cholinergic action may promote Aβ clearance.

7.3.4.2 Stress response

The hypothalamic-pituitary-adrenocortical (HPA) axis forms a stress-response feedback system in the body. Stressful stimuli trigger a cascade of events in the hypothalamus, pituitary gland, and adrenal gland, that lead to the release of glucocorticoid hormones. The latter affects multiple bodily functions, as well as bind to antecedent areas of the HPA axis to form a feedback loop. Hippocampal neurons are one of the primary target tissues of glucocorticoid hormones. The hippocampal sensitivity to glucocorticoids makes this brain region especially susceptible to prolonged stress which has well-known negative effects on cognitive function (Paul et al., 2015). The cholinergic system is also involved in the regulation of stress. Acute stress (Finkelstein et al., 1985; Gilad et al., 1985; Kaufer et al., 1998; Mark et al., 1996) as well as experimental glucocorticoid administration (Imperato et al., 1989) enhances the release of ACh in the rodent hippocampus, suggesting a role for ACh in the regulation of the HPA axis. Thus, some have hypothesised (Risacher et al., 2016; Wang et al., 2019) that a reduction in ACh through administration of anticholinergics might dampen the negative feedback of the HPA axis, increase the levels of serum glucocorticoids, and lead to cognitive decline through stress-induced hippocampal damage.

7.3.4.3 Inflammation

Proinflammatory cytokines mediate inflammation in the body, triggering a cascade of events that protects from infection and cancer. This process is usually self-limiting, with regulatory mechanisms that include the actions of adrenal glucocorticoids and anti-inflammatory cytokines inhibiting the generation of an exaggerated and dangerous immune response (Scheinman et al., 1995; Tsunawaki et al., 1988; van der Poll et al., 1996). The balance between pro-inflammatory and anti-inflammatory signals is controlled by the brain. Inflammatory stimuli are detected and relayed by sensory pathways to the hypothalamus, where neurons alter their firing in response (Besedovsky et al., 1977; Tracey, 2002). This leads to activation of the well-known hypothalamic pituitary-adrenal anti-inflammatory response (Bellavance & Rivest, 2014; Sternberg, 1997). Additionally, the hypothalamus stimulates the vagus nerve of the PNS, thus activating the cholinergic anti-inflammatory pathway. This leads

to the inhibition of tumour necrosis factor (TNF; a proinflammatory cytokine) synthesis in the liver, spleen, and heart (Borovikova et al., 2000). Ample evidence exists for vagal cholinergic transmission as the main conduit of this anti-inflammatory response. In rodents, stimulation of the cholinergic anti-inflammatory pathway through pharmacological or electrical means reduces systemic levels of TNF during endotoxemia (the presence in the bloodstream of toxic chemicals released by destroyed bacteria) (Gallowitsch-Puerta & Pavlov, 2007; Pavlov et al., 2003). Furthermore, surgical transection of the vagal nerve eliminates the suppression of systemic TNF triggered by endotoxins (Bernik et al., 2002). Evidence suggests that there is a role for the cholinergic basal forebrain in this anti-inflammatory axis. First, muscarinic agonists (Lee et al., 2010; Munyaka et al., 2014; Pavlov et al., 2006), ACh-I activity (Ji et al., 2014; Lee et al., 2010; Pavlov et al., 2009), and direct central muscarinic stimulation (Pavlov et al., 2006) in the brain suppress systemic levels of TNF and other circulating cytokines in the periphery. Second, genetic ablation of VAChT, which eliminates the release of ACh from the basal forebrain (Al-Onaizi et al., 2017), abolishes the anti-inflammatory effect of the AChE-I galantamine (Lehner et al., 2019). Thus, muscarinic networks may play an important role in the vagal cholinergic anti-inflammatory pathway. Combined with evidence that inflammation may play a role in the pathogenesis of AD (Yoshiyama et al., 2015), this suggests that anticholinergic activity could inhibit the cholinergic anti-inflammatory pathway, thus increasing inflammation and the risk of cognitive decline. To my knowledge, only one study has tested the association between anticholinergic use and inflammation. Sanghavi et al. (2022) found associations between AChB and higher concentrations of inflammatory markers, including fibrinogen, TNF-α, interleukin 6, and C-reactive protein. The authors hypothesised that anticholinergic activity may affect cognitive decline through vagal inhibition. However, the changes in inflammation may have also been caused by underlying disorders for which the drugs were prescribed (i.e., confounding by indication).

7.4 Conclusion

This thesis investigated the long-term trends in anticholinergic prescribing in UK Biobank, and the associations of AChB with dementia, cognitive ability, and brain structural MRI. The results suggest that anticholinergic prescribing steadily rose in the sample from the year 1990 to 2015 and was not attributable solely to ageing of the sample. I also found associations of AChB with the risk of dementia and lower cognitive ability across several anticholinergic scales. In line with other recent evidence, I found these associations to strongly depend on drug class. There was no evidence for an effect of anticholinergic drugs on brain structure. While the effect sizes reported in my results were generally small, large-scale interventions that incorporate such findings could still have a sizeable impact on public health. I hope that my work as presented in this thesis will help inform future research and contribute to clarity as opposed to confusion in the field. Even in that case, there is ample need and opportunity for future work. The entire body of evidence for a role of anticholinergic use in cognition is conflicting, and the field is rife with methodological obstacles, including questionable validity in the measurement of anticholinergic potency and the determination of causal pathways. I encourage further work to confront these challenges.

8 References

Aalto, U. L., Roitto, H. M., Finne-Soveri, H., Kautiainen, H., & Pitkala, K. (2018). Use of Anticholinergic Drugs and its Relationship With Psychological Well-Being and Mortality in Long-Term Care Facilities in Helsinki. *J Am Med Dir Assoc*, *19*(6), 511-515. https://doi.org/10.1016/j.jamda.2017.11.013

Aalto, U. L., Roitto, H. M., Finne-Soveri, H., Kautiainen, H., & Pitkälä, K. H. (2020). Temporal Trends in the Use of Anticholinergic Drugs Among Older People Living in Long-Term Care Facilities in Helsinki. *Drugs Aging*, *37*(1), 27-34. https://doi.org/10.1007/s40266-019-00720-6

Abdel-Rahman, A., Abou-Donia, S., El-Masry, E., Shetty, A., & Abou-Donia, M. (2004). Stress and combined exposure to low doses of pyridostigmine bromide, DEET, and permethrin produce neurochemical and neuropathological alterations in cerebral cortex, hippocampus, and cerebellum. *J Toxicol Environ Health A*, *67*(2), 163-192. https://doi.org/10.1080/15287390490264802

Ablett, A. D., Wood, A. D., Barr, R., Guillot, J., Black, A. J., Macdonald, H. M., Reid, D. M., & Myint, P. K. (2018). A high anticholinergic burden is associated with a history of falls in the previous year in middle-aged women: findings from the Aberdeen Prospective Osteoporosis Screening Study. *Ann Epidemiol*, *28*(8), 557-562 e552. https://doi.org/10.1016/j.annepidem.2018.05.011

Abrams, P., Andersson, K. E., Buccafusco, J. J., Chapple, C., de Groat, W. C., Fryer, A. D., Kay, G., Laties, A., Nathanson, N. M., Pasricha, P. J., & Wein, A. J. (2006). Muscarinic receptors: their distribution and function in body systems, and the implications for treating overactive bladder. *Br J Pharmacol*, *148*(5), 565-578. https://doi.org/10.1038/sj.bjp.0706780

Aizenberg, D., Sigler, M., Weizman, A., & Barak, Y. (2002). Anticholinergic burden and the risk of falls among elderly psychiatric inpatients: a 4-year case-control study. *Int Psychogeriatr*, *14*(3), 307-310. https://doi.org/10.1017/s1041610202008505

Akgün, Ö., Oudshoorn, C., Mattace-Raso, F. U. S., & Egberts, A. (2022). Anticholinergic Drug Use on Admission and the Risk of In-Hospital Falls in Older Hospitalized Patients. *Clin Interv Aging*, *17*, 277-285. https://doi.org/10.2147/CIA.S357818

Al-Onaizi, M. A., Parfitt, G. M., Kolisnyk, B., Law, C. S., Guzman, M. S., Barros, D. M., Leung, L. S., Prado, M. A., & Prado, V. F. (2017). Regulation of Cognitive Processing by Hippocampal Cholinergic Tone. *Cereb Cortex*, *27*(2), 1615-1628. https://doi.org/10.1093/cercor/bhv349

Al Rihani, S. B., Deodhar, M., Darakjian, L. I., Dow, P., Smith, M. K., Bikmetov, R., Turgeon, J., & Michaud, V. (2021). Quantifying Anticholinergic Burden and Sedative Load in Older Adults with Polypharmacy: A Systematic Review of Risk Scales and Models. *Drugs Aging*, *38*(11), 977-994. https://doi.org/10.1007/s40266-021-00895-x

Alagiakrishnan, K., & Wiens, C. A. (2004). An approach to drug induced delirium in the elderly. *Postgrad Med J*, *80*(945), 388-393. https://doi.org/10.1136/pgmj.2003.017236

Aldebert, G., Faillie, J. L., Hillaire-Buys, D., Mura, T., Carrière, I., Delcourt, C., Creuzot-Garcher, C., Villain, M., & Daien, V. (2018). Association of Anticholinergic Drug Use With Risk for Late Age-Related Macular Degeneration. *JAMA Ophthalmol*, *136*(7), 770-778. https://doi.org/10.1001/jamaophthalmol.2018.1719

Alfaro-Almagro, F., Jenkinson, M., Bangerter, N. K., Andersson, J. L. R., Griffanti, L., Douaud, G., Sotiropoulos, S. N., Jbabdi, S., Hernandez-Fernandez, M., Vallee, E., Vidaurre, D., Webster, M., McCarthy, P., Rorden, C., Daducci, A., Alexander, D. C., Zhang, H., Dragonu, I., Matthews, P. M., . . . Smith, S. M. (2018). Image processing and Quality Control for the first 10,000 brain

imaging datasets from UK Biobank. *Neuroimage*, *166*, 400-424. https://doi.org/10.1016/j.neuroimage.2017.10.034

Alfaro-Almagro, F., McCarthy, P., Afyouni, S., Andersson, J. L. R., Bastiani, M., Miller, K. L., Nichols, T. E., & Smith, S. M. (2021). Confound modelling in UK Biobank brain imaging. *Neuroimage*, *224*, 117002. https://doi.org/10.1016/j.neuroimage.2020.117002

Ali, S., Peterson, G. M., Bereznicki, L. R., & Salahudeen, M. S. (2020). Association between anticholinergic drug burden and mortality in older people: a systematic review. *Eur J Clin Pharmacol*, *76*(3), 319-335. https://doi.org/10.1007/s00228-019-02795-x

Allen, N., Sudlow, C., Downey, P., Peakman, T., Danesh, J., Elliott, P., Gallacher, J., Green, J., Matthews, P., Pell, J., Sprosen, T., & Collins, R. (2012). UK Biobank: Current status and what it means for epidemiology. *Health Policy and Technology*, *1*(3), 123-126. https://doi.org/10.1016/j.hlpt.2012.07.003

Alvarez-Jimenez, R., Groeneveld, G. J., van Gerven, J. M., Goulooze, S. C., Baakman, A. C., Hay, J. L., & Stevens, J. (2016). Model-based exposure-response analysis to quantify age related differences in the response to scopolamine in healthy subjects. *Br J Clin Pharmacol*, *82*(4), 1011-1021. https://doi.org/10.1111/bcp.13031

Alvarez, A., Opazo, C., Alarcón, R., Garrido, J., & Inestrosa, N. C. (1997). Acetylcholinesterase promotes the aggregation of amyloid-beta-peptide fragments by forming a complex with the growing fibrils. *J Mol Biol*, *272*(3), 348-361. https://doi.org/10.1006/jmbi.1997.1245

Alzheimer's Association. (2015). 2015 Alzheimer's disease facts and figures. *Alzheimers Dement*, *11*(3), 332-384. https://doi.org/10.1016/j.jalz.2015.02.003

Amenta, F., Parnetti, L., Gallai, V., & Wallin, A. (2001). Treatment of cognitive dysfunction associated with Alzheimer's disease with cholinergic precursors. Ineffective treatments or inappropriate approaches? *Mech Ageing Dev*, *122*(16), 2025-2040. https://doi.org/10.1016/s0047-6374(01)00310-4

Anagnostaras, S. G., Murphy, G. G., Hamilton, S. E., Mitchell, S. L., Rahnama, N. P., Nathanson, N. M., & Silva, A. J. (2003). Selective cognitive dysfunction in acetylcholine M1 muscarinic receptor mutant mice. *Nat Neurosci*, *6*(1), 51-58. https://doi.org/10.1038/nn992

Ancelin, M. L., Artero, S., Portet, F., Dupuy, A. M., Touchon, J., & Ritchie, K. (2006). Non-degenerative mild cognitive impairment in elderly people and use of anticholinergic drugs: longitudinal cohort study. *BMJ*, *332*(7539), 455-459. https://doi.org/10.1136/bmj.38740.439664.DE

Andre, L., Gallini, A., Montastruc, F., Montastruc, J. L., Piau, A., Lapeyre-Mestre, M., & Gardette, V. (2019). Association between anticholinergic (atropinic) drug exposure and cognitive function in longitudinal studies among individuals over 50 years old: a systematic review. *Eur J Clin Pharmacol*, *75*(12), 1631-1644. https://doi.org/10.1007/s00228-019-02744-8

Anglemyer, A., Horvath, H. T., & Bero, L. (2014). Healthcare outcomes assessed with observational study designs compared with those assessed in randomized trials. *Cochrane Database Syst Rev*, *2014*(4), MR000034. https://doi.org/10.1002/14651858.MR000034.pub2

Anticholinergic Cognitive Burden Scale: 2012 Update. (2012). In R. I. Inc. (Ed.), (pp. 1).

Apelt, J., Kumar, A., & Schliebs, R. (2002). Impairment of cholinergic neurotransmission in adult and aged transgenic Tg2576 mouse brain expressing the Swedish mutation of human beta-amyloid precursor protein. *Brain Res*, *953*(1-2), 17-30. https://doi.org/10.1016/s0006-8993(02)03262-6

Arendt, T., Bigl, V., & Arendt, A. (1984). Neurone loss in the nucleus basalis of Meynert in Creutzfeldt-Jakob disease. *Acta Neuropathol*, *65*(1), 85-88. https://doi.org/10.1007/BF00689832

Arendt, T., Bigl, V., Arendt, A., & Tennstedt, A. (1983). Loss of neurons in the nucleus basalis of Meynert in Alzheimer's disease, paralysis agitans and Korsakoff's disease. *Acta Neuropathologica*, *61*(2), 101-108. https://doi.org/10.1007/BF00697388

Arendt, T., Brückner, M. K., Morawski, M., Jager, C., & Gertz, H. J. (2015). Early neurone loss in Alzheimer's disease: cortical or subcortical? *Acta Neuropathol Commun*, *3*, 10. https://doi.org/10.1186/s40478-015-0187-1

Aromataris, E., & Munn, Z. (2020). *JBI Manual for Evidence Synthesis*. https://doi.org/10.46658/JBIMES-20-01

ATC/DDD Index 2022. (December 14th 2021). WHO Collaborating Centre for Drug Statistics Methodology. Retrieved February 9th 2022 from https://www.whocc.no/atc_ddd_index/

Ates Bulut, E., Erken, N., Kaya, D., Dost, F. S., & Isik, A. T. (2022). An Increased Anticholinergic Drug Burden Index Score Negatively Affect Nutritional Status in Older Patients Without Dementia. *Front Nutr*, *9*, 789986. https://doi.org/10.3389/fnut.2022.789986

Attoh-Mensah, E., Loggia, G., Schumann-Bard, P., Morello, R., Descatoire, P., Marcelli, C., & Chavoix, C. (2020). Adverse Effects of Anticholinergic Drugs on Cognition and Mobility: Cutoff for Impairment in a Cross-Sectional Study in Young-Old and Old-Old Adults. *Drugs Aging*, *37*(4), 301-310. https://doi.org/10.1007/s40266-019-00743-z

Audit Commission. (2010). *Improving data quality in the NHS: Annual report on the PbR assurance programme*.

Auld, D. S., Kornecook, T. J., Bastianetto, S., & Quirion, R. (2002). Alzheimer's disease and the basal forebrain cholinergic system. *Progress in Neurobiology*, *68*(3), 209-245. https://doi.org/10.1016/s0301-0082(02)00079-5

Bachman, D. L., Wolf, P. A., Linn, R. T., Knoefel, J. E., Cobb, J. L., Belanger, A. J., White, L. R., & D'Agostino, R. B. (1993). Incidence of dementia and probable Alzheimer's disease in a general population: the Framingham Study. *Neurology*, *43*(3 Pt 1), 515-519. https://doi.org/10.1212/wnl.43.3_part_1.515

Bachrach, W. H. (1958). Anticholinergic drugs. *The American Journal of Digestive Diseases*, *3*(10), 743-799. https://doi.org/10.1007/BF02231366

Baddeley, A. (1991). Working Memory Components of Working Memory The Slave Systems of Working Memory Individual Differences in Working Memory. *Science*, *255*(5044), 556-559. https://doi.org/10.1126/science.1736359

Bag Soytas, R., Arman, P., Suzan, V., Emiroglu Gedik, T., Unal, D., Suna Erdincler, D., Doventas, A., & Yavuzer, H. (2022). Association between anticholinergic drug burden with sarcopenia, anthropometric measurements, and comprehensive geriatric assessment parameters in older adults. *Arch Gerontol Geriatr*, *99*, 104618. https://doi.org/10.1016/j.archger.2021.104618

Baldessarini, R. J., & Willmuth, R. L. (1968). Psychotic reactions during amitriptyline therapy. *Canadian Psychiatric Association Journal*, *13*(6), 571-573. https://doi.org/10.1177/070674376801300620

Ballinger, E. C., Ananth, M., Talmage, D. A., & Role, L. W. (2016). Basal Forebrain Cholinergic Circuits and Signaling in Cognition and Cognitive Decline. *Neuron*, *91*(6), 1199-1218. https://doi.org/10.1016/j.neuron.2016.09.006

Bartolini, M., Bertucci, C., Cavrini, V., & Andrisano, V. (2003). beta-Amyloid aggregation induced by human acetylcholinesterase: inhibition studies. *Biochem Pharmacol*, *65*(3), 407-416. https://doi.org/10.1016/s0006-2952(02)01514-9

Bartus, R. T., Dean, R. L., 3rd, Beer, B., & Lippa, A. S. (1982). The cholinergic hypothesis of geriatric memory dysfunction. *Science*, *217*(4558), 408-414. https://doi.org/10.1126/science.7046051

Batty, G. D., Deary, I. J., & Gottfredson, L. S. (2007). Premorbid (early life) IQ and later mortality risk: systematic review. *Ann Epidemiol*, *17*(4), 278-288. https://doi.org/10.1016/j.annepidem.2006.07.010

Baumeister, A. A., & Hawkins, M. F. (2005). Continuity and discontinuity in the historical development of modern psychopharmacology. *J Hist Neurosci*, *14*(3), 199-209. https://doi.org/10.1080/096470490512562

Baxter, M. G., & Chiba, A. A. (1999). Cognitive functions of the basal forebrain. *Current Opinion in Neurobiology*, *9*(2), 178-183. https://doi.org/10.1016/s0959-4388(99)80024-5

Beach, T. G., Kuo, Y. M., Schwab, C., Walker, D. G., & Roher, A. E. (2001). Reduction of cortical amyloid beta levels in guinea pig brain after systemic administration of physostigmine. *Neurosci Lett*, *310*(1), 21-24. https://doi.org/10.1016/s0304-3940(01)02076-6

Beach, T. G., Walker, D. G., Potter, P. E., Sue, L. I., & Fisher, A. (2001). Reduction of cerebrospinal fluid amyloid beta after systemic administration of M1 muscarinic agonists. *Brain Res*, *905*(1-2), 220-223. https://doi.org/10.1016/s0006-8993(01)02484-2

Beatty, W. W., & Bierley, R. A. (1985). Scopolamine degrades spatial working memory but spares spatial reference memory: dissimilarity of anticholinergic effect and restriction of distal visual cues. *Pharmacol Biochem Behav*, *23*(1), 1-6. https://doi.org/10.1016/0091-3057(85)90120-0

Behçet, A. (2014). The Source-Synthesis- History and Use of Atropine. *Journal of Academic Emergency Medicine*, *13*(1), 2-3. https://doi.org/10.5152/jaem.2014.1120141

Bell, A., & Jones, K. (2013). The impossibility of separating age, period and cohort effects. *Soc Sci Med*, *93*, 163-165. https://doi.org/10.1016/j.socscimed.2013.04.029

Bell, A., & Jones, K. (2018). The hierarchical age-period-cohort model: Why does it find the results that it finds? *Qual Quant*, *52*(2), 783-799. https://doi.org/10.1007/s11135-017-0488-5

Bellavance, M. A., & Rivest, S. (2014). The HPA - Immune Axis and the Immunomodulatory Actions of Glucocorticoids in the Brain. *Front Immunol*, *5*, 136. https://doi.org/10.3389/fimmu.2014.00136

Bentley, P., Driver, J., & Dolan, R. J. (2008). Cholinesterase inhibition modulates visual and attentional brain responses in Alzheimer's disease and health. *Brain*, *131*(Pt 2), 409-424. https://doi.org/10.1093/brain/awm299

Bentley, P., Driver, J., & Dolan, R. J. (2009). Modulation of fusiform cortex activity by cholinesterase inhibition predicts effects on subsequent memory. *Brain*, *132*(Pt 9), 2356-2371. https://doi.org/10.1093/brain/awp176

Bentley, P., Driver, J., & Dolan, R. J. (2011). Cholinergic modulation of cognition: insights from human pharmacological functional neuroimaging. *Prog Neurobiol*, *94*(4), 360-388. https://doi.org/10.1016/j.pneurobio.2011.06.002

Bernik, T. R., Friedman, S. G., Ochani, M., DiRaimo, R., Ulloa, L., Yang, H., Sudan, S., Czura, C. J., Ivanova, S. M., & Tracey, K. J. (2002). Pharmacological stimulation of the cholinergic antiinflammatory pathway. *J Exp Med*, *195*(6), 781-788. https://doi.org/10.1084/jem.20011714

Bertrand, D., & Wallace, T. L. (2020). *Behavioral Pharmacology of the Cholinergic System* (Vol. 45). https://doi.org/10.1007/7854_2020_141

Besedovsky, H., Sorkin, E., Felix, D., & Haas, H. (1977). Hypothalamic changes during the immune response. *Eur J Immunol*, *7*(5), 323-325. https://doi.org/10.1002/eji.1830070516

Bhattacharya, R., Chatterjee, S., Carnahan, R. M., & Aparasu, R. R. (2011). Prevalence and predictors of anticholinergic agents in elderly outpatients with dementia. *Am J Geriatr Pharmacother*, *9*(6), 434-441. https://doi.org/10.1016/j.amjopharm.2011.10.001

Bierer, L. M., Haroutunian, V., Gabriel, S., Knott, P. J., Carlin, L. S., Purohit, D. P., Perl, D. P., Schmeidler, J., Kanof, P., & Davis, K. L. (1995). Neurochemical correlates of dementia severity in Alzheimer's disease: relative importance of the cholinergic deficits. *J Neurochem*, *64*(2), 749-760. https://doi.org/10.1046/j.1471-4159.1995.64020749.x

Bishara, D., Harwood, D., Sauer, J., & Taylor, D. M. (2017). Anticholinergic effect on cognition (AEC) of drugs commonly used in older people. *Int J Geriatr Psychiatry*, *32*(6), 650-656. https://doi.org/10.1002/gps.4507

Bishara, D., Perera, G., Harwood, D., Taylor, D., Sauer, J., Funnell, N., Gee, S., Stewart, R., & Mueller, C. (2021). Centrally-acting anticholinergic drugs- associations with mortality, hospitalisation and cognitive decline following dementia diagnosis in people receiving antidepressant and antipsychotic drugs. *Aging Ment Health*, 1-9. https://doi.org/10.1080/13607863.2021.1947967

Bishara, D., Perera, G., Harwood, D., Taylor, D., Sauer, J., Funnell, N., Stewart, R., & Mueller, C. (2021). Centrally Acting Anticholinergic Drugs Used for Urinary Conditions Associated with Worse Outcomes in Dementia. *J Am Med Dir Assoc*, *22*(12), 2547-2552. https://doi.org/10.1016/j.jamda.2021.08.011

Bishara, D., Perera, G., Harwood, D., Taylor, D., Sauer, J., Stewart, R., & Mueller, C. (2020). The anticholinergic effect on cognition (AEC) scale-Associations with mortality, hospitalisation and cognitive decline following dementia diagnosis. *Int J Geriatr Psychiatry*, *35*(9), 1069-1077. https://doi.org/10.1002/gps.5330

Bishop, K. I., Curran, H. V., & Lader, M. (1996). Do scopolamine and lorazepam have dissociable effects can human memory systems? A dose-response study with normal volunteers. *Experimental and Clinical Psychopharmacology*, *4*(3), 292-299. https://doi.org/10.1037/1064-1297.4.3.292

Blazer, D. G., Federspiel, C. F., Ray, W. A., & Schaffner, W. (1983). The risk of anticholinergic toxicity in the elderly: A study of prescribing practices in two populations. *Journals of Gerontology*, *38*(1), 31-35. https://doi.org/10.1093/geronj/38.1.31

Blumenthal, D. K. (2018). Pharmacodynamics: Molecular Mechanisms of Drug Action. In L. L. Brunton, R. Hilal-Dandan, & B. C. Knollmann (Eds.), *Goodman & Gilman's: The Pharmacological Basis of Therapeutics* (12 ed., pp. 31-54). McGraw-Hill.

Bodick, N. C., Offen, W. W., Levey, A. I., Cutler, N. R., Gauthier, S. G., Satlin, A., Shannon, H. E., Tollefson, G. D., Rasmussen, K., Bymaster, F. P., Hurley, D. J., Potter, W. Z., & Paul, S. M. (1997). The selective muscarinic agonist xanomeline improves both the cognitive deficits and behavioral symptoms of Alzheimer disease. *Alzheimer disease and associated disorders*, *11*, S16-22.

Boekholdt, S. M., Peters, R. J., Day, N. E., Luben, R., Bingham, S. A., Wareham, N. J., Hack, C. E., Reitsma, P. H., & Khaw, K. T. (2004). Macrophage migration inhibitory factor and the risk of myocardial infarction or death due to coronary artery disease in adults without prior myocardial infarction or stroke: the EPIC-Norfolk Prospective Population study. *Am J Med*, *117*(6), 390-397. https://doi.org/10.1016/j.amjmed.2004.04.010

Borghans, L. G., Blokland, A., & Sambeth, A. (2017). Effects of biperiden and acute tryptophan depletion and their combination on verbal word memory and EEG. *Psychopharmacology (Berl)*, *234*(7), 1135-1143. https://doi.org/10.1007/s00213-017-4549-1

Boriosi, J. P., Lasarev, M. R., Peters, M. E., Ferrazzano, P., & Hollman, G. A. (2022). Anticholinergics and serious adverse events in pediatric procedural sedation: a report of the Pediatric Sedation Research Consortium. *Paediatr Anaesth*. https://doi.org/10.1111/pan.14403

Borovikova, L. V., Ivanova, S., Zhang, M., Yang, H., Botchkina, G. I., Watkins, L. R., Wang, H., Abumrad, N., Eaton, J. W., & Tracey, K. J. (2000). Vagus nerve stimulation attenuates the

systemic inflammatory response to endotoxin. *Nature*, *405*(6785), 458-462. https://doi.org/10.1038/35013070

Bosboom, P. R., Alfonso, H., Almeida, O. P., & Beer, C. (2012). Use of Potentially Harmful Medications and Health-Related Quality of Life among People with Dementia Living in Residential Aged Care Facilities. *Dement Geriatr Cogn Dis Extra*, *2*(1), 361-371. https://doi.org/10.1159/000342172

Bostwick, W. W. (1897). Hyoscine in angina pectoris. *Medical Records (1866-1922)*, *51*(19), 679.

Bottiggi, K. A., Salazar, J. C., Yu, L., Caban-Holt, A. M., Ryan, M., Mendiondo, M. S., & Schmitt, F. A. (2006). Lone-term cognitive impact of anticholinergic medications in older adults. *American Journal of Geriatric Psychiatry*, *14*(11), 980-984. https://doi.org/10.1097/01.JGP.0000224619.87681.71

Boustani, M., Campbell, N., Munger, S., Maidment, I., & Fox, C. (2008). Impact of anticholinergics on the aging brain: A review and practical application. *Aging Health*, *4*(3), 311-320. https://doi.org/10.2217/1745509X.4.3.311

Bowen, D. M., Smith, C. B., White, P., & Davison, A. N. (1976). Neurotransmitter-related enzymes and indices of hypoxia in senile dementia and other abiotrophies. *Brain*, *99*(3), 459-496. https://doi.org/10.1093/brain/99.3.459

Brandeis, R., Sapir, M., Hafif, N., Abraham, S., Oz, N., Stein, E., & Fisher, A. (1995). AF150(S): A new functionally selective M1 agonist improves cognitive performance in rats. *Pharmacology Biochemistry and Behavior*, *51*(4), 667-674. https://doi.org/10.1016/0091-3057(94)00435-l

Brayfield, A. (2014). Atropine. In A. Brayfield (Ed.), *Martindale: The Complete Drug Reference* (38 ed., Vol. 2, pp. 1310-1313). Pharmaceutical Press.

Briet, J., Javelot, H., Heitzman, E., Weiner, L., Lameira, C., D'Athis, P., Corneloup, M., & Vailleau, J.-L. (2017). The anticholinergic impregnation scale: towards the elaboration of a scale adapted to prescriptions in French psychiatric settings. *Thérapie*, *72*, 427-437. https://doi.org/10.1016/j.therap.2016.12.010

Brockmöller, J., & Stingl, J. C. (2017). Multimorbidity, polypharmacy and pharmacogenomics in old age. *Pharmacogenomics*, *18*(6), 515-517. https://doi.org/10.2217/pgs-2017-0026

Broder, J. C., Ryan, J., Shah, R. C., Lockery, J. E., Orchard, S. G., Gilmartin-Thomas, J. F., Fravel, M. A., Owen, A. J., Woods, R. L., Wolfe, R., Storey, E., Murray, A. M., & Ernst, M. E. (2021). Anticholinergic medication burden and cognitive function in participants of the ASPREE study. *Pharmacotherapy*. https://doi.org/10.1002/phar.2652

Bruno, G. (1986). Muscarinic Agonist Therapy of Alzheimer's Disease. *Archives of Neurology*, *43*(7), 659. https://doi.org/10.1001/archneur.1986.00520070017009

Bubb, E. J., Metzler-Baddeley, C., & Aggleton, J. P. (2018). The cingulum bundle: Anatomy, function, and dysfunction. *Neurosci Biobehav Rev*, *92*, 104-127. https://doi.org/10.1016/j.neubiorev.2018.05.008

Bubser, M., Byun, N., Wood, M. R., & Jones, C. K. (2012). Muscarinic receptor pharmacology and circuitry for the modulation of cognition. *Handb Exp Pharmacol*(208), 121-166. https://doi.org/10.1007/978-3-642-23274-9_7

Buchanan, G., & Elzevir, L. (1643). *Rerum Scoticarvm historia*. Apud Ludovicum Elzevirium.

Bulley, F. (1842). Observations on the use of belladonna emploted as a douche in iritis and some other forms of ophthalmic disease. *Provincial Medical Journal and Retrospect of the Medical Sciences*, *4*(80), 3-5. https://doi.org/10.1136/bmj.s1-4.1.3

Burns, E. M., Rigby, E., Mamidanna, R., Bottle, A., Aylin, P., Ziprin, P., & Faiz, O. D. (2012). Systematic review of discharge coding accuracy. *J Public Health (Oxf)*, *34*(1), 138-148. https://doi.org/10.1093/pubmed/fdr054

Bycroft, C., Freeman, C., Petkova, D., Band, G., Elliott, L. T., Sharp, K., Motyer, A., Vukcevic, D., Delaneau, O., O'Connell, J., Cortes, A., Welsh, S., Young, A., Effingham, M., McVean, G., Leslie, S., Allen, N., Donnelly, P., & Marchini, J. (2018). The UK Biobank resource with deep phenotyping and genomic data. *Nature*, *562*(7726), 203-209. https://doi.org/10.1038/s41586-018-0579-z

Bymaster, F. P., Heath, I., Hendrix, J. C., & Shannon, H. E. (1993). Comparative behavioral and neurochemical activities of cholinergic antagonists in rats. *J Pharmacol Exp Ther*, *267*(1), 16-24. https://www.ncbi.nlm.nih.gov/pubmed/7901390

Byrne, C. J., Walsh, C., Cahir, C., Ryan, C., Williams, D. J., & Bennett, K. (2018). Anticholinergic and sedative drug burden in community-dwelling older people: a national database study. *BMJ Open*, *8*(7), e022500. https://doi.org/10.1136/bmjopen-2018-022500

Cain, L. E., Saag, M. S., Petersen, M., May, M. T., Ingle, S. M., Logan, R., Robins, J. M., Abgrall, S., Shepherd, B. E., Deeks, S. G., John Gill, M., Touloumi, G., Vourli, G., Dabis, F., Vandenhende, M. A., Reiss, P., van Sighem, A., Samji, H., Hogg, R. S., . . . the, H. I. V. C. C. (2016). Using observational data to emulate a randomized trial of dynamic treatment-switching strategies: an application to antiretroviral therapy. *Int J Epidemiol*, *45*(6), 2038-2049. https://doi.org/10.1093/ije/dyv295

Calandreau, L., Trifilieff, P., Mons, N., Costes, L., Marien, M., Marighetto, A., Micheau, J., Jaffard, R., & Desmedt, A. (2006). Extracellular hippocampal acetylcholine level controls amygdala function and promotes adaptive conditioned emotional response. *J Neurosci*, *26*(52), 13556-13566. https://doi.org/10.1523/JNEUROSCI.3713-06.2006

Caleyachetty, R., Littlejohns, T., Lacey, B., Besevic, J., Conroy, M., Collins, R., & Allen, N. (2021). United Kingdom Biobank (UK Biobank): JACC Focus Seminar 6/8. *J Am Coll Cardiol*, *78*(1), 56-65. https://doi.org/10.1016/j.jacc.2021.03.342

Callegari, E., Malhotra, B., Bungay, P. J., Webster, R., Fenner, K. S., Kempshall, S., LaPerle, J. L., Michel, M. C., & Kay, G. G. (2011). A comprehensive non-clinical evaluation of the CNS penetration potential of antimuscarinic agents for the treatment of overactive bladder. *Br J Clin Pharmacol*, *72*(2), 235-246. https://doi.org/10.1111/j.1365-2125.2011.03961.x

Calvin, C. M., Batty, G. D., Der, G., Brett, C. E., Taylor, A., Pattie, A., Čukić, I., & Deary, I. J. (2017). Childhood intelligence in relation to major causes of death in 68 year follow-up: prospective population study. *BMJ*, *357*, j2708. https://doi.org/10.1136/bmj.j2708

Campbell, N., Boustani, M., Limbil, T., Ott, C., Fox, C., Maidment, I., Schubert, C. C., Munger, S., Fick, D., Miller, D., & Gulati, R. (2009). The cognitive impact of anticholinergics: a clinical review. *Clinical interventions in aging*, *4*, 225-233. https://doi.org/10.2147/cia.s5358

Campbell, N. L., Hines, L., Epstein, A. J., Walker, D., Lockefeer, A., & Shiozawa, A. (2021). A 12-Year Retrospective Study of the Prevalence of Anticholinergic Polypharmacy and Associated Outcomes Among Medicare Patients with Overactive Bladder in the USA. *Drugs Aging*, *38*(12), 1075-1085. https://doi.org/10.1007/s40266-021-00901-2

Cancelli, I., Valentinis, L., Merlino, G., Valente, M., & Gigli, G. L. (2008). Drugs with anticholinergic properties as a risk factor for psychosis in patients affected by Alzheimer's disease. *Clinical Pharmacology and Therapeutics*, *84*(1), 63-68. https://doi.org/10.1038/sj.clpt.6100435

Cao, Y. J., Mager, D. E., Simonsick, E. M., Hilmer, S. N., Ling, S. M., Windham, B. G., Crentsil, V., Yasar, S., Fried, L. P., & Abernethy, D. R. (2008). Physical and cognitive performance and burden of anticholinergics, sedatives, and ACE inhibitors in older women. *Clin Pharmacol Ther*, *83*(3), 422-429. https://doi.org/10.1038/sj.clpt.6100303

Caparrotta, T. M., Dear, J. W., Colhoun, H. M., & Webb, D. J. (2019). Pharmacoepidemiology: Using randomised control trials and observational studies in clinical decision-making. *Br J Clin Pharmacol*, *85*(9), 1907-1924. https://doi.org/10.1111/bcp.14024

Capizzano, A. A., Ación, L., Bekinschtein, T., Furman, M., Gomila, H., Martínez, A., Mizrahi, R., & Starkstein, S. E. (2004). White matter hyperintensities are significantly associated with cortical atrophy in Alzheimer's disease. *J Neurol Neurosurg Psychiatry*, *75*(6), 822-827. https://doi.org/10.1136/jnnp.2003.019273

Cardwell, K., Kerse, N., Ryan, C., Teh, R., Moyes, S. A., Menzies, O., Rolleston, A., Broad, J., & Hughes, C. M. (2020). The Association Between Drug Burden Index (DBI) and Health-Related Outcomes: A Longitudinal Study of the 'Oldest Old' (LiLACS NZ). *Drugs Aging*, *37*(3), 205-213. https://doi.org/10.1007/s40266-019-00735-z

Carnahan, R. M., Lund, B. C., Perry, P. J., & Pollock, B. G. (2002). A critical appraisal of the utility of the serum anticholinergic activity assay in research and clinical practice. *Psychopharmacol Bull*, *36*(2), 24-39.

Carnahan, R. M., Lund, B. C., Perry, P. J., Pollock, B. G., & Culp, K. R. (2006). The Anticholinergic Drug Scale as a measure of drug-related anticholinergic burden: associations with serum anticholinergic activity. *J Clin Pharmacol*, *46*(12), 1481-1486. https://doi.org/10.1177/0091270006292126

Carrière, I., Fourrier-Reglat, A., Dartigues, J.-F., Rouaud, O., Pasquier, F., Ritchie, K., & Ancelin, M. L. (2009). Drugs with anticholinergic properties, cognitive decline, and dementia in an elderly general population: the 3-city study. *Archives of Internal Medicine*, *169*(14), 1317-1324. https://doi.org/10.1001/archinternmed.2009.229

Castier, S., Preda, C., Puisieux, F., Beuscart, J. B., Mahmoudi, R., & Visade, F. (2021). Association between anticholinergic burden and hospital readmission in older patients: a prospective analysis. *Hosp Pract (1995)*, *49*(2), 104-109. https://doi.org/10.1080/21548331.2020.1835264

Cavedo, E., Boccardi, M., Ganzola, R., Canu, E., Beltramello, A., Caltagirone, C., Thompson, P. M., & Frisoni, G. B. (2011). Local amygdala structural differences with 3T MRI in patients with Alzheimer disease. *Neurology*, *76*(8), 727-733. https://doi.org/10.1212/WNL.0b013e31820d62d9

Chancellor, M. B., Staskin, D. R., Kay, G. G., Sandage, B. W., Oefelein, M. G., & Tsao, J. W. (2012). Blood-brain barrier permeation and efflux exclusion of anticholinergics used in the treatment of overactive bladder. *Drugs Aging*, *29*(4), 259-273. https://doi.org/10.2165/11597530-000000000-00000

Chandler, D. J., Lamperski, C. S., & Waterhouse, B. D. (2013). Identification and distribution of projections from monoaminergic and cholinergic nuclei to functionally differentiated subregions of prefrontal cortex. *Brain Res*, *1522*, 38-58. https://doi.org/10.1016/j.brainres.2013.04.057

Chandra, A., Dervenoulas, G., Politis, M., & Alzheimer's Disease Neuroimaging, I. (2019). Magnetic resonance imaging in Alzheimer's disease and mild cognitive impairment. *J Neurol*, *266*(6), 1293-1302. https://doi.org/10.1007/s00415-018-9016-3

Chandramouleeshwaran, S., Ahsan, N., Raymond, R., Nobrega, J. N., Wang, W., Fischer, C. E., Flint, A. J., Herrmann, N., Kumar, S., Lanctot, K., Mah, L., Mulsant, B. H., Pollock, B. G., & Rajji, T. K. (2021). Relationships Between a New Cultured Cell-Based Serum Anticholinergic Activity Assay and Anticholinergic Burden Scales or Cognitive Performance in Older Adults. *Am J Geriatr Psychiatry*, *29*(12), 1239-1252. https://doi.org/10.1016/j.jagp.2021.03.002

Chandramouleeshwaran, S., Ghazala, Z., Nobrega, J. N., Raymond, R., Gambino, S., Pollock, B. G., & Rajji, T. K. (2022). Cell-based serum anticholinergic activity assay and working memory in cognitively healthy older adults before and after scopolamine: An exploratory study. *J Psychopharmacol*, 2698811221122019. https://doi.org/10.1177/02698811221122019

Chatterjee, S., Mehta, S., Sherer, J. T., & Aparasu, R. R. (2010). Prevalence and Predictors of Anticholinergic Medication Use in Elderly Nursing Home Residents with Dementia. *Drugs & aging*, *27*(12), 987-997. https://doi.org/10.2165/11584430-000000000-00000

Chen, N., Sugihara, H., & Sur, M. (2015). An acetylcholine-activated microcircuit drives temporal dynamics of cortical activity. *Nat Neurosci*, *18*(6), 892-902. https://doi.org/10.1038/nn.4002

Chen, X. Q., & Mobley, W. C. (2019). Exploring the Pathogenesis of Alzheimer Disease in Basal Forebrain Cholinergic Neurons: Converging Insights From Alternative Hypotheses. *Front Neurosci*, *13*, 446. https://doi.org/10.3389/fnins.2019.00446

Chew, M. L., Mulsant, B. H., Pollock, B. G., Lehman, M. E., Greenspan, A., Mahmoud, R. A., Kirshner, M. A., Sorisio, D. A., Bies, R. R., & Gharabawi, G. (2008). Anticholinergic activity of 107 medications commonly used by older adults. *J Am Geriatr Soc*, *56*(7), 1333-1341. https://doi.org/10.1111/j.1532-5415.2008.01737.x

Chhatwal, J. P., Schultz, A. P., Hedden, T., Boot, B. P., Wigman, S., Rentz, D., Johnson, K. A., & Sperling, R. A. (2019). Anticholinergic Amnesia is Mediated by Alterations in Human Network Connectivity Architecture. *Cereb Cortex*, *29*(8), 3445-3456. https://doi.org/10.1093/cercor/bhy214

Chiang, C. H., Wu, M. P., Ho, C. H., Weng, S. F., Huang, C. C., Hsieh, W. T., Hsu, Y. W., & Chen, P. J. (2015). Lower Urinary Tract Symptoms Are Associated with Increased Risk of Dementia among the Elderly: A Nationwide Study. *Biomed Res Int*, *2015*, 187819. https://doi.org/10.1155/2015/187819

Choi, J. Y., Kim, H., Jung, Y. I., Chun, S., Yoo, S., Lim, J. Y., Ko, J. Y., Park, K., & Kim, K. I. (2022). Factors associated with anticholinergic burden among older patients in long-term care hospitals in Korea. *Korean J Intern Med*. https://doi.org/10.3904/kjim.2021.457

Chuang, Y. F., Elango, P., Gonzalez, C. E., & Thambisetty, M. (2017). Midlife anticholinergic drug use, risk of Alzheimer's disease, and brain atrophy in community-dwelling older adults. *Alzheimers Dement (N Y)*, *3*(3), 471-479. https://doi.org/10.1016/j.trci.2017.06.004

Chubak, J., Pocobelli, G., & Weiss, N. S. (2012). Tradeoffs between accuracy measures for electronic health care data algorithms. *J Clin Epidemiol*, *65*(3), 343-349 e342. https://doi.org/10.1016/j.jclinepi.2011.09.002

Clowes, N. (1896). A case of puerperal eclampsia treated by hyoscine. *The Lancet*, *148*(3814), 940-941. https://doi.org/10.1016/S0140-6736(01)75811-4

Cohen, N. J., & Squire, L. R. (1980). Preserved learning and retention of pattern-analyzing skill in amnesia: Dissociation of knowing how and knowing that. *Science*, *210*(4466), 207-210. https://doi.org/10.1126/science.7414331

Collamati, A., Martone, A. M., Poscia, A., Brandi, V., Celi, M., Marzetti, E., Cherubini, A., & Landi, F. (2016). Anticholinergic drugs and negative outcomes in the older population: from biological plausibility to clinical evidence. *Aging Clin Exp Res*, *28*(1), 25-35. https://doi.org/10.1007/s40520-015-0359-7

Collin, B. G., Raju, D., & Katsikas, S. (2021). The cognitive effects of anticholinergic drugs on apolipoprotein epsilon4 carriers and noncarriers in the Wisconsin Registry for Alzheimer's Prevention study. *Neuropsychology*, *35*(2), 220-231. https://doi.org/10.1037/neu0000713

Colman, W., & Taylor, J. (1889). Some notes on the value of hyoscine as a sedative. *The Lancet*, *134*(3450), 736-737. https://doi.org/10.1016/S0140-6736(02)29908-0

Consortium, A. N. S. (2017). The AGREE II Instrument [Updated 2017]. In.

Contant, C., Umbriaco, D., Garcia, S., Watkins, K. C., & Descarries, L. (1996). Ultrastructural characterization of the acetylcholine innervation in adult rat neostriatum. *Neuroscience*, *71*(4), 937-947. https://doi.org/10.1016/0306-4522(95)00507-2

Corson, H. (1852). ART. VI.—On the Efficacy of Belladonna as a Remedy in Pertussis. *The American Journal of the Medical Sciences (1827-1924)*, *48*, 353-357.
Coupland, C. A. C., Hill, T., Dening, T., Morriss, R., Moore, M., & Hippisley-Cox, J. (2019). Anticholinergic Drug Exposure and the Risk of Dementia: A Nested Case-Control Study. *JAMA Intern Med*, *179*(8), 1084-1093. https://doi.org/10.1001/jamainternmed.2019.0677
Cox, E. A., Kwatra, S. G., Shetty, S. Kwatra, M. M. (2009). Flaws in the Serum Anticholinergic Activity Assay: Implications for the Study of Delirium. *Journal of the American Geriatrics Society*, *57*(9), 1707-1708. https://doi.org/10.1111/j.1532-5415.2009.02411.x
Cox, M. A., Bassi, C., Saunders, M. E., Nechanitzky, R., Morgado-Palacin, I., Zheng, C., & Mak, T. W. (2020). Beyond neurotransmission: acetylcholine in immunity and inflammation. *J Intern Med*, *287*(2), 120-133. https://doi.org/10.1111/joim.13006
Coyle, J. T., Price, D. L., & DeLong, M. R. (1983). Alzheimer's disease: a disorder of cortical cholinergic innervation. *Science*, *219*(4589), 1184-1190. https://doi.org/10.1126/science.6338589
Croxson, P. L., Kyriazis, D. A., & Baxter, M. G. (2011). Cholinergic modulation of a specific memory function of prefrontal cortex. *Nat Neurosci*, *14*(12), 1510-1512. https://doi.org/10.1038/nn.2971
Cunningham, Y., Wood, K., Stewart, C., Nakham, A., Newlands, R., Gallacher, K. I., Quinn, T. J., Ellis, G., Lowrie, R., Myint, P. K., Bond, C., & Mair, F. S. (2021). Understanding Stakeholder Views Regarding the Design of an Intervention Trial to Reduce Anticholinergic Burden: A Qualitative Study. *Front Pharmacol*, *12*, 608208. https://doi.org/10.3389/fphar.2021.608208
Dale, A. M., Fischl, B., & Sereno, M. I. (1999). Cortical surface-based analysis. I. Segmentation and surface reconstruction. *Neuroimage*, *9*(2), 179-194. https://doi.org/10.1006/nimg.1998.0395
Dale, H. (1914). The action of certain esters and ethers ov choline, and their relation to muscarine. *Journal of Pharmacology and Experimental Therapeutics*, *6*(2), 147-190.
Dale, H. H., & Dudley, H. (1929). The presence of histamine and acetylcholine in the spleen of the ox and the horse. *The Journal of Physiology*, *68*(2), 97-123. https://doi.org/10.1113/jphysiol.1929.sp002598
Dale, H. H., & Gaddum, J. H. (1930). Reactions of denervated volunatry muscle, and their bearing on the mode of action of parasympathetic and related nerves. *The Journal of Physiology*, *70*(2), 109-144. https://doi.org/10.1113/jphysiol.1930.sp002682
Dannenberg, H., Pabst, M., Braganza, O., Schoch, S., Niediek, J., Bayraktar, M., Mormann, F., & Beck, H. (2015). Synergy of direct and indirect cholinergic septo-hippocampal pathways coordinates firing in hippocampal networks. *J Neurosci*, *35*(22), 8394-8410. https://doi.org/10.1523/JNEUROSCI.4460-14.2015
Dantas, L. P., Forte, A., Lima, B. C., Sousa, C. N. S., Vasconcelos, E. C., Lessa, P. H. C., Vieira, R. F., Patrocinio, M. C. A., & Vasconcelos, S. M. M. (2022). Treatment of bladder dysfunction with solifenacin: is there a risk of dementia or cognitive impairment? *Braz J Med Biol Res*, *55*, e11721. https://doi.org/10.1590/1414-431X2021e11721
Darke, P., Cassidy, S., Catt, M., Taylor, R., Missier, P., & Bacardit, J. (2022). Curating a longitudinal research resource using linked primary care EHR data-a UK Biobank case study. *J Am Med Inform Assoc*, *29*(3), 546-552. https://doi.org/10.1093/jamia/ocab260
Davies, P., & Maloney, A. J. (1976). Selective loss of central cholinergic neurons in Alzheimer's disease. *Lancet*, *2*(8000), 1403. https://doi.org/10.1016/s0140-6736(76)91936-x
Davis, A. A., Fritz, J. J., Wess, J., Lah, J. J., & Levey, A. I. (2010). Deletion of M1 muscarinic acetylcholine receptors increases amyloid pathology in vitro and in vivo. *J Neurosci*, *30*(12), 4190-4196. https://doi.org/10.1523/JNEUROSCI.6393-09.2010

Davis, K. A., Sudlow, C. L., & Hotopf, M. (2016). Can mental health diagnoses in administrative data be used for research? A systematic review of the accuracy of routinely collected diagnoses. *BMC Psychiatry*, *16*, 263. https://doi.org/10.1186/s12888-016-0963-x

de Jong, L. W., van der Hiele, K., Veer, I. M., Houwing, J. J., Westendorp, R. G., Bollen, E. L., de Bruin, P. W., Middelkoop, H. A., van Buchem, M. A., & van der Grond, J. (2008). Strongly reduced volumes of putamen and thalamus in Alzheimer's disease: an MRI study. *Brain*, *131*(Pt 12), 3277-3285. https://doi.org/10.1093/brain/awn278

de Leeuw, F. E., Barkhof, F., & Scheltens, P. (2004). White matter lesions and hippocampal atrophy in Alzheimer's disease. *Neurology*, *62*(2), 310-312. https://doi.org/10.1212/01.wnl.0000103289.03648.ad

Degroot, A., & Parent, M. B. (2001). Infusions of physostigmine into the hippocampus or the entorhinal cortex attenuate avoidance retention deficits produced by intra-septal infusions of the GABA agonist muscimol. *Brain Research*, *920*(1-2), 10-18. https://doi.org/10.1016/S0006-8993(01)02798-6

Derby, H. (1874). On the Atropine Treatment of Acquired and Progressive Myopia. With a Table of Cases. *Transactions of the American Ophthalmological Society*, *2*, 139-154.

Deutsch, J. A. (1971). The cholinergic synapse and the site of memory. *Science*, *174*(4011), 788-794. https://doi.org/10.1126/science.174.4011.788

Didelez, V. (2016). Commentary: Should the analysis of observational data always be preceded by specifying a target experimental trial? *Int J Epidemiol*, *45*(6), 2049-2051. https://doi.org/10.1093/ije/dyw032

Diniz, B. S., Butters, M. A., Albert, S. M., Dew, M. A., & Reynolds, C. F., 3rd. (2013). Late-life depression and risk of vascular dementia and Alzheimer's disease: systematic review and meta-analysis of community-based cohort studies. *Br J Psychiatry*, *202*(5), 329-335. https://doi.org/10.1192/bjp.bp.112.118307

Dmochowski, R. R., Thai, S., Iglay, K., Enemchukwu, E., Tee, S., Varano, S., Girman, C., Radican, L., Mudd, P. N., Jr., & Poole, C. (2021). Increased risk of incident dementia following use of anticholinergic agents: A systematic literature review and meta-analysis. *Neurourol Urodyn*, *40*(1), 28-37. https://doi.org/10.1002/nau.24536

Doležal, V., & Kašparová, J. (2003). B-Amyloid and Cholinergic Neurons. *Neurochemical Research*, *28*(3-4), 499-506. https://doi.org/10.1023/A:1022865121743

Doroshyenko, O., & Fuhr, U. (2009). Clinical Pharmacokinetics and Pharmacodynamics of Solifenacin. *Clinical Pharmacokinetics*, *48*(5), 281-302. https://doi.org/10.2165/00003088-200948050-00001

Dos Santos, A. N. M., Junior, G., Bensenor, I. M., Goulart, A. C., Brunoni, A. R., Viana, M. C., Lotufo, P. A., & Suemoto, C. K. (2022). Anticholinergic burden and cognitive performance: cross-sectional results from the ELSA-Brasil study. *Eur J Clin Pharmacol*. https://doi.org/10.1007/s00228-022-03361-8

Douchamps, V., Jeewajee, A., Blundell, P., Burgess, N., & Lever, C. (2013). Evidence for encoding versus retrieval scheduling in the hippocampus by theta phase and acetylcholine. *J Neurosci*, *33*(20), 8689-8704. https://doi.org/10.1523/JNEUROSCI.4483-12.2013

Dowd, F. J. (2017). Cholinergic Agonists and Muscarinic Receptor Antagonists. In *Pharmacology and Therapeutics for Dentistry* (pp. 82-97). https://doi.org/10.1016/b978-0-323-39307-2.00006-0

Drachman, D. A., & Leavitt, J. (1974). Human memory and the cholinergic system. A relationship to aging? *Arch Neurol*, *30*(2), 113-121. https://doi.org/10.1001/archneur.1974.00490320001001

Du, A. T., Schuff, N., Kramer, J. H., Ganzer, S., Zhu, X. P., Jagust, W. J., Miller, B. L., Reed, B. R., Mungas, D., Yaffe, K., Chui, H. C., & Weiner, M. W. (2004). Higher atrophy rate of entorhinal

cortex than hippocampus in AD. *Neurology*, *62*(3), 422-427. https://doi.org/10.1212/01.wnl.0000106462.72282.90

Duarte, A., Hayasaka, S., Du, A., Schuff, N., Jahng, G. H., Kramer, J., Miller, B., & Weiner, M. (2006). Volumetric correlates of memory and executive function in normal elderly, mild cognitive impairment and Alzheimer's disease. *Neurosci Lett*, *406*(1-2), 60-65. https://doi.org/10.1016/j.neulet.2006.07.029

Duncan, A. (1803). *The Edinburgh new dispensatory*. T. Dobson.

Duong, V., Iwamoto, A., Pennycuff, J., Kudish, B., & Iglesia, C. (2021). A systematic review of neurocognitive dysfunction with overactive bladder medications. *Int Urogynecol J*, *32*(10), 2693-2702. https://doi.org/10.1007/s00192-021-04909-5

Durán, C. E., Azermai, M., & Vander Stichele, R. H. (2013). Systematic review of anticholinergic risk scales in older adults. *Eur J Clin Pharmacol*, *69*(7), 1485-1496. https://doi.org/10.1007/s00228-013-1499-3

Duvoisin, R. C., & Katz, R. (1968). Reversal of Central Anticholinergic Syndrome in Man by Physostigmine. *JAMA*, *206*(9), 1963-1965. https://doi.org/10.1001/jama.1968.03150090039008

Egberts, A., Moreno-Gonzalez, R., Alan, H., Ziere, G., & Mattace-Raso, F. U. S. (2021). Anticholinergic Drug Burden and Delirium: A Systematic Review. *J Am Med Dir Assoc*, *22*(1), 65-73 e64. https://doi.org/10.1016/j.jamda.2020.04.019

Eglen, R. M. (2005). Muscarinic Receptor Subtype Pharmacology and Physiology. In (pp. 105-136). https://doi.org/10.1016/s0079-6468(05)43004-0

Eglen, R. M. (2012). Overview of muscarinic receptor subtypes. *Handb Exp Pharmacol*(208), 3-28. https://doi.org/10.1007/978-3-642-23274-9_1

Ehrt, U., Broich, K., Larsen, J. P., Ballard, C., & Aarsland, D. (2010). Use of drugs with anticholinergic effect and impact on cognition in Parkinson's disease: a cohort study. *J Neurol Neurosurg Psychiatry*, *81*(2), 160-165. https://doi.org/10.1136/jnnp.2009.186239

Ellis, J. R., Ellis, K. A., Bartholomeusz, C. F., Harrison, B. J., Wesnes, K. A., Erskine, F. F., Vitetta, L., & Nathan, P. J. (2006). Muscarinic and nicotinic receptors synergistically modulate working memory and attention in humans. *Int J Neuropsychopharmacol*, *9*(2), 175-189. https://doi.org/10.1017/S1461145705005407

Evans, D. A., Bennett, D. A., Wilson, R. S., Bienias, J. L., Morris, M. C., Scherr, P. A., Hebert, L. E., Aggarwal, N., Beckett, L. A., Joglekar, R., Berry-Kravis, E., & Schneider, J. (2003). Incidence of Alzheimer disease in a biracial urban community: relation to apolipoprotein E allele status. *Arch Neurol*, *60*(2), 185-189. https://doi.org/10.1001/archneur.60.2.185

Fadare, J. O., Obimakinde, A. M., Aina, F. O., Araromi, E. J., Adegbuyi, T. A., Osasona, O. E., & Agbesanwa, T. A. (2021). Anti-Cholinergic Drug Burden Among Ambulatory Elderly Patients in a Nigerian Tertiary Healthcare Facility. *Front Pharmacol*, *12*, 580152. https://doi.org/10.3389/fphar.2021.580152

Farlow, J. (1889). On the use of belladonna and cannabid indica by the rectum in gynecological practice. *New England Journal of Medicine*, *120*, 507-509. https://doi.org/10.1056/NEJM188905231202103

Farmer, R. L., Floyd, R. G., Reynolds, M. R., & Berlin, K. S. (2019). How Can General Intelligence Composites Most Accurately Index Psychometric g and What Might Be Good Enough? *Contemporary School Psychology*, *24*(1), 52-67. https://doi.org/10.1007/s40688-019-00244-1

Farrer, L. A. (1997). Effects of Age, Sex, and Ethnicity on the Association Between Apolipoprotein E Genotype and Alzheimer Disease. *JAMA*, *278*(16). https://doi.org/10.1001/jama.1997.03550160069041

Faure, R., Dauphinot, V., Krolak-Salmon, P., & Mouchoux, C. (2013). A standard international version of the drug burden index for cross-national comparison of the funcional burden of medications in older people. *Journal of the American Geriatrics Society*, *61*(7), 1227-1228. https://doi.org/10.1111/jgs.12343

Fawns-Ritchie, C., & Deary, I. J. (2020). Reliability and validity of the UK Biobank cognitive tests. *PLoS One*, *15*(4), e0231627. https://doi.org/10.1371/journal.pone.0231627

Feinberg, M. (1993). The problems of anticholinergic adverse effects in older patients. *Drugs & aging*, *3*(4), 335-348. https://doi.org/10.2165/00002512-199303040-00004

Feldberg, W., & Gaddum, J. H. (1934). The chemical transmitter at synapses in a sympathetic ganglion. *Journal of Physiology*, *81*(3), 305-319. https://doi.org/10.1113/jphysiol.1934.sp003137

Felder, C. C., Goldsmith, P. J., Jackson, K., Sanger, H. E., Evans, D. A., Mogg, A. J., & Broad, L. M. (2018). Current status of muscarinic M1 and M4 receptors as drug targets for neurodegenerative diseases. *Neuropharmacology*, *136*(Pt C), 449-458. https://doi.org/10.1016/j.neuropharm.2018.01.028

Fernández-Cabello, S., Kronbichler, M., Van Dijk, K. R. A., Goodman, J. A., Spreng, R. N., Schmitz, T. W., & Alzheimer's Disease Neuroimaging Initiative. (2020). Basal forebrain volume reliably predicts the cortical spread of Alzheimer's degeneration. *Brain*, *143*(3), 993-1009. https://doi.org/10.1093/brain/awaa012

Fick, D. M., Semla, T. P., Steinman, M., Beizer, J., Brandt, N., Dombrowski, R., DuBeau, C. E., Pezzullo, L., Epplin, J. J., Flanagan, N., Morden, E., Hanlon, J., Hollmann, P., Laird, R., Linnebur, S., & Sandhu, S. (2019). American Geriatrics Society 2019 Updated AGS Beers Criteria® for Potentially Inappropriate Medication Use in Older Adults. *J Am Geriatr Soc*, *67*(4), 674-694. https://doi.org/10.1111/jgs.15767

Fink-Jensen, A., Fedorova, I., Wörtwein, G., Woldbye, D. P., Rasmussen, T., Thomsen, M., Bolwig, T. G., Knitowski, K. M., McKinzie, D. L., Yamada, M., Wess, J., & Basile, A. (2003). Role for M5 muscarinic acetylcholine receptors in cocaine addiction. *J Neurosci Res*, *74*(1), 91-96. https://doi.org/10.1002/jnr.10728

Finkelstein, Y., Koffler, B., Rabey, J. M., & Gilad, G. M. (1985). Dynamics of cholinergic synaptic mechanisms in rat hippocampus after stress. *Brain Res*, *343*(2), 314-319. https://doi.org/10.1016/0006-8993(85)90749-8

Fischer, C. E., & Agüera-Ortiz, L. (2018). Psychosis and dementia: risk factor, prodrome, or cause? *Int Psychogeriatr*, *30*(2), 209-219. https://doi.org/10.1017/S1041610217000874

Fisher, S. K., & Wonnacott, S. (2012). Acetylcholine. In *Basic Neurochemistry* (pp. 258-282). https://doi.org/10.1016/b978-0-12-374947-5.00013-4

Flacker, J. M., & Lipsitz, L. A. (1999). Serum anticholinergic activity changes with acute illness in elderly medical patients. *J Gerontol A Biol Sci Med Sci*, *54*(1), M12-16. https://doi.org/10.1093/gerona/54.1.m12

Flacker, J. M., & Wei, J. Y. (2001). Endogenous anticholinergic substances may exist during acute illness in elderly medical patients. *J Gerontol A Biol Sci Med Sci*, *56*(6), M353-355. https://doi.org/10.1093/gerona/56.6.m353

Flicker, C., Ferris, S. H., & Serby, M. (1992). Hypersensitivity to scopolamine in the elderly. *Psychopharmacology*, *107*(2-3). https://doi.org/10.1007/BF02245172

Flynn, D. D., Ferrari-DiLeo, G., Mash, D. C., & Levey, A. I. (1995). Differential regulation of molecular subtypes of muscarinic receptors in Alzheimer's disease. *J Neurochem*, *64*(4), 1888-1891. https://doi.org/10.1046/j.1471-4159.1995.64041888.x

Forbes, T. R. (1977). Why is it called 'beautiful lady'? A note on belladonna. *Bull N Y Acad Med*, *53*(4), 403-406.

Fornari, R. V., Moreira, K. M., & Oliveira, M. G. (2000). Effects of the selective M1 muscarinic receptor antagonist dicyclomine on emotional memory. *Learn Mem*, *7*(5), 287-292. https://doi.org/10.1101/lm.34900

Fox, C., Richardson, K., Maidment, I. D., Savva, G. M., Matthews, F. E., Smithard, D., Coulton, S., Katona, C., Boustani, M. A., & Brayne, C. (2011). Anticholinergic medication use and cognitive impairment in the older population: the medical research council cognitive function and ageing study. *J Am Geriatr Soc*, *59*(8), 1477-1483. https://doi.org/10.1111/j.1532-5415.2011.03491.x

Fox, C., Smith, T., Maidment, I., Chan, W. Y., Bua, N., Myint, P. K., Boustani, M., Kwok, C. S., Glover, M., Koopmans, I., & Campbell, N. (2014). Effect of medications with anti-cholinergic properties on cognitive function, delirium, physical function and mortality: a systematic review. *Age Ageing*, *43*(5), 604-615. https://doi.org/10.1093/ageing/afu096

Francis, P. T., Palmer, A. M., Snape, M., & Wilcock, G. K. (1999). The cholinergic hypothesis of Alzheimer's disease: A review of progress. *Journal of Neurology Neurosurgery and Psychiatry*, *66*(2), 137-147. https://doi.org/10.1136/jnnp.66.2.137

Franco-Marina, F., Garcia-Gonzalez, J. J., Wagner-Echeagaray, F., Gallo, J., Ugalde, O., Sanchez-Garcia, S., Espinel-Bermudez, C., Juarez-Cedillo, T., Rodriguez, M. A., & Garcia-Pena, C. (2010). The Mini-mental State Examination revisited: ceiling and floor effects after score adjustment for educational level in an aging Mexican population. *Int Psychogeriatr*, *22*(1), 72-81. https://doi.org/10.1017/S1041610209990822

Fried, T. R., O'Leary, J., Towle, V., Goldstein, M. K., Trentalange, M., & Martin, D. K. (2014). Health outcomes associated with polypharmacy in community-dwelling older adults: a systematic review. *J Am Geriatr Soc*, *62*(12), 2261-2272. https://doi.org/10.1111/jgs.13153

Fry, A., Littlejohns, T. J., Sudlow, C., Doherty, N., Adamska, L., Sprosen, T., Collins, R., & Allen, N. E. (2017). Comparison of Sociodemographic and Health-Related Characteristics of UK Biobank Participants With Those of the General Population. *Am J Epidemiol*, *186*(9), 1026-1034. https://doi.org/10.1093/aje/kwx246

Furey, M. L., Pietrini, P., Haxby, J. V., Alexander, G. E., Lee, H. C., VanMeter, J., Grady, C. L., Shetty, U., Rapoport, S. I., Schapiro, M. B., & Freo, U. (1997). Cholinergic stimulation alters performance and task-specific regional cerebral blood flow during working memory. *Proc Natl Acad Sci U S A*, *94*(12), 6512-6516. https://doi.org/10.1073/pnas.94.12.6512

Gais, S., & Born, J. (2004). Declarative memory consolidation: mechanisms acting during human sleep. *Learn Mem*, *11*(6), 679-685. https://doi.org/10.1101/lm.80504

Gallowitsch-Puerta, M., & Pavlov, V. A. (2007). Neuro-immune interactions via the cholinergic anti-inflammatory pathway. *Life Sci*, *80*(24-25), 2325-2329. https://doi.org/10.1016/j.lfs.2007.01.002

Galvin, V. C., Arnsten, A. F. T., & Wang, M. (2020). Involvement of Nicotinic Receptors in Working Memory Function. *Curr Top Behav Neurosci*, *45*, 89-99. https://doi.org/10.1007/7854_2020_142

Garraway, E. (1865). Oil the Treatment of Whooping- Cough by Belladonna and Sulphate of Zinc. *Confederate States Medical and Surgical Journal*, *2*(1), 21-22.

Gaulton, A., Bellis, L. J., Bento, A. P., Chambers, J., Davies, M., Hersey, A., Light, Y., McGlinchey, S., Michalovich, D., Al-Lazikani, B., & Overington, J. P. (2012). ChEMBL: a large-scale bioactivity database for drug discovery. *Nucleic Acids Res*, *40*(Database issue), D1100-1107. https://doi.org/10.1093/nar/gkr777

Gautam, D., Jeon, J., Starost, M. F., Han, S. J., Hamdan, F. F., Cui, Y., Parlow, A. F., Gavrilova, O., Szalayova, I., Mezey, E., & Wess, J. (2009). Neuronal M3 muscarinic acetylcholine receptors

are essential for somatotroph proliferation and normal somatic growth. *Proc Natl Acad Sci U S A*, *106*(15), 6398-6403. https://doi.org/10.1073/pnas.0900977106
Geiger, P. L., Hesse. (1833). Darstellung des Atropins. *Annalen der Pharmacie*, *5*(1), 43-81. https://doi.org/10.1002/jlac.18330050108
Geldenhuys, W. J., Mohammad, A. S., Adkins, C. E., & Lockman, P. R. (2015). Molecular determinants of blood-brain barrier permeation. *Ther Deliv*, *6*(8), 961-971. https://doi.org/10.4155/tde.15.32
Geyer, J., Gavrilova, O., & Petzinger, E. (2009). The role of p-glycoprotein in limiting brain penetration of the peripherally acting anticholinergic overactive bladder drug trospium chloride. *Drug Metab Dispos*, *37*(7), 1371-1374. https://doi.org/10.1124/dmd.109.027144
Ghezzi, E., Chan, M., Kalisch Ellett, L. M., Ross, T. J., Richardson, K., Ho, J. N., Copley, D., Steele, C., & Keage, H. A. D. (2021). The effects of anticholinergic medications on cognition in children: a systematic review and meta-analysis. *Sci Rep*, *11*(1), 219. https://doi.org/10.1038/s41598-020-80211-6
Gilad, G. M., Mahon, B. D., Finkelstein, Y., Koffler, B., & Gilad, V. H. (1985). Stress-induced activation of the hippocampal cholinergic system and the pituitary-adrenocortical axis. *Brain Res*, *347*(2), 404-408. https://doi.org/10.1016/0006-8993(85)90209-4
Ginsberg, S. D., Che, S., Counts, S. E., & Mufson, E. J. (2006). Shift in the ratio of three-repeat tau and four-repeat tau mRNAs in individual cholinergic basal forebrain neurons in mild cognitive impairment and Alzheimer's disease. *J Neurochem*, *96*(5), 1401-1408. https://doi.org/10.1111/j.1471-4159.2005.03641.x
Glass, T. A., Goodman, S. N., Hernán, M. A., & Samet, J. M. (2013). Causal inference in public health. *Annu Rev Public Health*, *34*, 61-75. https://doi.org/10.1146/annurev-publhealth-031811-124606
Glickstein, M. (2014). *Neuroscience: a historical introduction*. MIT Press.
Gnjidic, D., Bell, J. S., Hilmer, S. N., Lönnroos, E., Sulkava, R., & Hartikainen, S. (2012). Drug Burden Index associated with function in community-dwelling older people in Finland: a cross-sectional study. *Ann Med*, *44*(5), 458-467. https://doi.org/10.3109/07853890.2011.573499
Gnjidic, D., Hilmer, S. N., Blyth, F. M., Naganathan, V., Cumming, R. G., Handelsman, D. J., McLachlan, A. J., Abernethy, D. R., Banks, E., & Le Couteur, D. G. (2012). High-risk prescribing and incidence of frailty among older community-dwelling men. *Clin Pharmacol Ther*, *91*(3), 521-528. https://doi.org/10.1038/clpt.2011.258
Goard, M., & Dan, Y. (2009). Basal forebrain activation enhances cortical coding of natural scenes. *Nat Neurosci*, *12*(11), 1444-1449. https://doi.org/10.1038/nn.2402
Gomeza, J., Zhang, L., Kostenis, E., Felder, C., Bymaster, F., Brodkin, J., Shannon, H., Xia, B., Deng, C. X., & Wess, J. (1999). Enhancement of D1 dopamine receptor-mediated locomotor stimulation in M4 muscarinic acetylcholine receptor knockout mice. *Proceedings of the National Academy of Sciences of the United States of America*, *96*(18), 10483-10488. https://doi.org/10.1073/pnas.96.18.10483
Gonzales, K. K., & Smith, Y. (2015). Cholinergic interneurons in the dorsal and ventral striatum: anatomical and functional considerations in normal and diseased conditions. *Ann N Y Acad Sci*, *1349*, 1-45. https://doi.org/10.1111/nyas.12762
Goodman, L. S., Gilman, A., Brunton, L. L., Lazo, J. S., & Parker, K. L. (2006). *Goodman & Gilman's the pharmacological basis of therapeutics* (11th ed.). McGraw-Hill.
Gould, R. W., Dencker, D., Grannan, M., Bubser, M., Zhan, X., Wess, J., Xiang, Z., Locuson, C., Lindsley, C. W., Conn, P. J., & Jones, C. K. (2015). Role for the M1 Muscarinic Acetylcholine Receptor in Top-Down Cognitive Processing Using a Touchscreen Visual Discrimination Task

in Mice. *ACS Chem Neurosci*, *6*(10), 1683-1695. https://doi.org/10.1021/acschemneuro.5b00123

Gradek-Kwinta, E., Slowik, A., & Dziedzic, T. (2022). The use of anticholinergic medication is associated with an increased risk of stroke-associated pneumonia. *Aging Clin Exp Res*. https://doi.org/10.1007/s40520-022-02123-x

Graef, S., Schönknecht, P., Sabri, O., & Hegerl, U. (2011). Cholinergic receptor subtypes and their role in cognition, emotion, and vigilance control: an overview of preclinical and clinical findings. *Psychopharmacology (Berl)*, *215*(2), 205-229. https://doi.org/10.1007/s00213-010-2153-8

Granacher, R. P., & Baldessarini, R. J. (1975). Physostigmine: its use in acute anticholinergic syndrome with antidepressant and antiparkinson drugs. *Archives of General Psychiatry*, *32*(3), 375-380. https://doi.org/10.1001/archpsyc.1975.01760210109008

Granon, S., Poucet, B., Thinus-Blanc, C., Changeux, J. P., & Vidal, C. (1995). Nicotinic and muscarinic receptors in the rat prefrontal cortex: differential roles in working memory, response selection and effortful processing. *Psychopharmacology (Berl)*, *119*(2), 139-144. https://doi.org/10.1007/BF02246154

Graves-Morris, K., Stewart, C., Soiza, R. L., Taylor-Rowan, M., Quinn, T. J., Loke, Y. K., & Myint, P. K. (2020). The Prognostic Value of Anticholinergic Burden Measures in Relation to Mortality in Older Individuals: A Systematic Review and Meta-Analysis. *Front Pharmacol*, *11*, 570. https://doi.org/10.3389/fphar.2020.00570

Graves, R., & Morton, H. V. (1955). *The Greek myths*. In *Penguin books 1026-1027.* Penguin Books.

Gray, S. L., Anderson, M. L., Dublin, S., Hanlon, J. T., Hubbard, R., Walker, R., Yu, O., Crane, P. K., & Larson, E. B. (2015). Cumulative use of strong anticholinergics and incident dementia: a prospective cohort study. *JAMA Intern Med*, *175*(3), 401-407. https://doi.org/10.1001/jamainternmed.2014.7663

Gray, S. L., Anderson, M. L., Hanlon, J. T., Dublin, S., Walker, R. L., Hubbard, R. A., Yu, O., Montine, T. J., Crane, P. K., Sonnen, J. A., Keene, C. D., & Larson, E. B. (2018). Exposure to Strong Anticholinergic Medications and Dementia-Related Neuropathology in a Community-Based Autopsy Cohort. *J Alzheimers Dis*, *65*(2), 607-616. https://doi.org/10.3233/JAD-171174

Gray, S. L., Dublin, S., Yu, O., Walker, R., Anderson, M., Hubbard, R. A., Crane, P. K., & Larson, E. B. (2016). Benzodiazepine use and risk of incident dementia or cognitive decline: prospective population based study. *BMJ*, *352*, i90. https://doi.org/10.1136/bmj.i90

Green, A., Ellis, K. A., Ellis, J., Bartholomeusz, C. F., Ilic, S., Croft, R. J., Phan, K. L., & Nathan, P. J. (2005). Muscarinic and nicotinic receptor modulation of object and spatial n-back working memory in humans. *Pharmacol Biochem Behav*, *81*(3), 575-584. https://doi.org/10.1016/j.pbb.2005.04.010

Gritton, H. J., Howe, W. M., Mallory, C. S., Hetrick, V. L., Berke, J. D., & Sarter, M. (2016). Cortical cholinergic signaling controls the detection of cues. *Proc Natl Acad Sci U S A*, *113*(8), E1089-1097. https://doi.org/10.1073/pnas.1516134113

Grossi, C. M., Richardson, K., Savva, G. M., Fox, C., Arthur, A., Loke, Y. K., Steel, N., Brayne, C., Matthews, F. E., Robinson, L., Myint, P. K., & Maidment, I. D. (2020). Increasing prevalence of anticholinergic medication use in older people in England over 20 years: cognitive function and ageing study I and II. *BMC Geriatr*, *20*(1), 267. https://doi.org/10.1186/s12877-020-01657-x

Grothe, M., Heinsen, H., & Teipel, S. (2013). Longitudinal measures of cholinergic forebrain atrophy in the transition from healthy aging to Alzheimer's disease. *Neurobiol Aging*, *34*(4), 1210-1220. https://doi.org/10.1016/j.neurobiolaging.2012.10.018

Gu, Z., Lamb, P. W., & Yakel, J. L. (2012). Cholinergic coordination of presynaptic and postsynaptic activity induces timing-dependent hippocampal synaptic plasticity. *J Neurosci*, *32*(36), 12337-12348. https://doi.org/10.1523/JNEUROSCI.2129-12.2012

Gu, Z., & Yakel, J. L. (2011). Timing-dependent septal cholinergic induction of dynamic hippocampal synaptic plasticity. *Neuron*, *71*(1), 155-165. https://doi.org/10.1016/j.neuron.2011.04.026

Guo, X., Wang, Z., Li, K., Li, Z., Qi, Z., Jin, Z., Yao, L., & Chen, K. (2010). Voxel-based assessment of gray and white matter volumes in Alzheimer's disease. *Neurosci Lett*, *468*(2), 146-150. https://doi.org/10.1016/j.neulet.2009.10.086

Guthrie, B., Makubate, B., Hernandez-Santiago, V., & Dreischulte, T. (2015). The rising tide of polypharmacy and drug-drug interactions: population database analysis 1995-2010. *BMC Med*, *13*, 74. https://doi.org/10.1186/s12916-015-0322-7

Guyatt, G. H., Oxman, A. D., Vist, G. E., Kunz, R., Falck-Ytter, Y., Alonso-Coello, P., Schünemann, H. J., & Group, G. W. (2008). GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. *BMJ*, *336*(7650), 924-926. https://doi.org/10.1136/bmj.39489.470347.AD

Haasum, Y., Fastbom, J., & Johnell, K. (2012). Institutionalization as a Risk Factor for Inappropriate Drug Use in the Elderly: A Swedish Nationwide Registe-Based Study. *The Annals of Pharmacotherapy*, *46*(3), 339-346. https://doi.org/10.1345/aph.1Q597

Haenisch, B., von Holt, K., Wiese, B., Prokein, J., Lange, C., Ernst, A., Brettschneider, C., Konig, H. H., Werle, J., Weyerer, S., Luppa, M., Riedel-Heller, S. G., Fuchs, A., Pentzek, M., Weeg, D., Bickel, H., Broich, K., Jessen, F., Maier, W., & Scherer, M. (2015). Risk of dementia in elderly patients with the use of proton pump inhibitors. *Eur Arch Psychiatry Clin Neurosci*, *265*(5), 419-428. https://doi.org/10.1007/s00406-014-0554-0

Hafdi, M., Hoevenaar-Blom, M. P., Beishuizen, C. R. L., Moll van Charante, E. P., Richard, E., & van Gool, W. A. (2020). Association of Benzodiazepine and Anticholinergic Drug Usage With Incident Dementia: A Prospective Cohort Study of Community-Dwelling Older Adults. *J Am Med Dir Assoc*, *21*(2), 188-193 e183. https://doi.org/10.1016/j.jamda.2019.05.010

Haijma, S. V., Van Haren, N., Cahn, W., Koolschijn, P. C., Hulshoff Pol, H. E., & Kahn, R. S. (2013). Brain volumes in schizophrenia: a meta-analysis in over 18 000 subjects. *Schizophr Bull*, *39*(5), 1129-1138. https://doi.org/10.1093/schbul/sbs118

Hajjar, E. R., Hanlon, J. T., Sloane, R. J., Lindblad, C. I., Pieper, C. F., Ruby, C. M., Branch, L. C., & Schmader, K. E. (2005). Unnecessary drug use in frail older people at hospital discharge. *J Am Geriatr Soc*, *53*(9), 1518-1523. https://doi.org/10.1111/j.1532-5415.2005.53523.x

Hampel, H., Mesulam, M. M., Cuello, A. C., Farlow, M. R., Giacobini, E., Grossberg, G. T., Khachaturian, A. S., Vergallo, A., Cavedo, E., Snyder, P. J., & Khachaturian, Z. S. (2018). The cholinergic system in the pathophysiology and treatment of Alzheimer's disease. *Brain*, *141*(7), 1917-1933. https://doi.org/10.1093/brain/awy132

Han, L., Agostini, J. V., & Allore, H. G. (2008). Cumulative anticholinergic exposure is associated with poor memory and executive function in older men. *J Am Geriatr Soc*, *56*(12), 2203-2210. https://doi.org/10.1111/j.1532-5415.2008.02009.x

Han, L., McCusker, J., Cole, M., Abrahamowicz, M., Primeau, F., & Élie, M. (2001). Use of medications with anticholinergic effect predicts clinical severity of delirium symptoms in older medical inpatients. *Archives of Internal Medicine*, *161*(8), 1099-1105. https://doi.org/10.1001/archinte.161.8.1099

Hanlon, P., Quinn, T. J., Gallacher, K. I., Myint, P. K., Jani, B. D., Nicholl, B. I., Lowrie, R., Soiza, R. L., Neal, S. R., Lee, D., & Mair, F. S. (2020). Assessing Risks of Polypharmacy Involving Medications With Anticholinergic Properties. *Ann Fam Med*, *18*(2), 148-155. https://doi.org/10.1370/afm.2501

Hansen, R. A., Gartlehner, G., Webb, A. P., Morgan, L. C., Moore, C. G., & Jonas, D. E. (2008). Efficacy and safety of donepezil, galantamine, and rivastigmine for the treatment of Alzheimer's disease: A systematic review and meta-analysis. *Clinical interventions in aging*, *3*(2), 211-225. https://doi.org/10.17615/ae8c-2p76

Harnod, T., Yang, Y. C., Chiu, L. T., Wang, J. H., Lin, S. Z., & Ding, D. C. (2021). Use of bladder antimuscarinics is associated with an increased risk of dementia: a retrospective population-based case-control study. *Sci Rep*, *11*(1), 4827. https://doi.org/10.1038/s41598-021-84229-2

Harvey, R. D., & Belevych, A. E. (2003). Muscarinic regulation of cardiac ion channels. *Br J Pharmacol*, *139*(6), 1074-1084. https://doi.org/10.1038/sj.bjp.0705338

Hasselmo, M. E., & Sarter, M. (2011). Modes and models of forebrain cholinergic neuromodulation of cognition. *Neuropsychopharmacology*, *36*(1), 52-73. https://doi.org/10.1038/npp.2010.104

Hasselmo, M. E., & Stern, C. E. (2014). Theta rhythm and the encoding and retrieval of space and time. *Neuroimage*, *85 Pt 2*, 656-666. https://doi.org/10.1016/j.neuroimage.2013.06.022

Hawker, S., Payne, S., Kerr, C., Hardey, M., & Powell, J. (2002). Appraising the evidence: reviewing disparate data systematically. *Qual Health Res*, *12*(9), 1284-1299. https://doi.org/10.1177/1049732302238251

Hebert, L. E. (1995). Age-Specific Incidence of Alzheimer's Disease in a Community Population. *JAMA: The Journal of the American Medical Association*, *273*(17). https://doi.org/10.1001/jama.1995.03520410048025

Heeschen, C., Weis, M., Aicher, A., Dimmeler, S., & Cooke, J. P. (2002). A novel angiogenic pathway mediated by non-neuronal nicotinic acetylcholine receptors. *Journal of Clinical Investigation*, *110*(4), 527-536. https://doi.org/10.1172/jci0214676

Hefner, G., Shams, M. E. E., Wenzel-Seifert, K., Fellgiebel, A., Falter, T., Haen, E., & Hiemke, C. (2015). Rating The Delirogenic Potential of Drugs for Prediction of Side Effects in Elderly Psychiatric Inpatients. *Jacobs Journal of Pharmacology and Pharmacovigilance*, *1*(1).

Heller Brown, J., Brandl, K., & Wess, J. (2018). Muscarinic receptor agonists and antagonists. In L. L. Brunton, R. Hilal-Dandan, & B. C. Knollmann (Eds.), *The Pharmacological Basis of Therapeutics* (13 ed., pp. 149-161). McGraw-Hill. https://doi.org/10.1517/13543776.9.8.1029

Herkenham, M., & Nauta, W. J. (1979). Efferent connections of the habenular nuclei in the rat. *J Comp Neurol*, *187*(1), 19-47. https://doi.org/10.1002/cne.901870103

Hernán, M. A., Alonso, A., Logan, R., Grodstein, F., Michels, K. B., Willett, W. C., Manson, J. E., & Robins, J. M. (2008). Observational studies analyzed like randomized experiments: an application to postmenopausal hormone therapy and coronary heart disease. *Epidemiology*, *19*(6), 766-779. https://doi.org/10.1097/EDE.0b013e3181875e61

Hernán, M. A., Brumback, B., & Robins, J. M. (2001). Marginal structural models to estimate the joint causal effect of nonrandomized treatments. *Journal of the American Statistical Association*, *96*(454), 440-448. https://doi.org/Doi 10.1198/016214501753168154

Hernán, M. A., Cole, S. R., Margolick, J., Cohen, M., & Robins, J. M. (2005). Structural accelerated failure time models for survival analysis in studies with time-varying treatments. *Pharmacoepidemiol Drug Saf*, *14*(7), 477-491. https://doi.org/10.1002/pds.1064

Hernán, M. A., Hsu, J., & Healy, B. (2019). A Second Chance to Get Causal Inference Right: A Classification of Data Science Tasks. *Chance*, *32*(1), 42-49. https://doi.org/10.1080/09332480.2019.1579578

Hernán, M. A., & Robins, J. M. (2016). Using Big Data to Emulate a Target Trial When a Randomized Trial Is Not Available. *Am J Epidemiol*, *183*(8), 758-764. https://doi.org/10.1093/aje/kwv254

Herrero-Zazo, M., Berry, R., Bines, E., Bhattacharya, D., Myint, P. K., & Keevil, V. L. (2021). Anticholinergic burden in older adult inpatients: patterns from admission to discharge and associations with hospital outcomes. *Ther Adv Drug Saf*, *12*, 20420986211012592. https://doi.org/10.1177/20420986211012592

Herrett, E., Gallagher, A. M., Bhaskaran, K., Forbes, H., Mathur, R., van Staa, T., & Smeeth, L. (2015). Data Resource Profile: Clinical Practice Research Datalink (CPRD). *Int J Epidemiol*, *44*(3), 827-836. https://doi.org/10.1093/ije/dyv098

Hersman, S., Cushman, J., Lemelson, N., Wassum, K., Lotfipour, S., & Fanselow, M. S. (2017). Optogenetic excitation of cholinergic inputs to hippocampus primes future contextual fear associations. *Sci Rep*, *7*(1), 2333. https://doi.org/10.1038/s41598-017-02542-1

Higley, M. J., & Picciotto, M. R. (2014). Neuromodulation by acetylcholine: examples from schizophrenia and depression. *Curr Opin Neurobiol*, *29*, 88-95. https://doi.org/10.1016/j.conb.2014.06.004

Hilmer, S. N., Mager, D. E., Simonsick, E. M., Cao, Y., Ling, S. M., Windham, B. G., Harris, T. B., Hanlon, J. T., Rubin, S. M., Shorr, R. I., Bauer, D. C., & Abernethy, D. R. (2007). A drug burden index to define the functional burden of medications in older people. *Arch Intern Med*, *167*(8), 781-787. https://doi.org/10.1001/archinte.167.8.781

Hilmer, S. N., Mager, D. E., Simonsick, E. M., Ling, S. M., Windham, B. G., Harris, T. B., Shorr, R. I., Bauer, D. C., Abernethy, D. R., & Health, A. B. C. S. (2009). Drug burden index score and functional decline in older people. *Am J Med*, *122*(12), 1142-1149 e1141-1142. https://doi.org/10.1016/j.amjmed.2009.02.021

The history of atropine. (1902). *The Lancet*, *159*(4101), 983-984. https://doi.org/10.1016/S0140-6736(01)83573-X

Hock, C., Maddalena, A., Raschig, A., Muller-Spahn, F., Eschweiler, G., Hager, K., Heuser, I., Hampel, H., Muller-Thomsen, T., Oertel, W., Wienrich, M., Signorell, A., Gonzalez-Agosti, C., & Nitsch, R. M. (2003). Treatment with the selective muscarinic m1 agonist talsaclidine decreases cerebrospinal fluid levels of A beta 42 in patients with Alzheimer's disease. *Amyloid*, *10*(1), 1-6. https://doi.org/10.3109/13506120308995249

Hook, A., Randall, J. L., Grubb, C. M., Ellis, N., Wellington, J., Hemmad, A., Zerdelis, A., Winnett, A. R. D., Geers, B. D. W., Sykes, B., Auty, C. N., Vinchenzo, C., Thorburn, C. E., Asogbon, D., Granger, E., Boagey, H., Raphael, J., Patel, K., Bhargava, K., . . . Harrison, J. R. (2022). Anti-cholinergic drug burden in patients with dementia increases after hospital admission: a multicentre cross-sectional study. *BMC Geriatr*, *22*(1), 783. https://doi.org/10.1186/s12877-022-03235-9

Horn, J. P., & Swanson, L. W. (2013). The Autonomic Motor System and the Hypothalamus. In E. R. Kandel, J. H. Schwartz, T. M. Jessel, S. A. Siegelbaum, & A. J. Hudspeth (Eds.), *Principles of Neural Science* (5 ed., pp. 1056-1078). McGraw-Hill.

Hovstadius, B., Petersson, G., Hellström, L., & Ericson, L. (2014). Trends in inappropriate drug therapy prescription in the elderly in Sweden from 2006 to 2013: assessment using national indicators. *Drugs Aging*, *31*(5), 379-386. https://doi.org/10.1007/s40266-014-0165-5

Howe, W. M., Berry, A. S., Francois, J., Gilmour, G., Carp, J. M., Tricklebank, M., Lustig, C., & Sarter, M. (2013). Prefrontal cholinergic mechanisms instigating shifts from monitoring for cues to cue-guided performance: converging electrochemical and fMRI evidence from rats and humans. *J Neurosci*, *33*(20), 8742-8752. https://doi.org/10.1523/JNEUROSCI.5809-12.2013

Hsu, W. H., Huang, S. T., Lu, W. H., Wen, Y. W., Chen, L. K., & Hsiao, F. Y. (2021). Impact of Multiple Prescriptions With Anticholinergic Properties on Adverse Clinical Outcomes in the Elderly: A Longitudinal Cohort Study in Taiwan. *Clin Pharmacol Ther*, *110*(4), 966-974. https://doi.org/10.1002/cpt.2217

Hsu, W. H., Wen, Y. W., Chen, L. K., & Hsiao, F. Y. (2017). Comparative Associations Between Measures of Anti-cholinergic Burden and Adverse Clinical Outcomes. *Ann Fam Med*, *15*(6), 561-569. https://doi.org/10.1370/afm.2131

Hughes, K. M., Lang, J. C. T., Lazare, R., Gordon, D., Stanton, S. L., Malone-Lee, J., & Geraint, M. (1992). Measurement of oxybutynin and its N-desethyl metabolite in plasma , and its application to pharmacokinetic sty dies in young , elderly and frail elderly volunteers. *Xenobiotica*, *22*(7), 859-869. https://doi.org/10.3109/00498259209053145

Hung, A. Y., Haass, C., Nitsch, R. M., Qiu, W. Q., Citron, M., Wurtman, R. J., Growdon, J. H., & Selkoe, D. J. (1993). Activation of protein kinase C inhibits cellular production of the amyloid beta-protein. *Journal of Biological Chemistry*, *268*(31), 22959-22962. https://doi.org/10.1016/s0021-9258(19)49409-x

Imperato, A., Puglisi-Allegra, S., Casolini, P., Zocchi, A., & Angelucci, L. (1989). Stress-induced enhancement of dopamine and acetylcholine release in limbic structures: role of corticosterone. *Eur J Pharmacol*, *165*(2-3), 337-338. https://doi.org/10.1016/0014-2999(89)90735-8

Ing, H. R. (1946). LACHESINE, A NEW MYDRIATIC. *Br J Ophthalmol.*, *30*(4), 247-250.

Inouye, S. K., Westendorp, R. G. J., & Saczynski, J. S. (2014). Delirium in elderly people. *The Lancet*, *383*(9920), 911-922. https://doi.org/10.1016/s0140-6736(13)60688-1

Ioannidis, J. P., Haidich, A. B., Pappa, M., Pantazis, N., Kokori, S. I., Tektonidou, M. G., Contopoulos-Ioannidis, D. G., & Lau, J. (2001). Comparison of evidence of treatment effects in randomized and nonrandomized studies. *JAMA*, *286*(7), 821-830. https://doi.org/10.1001/jama.286.7.821

Ismail, Z., Black, S. E., Camicioli, R., Chertkow, H., Herrmann, N., Laforce, R., Jr., Montero-Odasso, M., Rockwood, K., Rosa-Neto, P., Seitz, D., Sivananthan, S., Smith, E. E., Soucy, J. P., Vedel, I., Gauthier, S., & participants, C. (2020). Recommendations of the 5th Canadian Consensus Conference on the diagnosis and treatment of dementia. *Alzheimers Dement*, *16*(8), 1182-1195. https://doi.org/10.1002/alz.12105

Iwata, N., Kozuka, M., Hara, T., Kanek, T., Tonohiro, T., Sugimoto, M., Niitsu, Y., Kondo, Y., Yamamoto, T., Sakai, J., & Nagano, M. (2000). Activation of cerebral function by CS-932, a functionally selective M1 partial agonist: neurochemical characterization and pharmacological studies. *Jpn J Pharmacol*, *84*(3), 266-280. https://doi.org/10.1254/jjp.84.266

Izquierdo, I., da Cunha, C., Rosat, R., Jerusalinsky, D., Ferreira, M. B., & Medina, J. H. (1992). Neurotransmitter receptors involved in post-training memory processing by the amygdala, medial septum, and hippocampus of the rat. *Behav Neural Biol*, *58*(1), 16-26. https://doi.org/10.1016/0163-1047(92)90847-w

Jackson, T., Pinnock, H., Liew, S. M., Horne, E., Ehrlich, E., Fulton, O., Worth, A., Sheikh, A., & De Simoni, A. (2020). Patient and public involvement in research: from tokenistic box ticking to valued team members. *BMC Med*, *18*(1), 79. https://doi.org/10.1186/s12916-020-01544-7

Jaïdi, Y., Guilloteau, A., Nonnonhou, V., Bertholon, L. A., Badr, S., Morrone, I., Novella, J. L., & Mahmoudi, R. (2019). Threshold for a Reduction in Anticholinergic Burden to Decrease Behavioral and Psychological Symptoms of Dementia. *J Am Med Dir Assoc*, *20*(2), 159-164 e153. https://doi.org/10.1016/j.jamda.2018.10.015

Jaïdi, Y., Nonnonhou, V., Kanagaratnam, L., Bertholon, L. A., Badr, S., Noël, V., Novella, J. L., & Mahmoudi, R. (2018). Reduction of the Anticholinergic Burden Makes It Possible to Decrease Behavioral and Psychological Symptoms of Dementia. *Am J Geriatr Psychiatry*, *26*(3), 280-288. https://doi.org/10.1016/j.jagp.2017.08.005

Jakobsen, S. M., Kersten, H., & Molden, E. (2011). Evaluation of brain anticholinergic activities of urinary spasmolytic drugs using a high-throughput radio receptor bioassay. *J Am Geriatr Soc*, *59*(3), 501-505. https://doi.org/10.1111/j.1532-5415.2010.03307.x

Jamieson, H., Nishtala, P. S., Bergler, H. U., Weaver, S. K., Pickering, J. W., Ailabouni, N. J., Abey-Nesbit, R., Gullery, C., Deely, J., Gee, S. B., Hilmer, S. N., & Mangin, D. (2023). Deprescribing anticholinergic and sedative drugs to reduce polypharmacy in frail older adults living in the community: a randomized controlled trial. *J Gerontol A Biol Sci Med Sci*. https://doi.org/10.1093/gerona/glac249

Jellinger, K. A. (1991). Pathology of Parkinson's disease - Changes other than the nigrostriatal pathway. *Molecular and Chemical Neuropathology*, *14*(3), 153-197. https://doi.org/10.1007/BF03159935

Jenkinson, M., Beckmann, C. F., Behrens, T. E., Woolrich, M. W., & Smith, S. M. (2012). Fsl. *Neuroimage*, *62*(2), 782-790. https://doi.org/10.1016/j.neuroimage.2011.09.015

Jett, D. A. (1998). Central Cholinergic Neurobiology. In *Handbook of Developmental Neurotoxicology* (pp. 257-274). https://doi.org/10.1016/b978-012648860-9.50018-2

Ji, H., Rabbi, M. F., Labis, B., Pavlov, V. A., Tracey, K. J., & Ghia, J. E. (2014). Central cholinergic activation of a vagus nerve-to-spleen circuit alleviates experimental colitis. *Mucosal Immunol*, *7*(2), 335-347. https://doi.org/10.1038/mi.2013.52

Jiang, J., Johnson, J. C. S., Requena-Komuro, M. C., Benhamou, E., Sivasathiaseelan, H., Sheppard, D. L., Volkmer, A., Crutch, S. J., Hardy, C. J. D., & Warren, J. D. (2022). Phonemic restoration in Alzheimer's disease and semantic dementia: a preliminary investigation. *Brain Commun*, *4*(3), fcac118. https://doi.org/10.1093/braincomms/fcac118

Jiang, L., Kundu, S., Lederman, J. D., López-Hernández, G. Y., Ballinger, E. C., Wang, S., Talmage, D. A., & Role, L. W. (2016). Cholinergic Signaling Controls Conditioned Fear Behaviors and Enhances Plasticity of Cortical-Amygdala Circuits. *Neuron*, *90*(5), 1057-1070. https://doi.org/10.1016/j.neuron.2016.04.028

Jiang, S., Li, Y., Zhang, C., Zhao, Y., Bu, G., Xu, H., & Zhang, Y. W. (2014). M1 muscarinic acetylcholine receptor in Alzheimer's disease. *Neurosci Bull*, *30*(2), 295-307. https://doi.org/10.1007/s12264-013-1406-z

Joung, K. I., Kim, S., Cho, Y. H., & Cho, S. I. (2019). Association of Anticholinergic Use with Incidence of Alzheimer's Disease: Population-based Cohort Study. *Sci Rep*, *9*(1), 6802. https://doi.org/10.1038/s41598-019-43066-0

Jun, K., Hwang, S., Ah, Y. M., Suh, Y., & Lee, J. Y. (2019). Development of an Anticholinergic Burden Scale specific for Korean older adults. *Geriatr Gerontol Int*, *19*(7), 628-634. https://doi.org/10.1111/ggi.13680

Kable, A., Fullerton, A., Fraser, S., Palazzi, K., Hullick, C., Oldmeadow, C., Pond, D., Searles, A., Edmunds, K., Attia, J., & On Behalf Of Sms Dementia Study Investigators, O. (2019). Comparison of Potentially Inappropriate Medications for People with Dementia at Admission and Discharge during An Unplanned Admission to Hospital: Results from the SMS Dementia Study. *Healthcare (Basel)*, *7*(1). https://doi.org/10.3390/healthcare7010008

Kachru, N., Carnahan, R. M., Johnson, M. L., & Aparasu, R. R. (2015a). Potentially inappropriate anticholinergic medication use in community-dwelling older adults: a national cross-sectional study. *Drugs Aging*, *32*(5), 379-389. https://doi.org/10.1007/s40266-015-0257-x

Kachru, N., Carnahan, R. M., Johnson, M. L., & Aparasu, R. R. (2015b). Potentially inappropriate anticholinergic medication use in older adults with dementia. *J Am Pharm Assoc (2003)*, *55*(6), 603-612. https://doi.org/10.1331/JAPhA.2015.14288

Kakkar, M., Barmak, A. B., & Arany, S. (2021). Anticholinergic medication and dental caries status in middle-aged xerostomia patients-a retrospective study. *Journal of Dental Sciences*. https://doi.org/10.1016/j.jds.2021.12.014

Kalmbach, A., & Waters, J. (2014). Modulation of high- and low-frequency components of the cortical local field potential via nicotinic and muscarinic acetylcholine receptors in anesthetized mice. *J Neurophysiol*, *111*(2), 258-272. https://doi.org/10.1152/jn.00244.2013

Kamboj, S. K., & Curran, H. V. (2006). Neutral and emotional episodic memory: global impairment after lorazepam or scopolamine. *Psychopharmacology (Berl)*, *188*(4), 482-488. https://doi.org/10.1007/s00213-006-0552-7

Kandel, E. R., & Siegelbaum, S. A. (2013). Signaling at the Nerve-Muscle Synapse: Directly Gated Transmission. In E. R. Kandel, J. H. Schwartz, T. M. Jessel, S. A. Siegelbaum, & A. J. Hudspeth (Eds.), *Principles of Neural Science* (5 ed., pp. 189-209). McGraw-Hill.

Kashyap, M., Belleville, S., Mulsant, B. H., Hilmer, S. N., Paquette, A., Tu le, M., & Tannenbaum, C. (2014). Methodological challenges in determining longitudinal associations between anticholinergic drug use and incident cognitive decline. *J Am Geriatr Soc*, *62*(2), 336-341. https://doi.org/10.1111/jgs.12632

Kaufer, D., Friedman, A., Seidman, S., & Soreq, H. (1998). Acute stress facilitates long-lasting changes in cholinergic gene expression. *Nature*, *393*(6683), 373-377. https://doi.org/10.1038/30741

Kawas, C., Gray, S., Brookmeyer, R., Fozard, J., & Zonderman, A. (2000). Age-specific incidence rates of Alzheimer's disease: the Baltimore Longitudinal Study of Aging. *Neurology*, *54*(11), 2072-2077. https://doi.org/10.1212/wnl.54.11.2072

Kay, C., Harper, D. N., & Hunt, M. (2010). Differential effects of MDMA and scopolamine on working versus reference memory in the radial arm maze task. *Neurobiol Learn Mem*, *93*(2), 151-156. https://doi.org/10.1016/j.nlm.2009.09.005

Kay, G. G., Abou-Donia, M. B., Messer, W. S., Jr., Murphy, D. G., Tsao, J. W., & Ouslander, J. G. (2005). Antimuscarinic drugs for overactive bladder and their potential effects on cognitive function in older patients. *J Am Geriatr Soc*, *53*(12), 2195-2201. https://doi.org/10.1111/j.1532-5415.2005.00537.x

Kempton, M., J., Salvador, Z., Munafò, M., R., Geddes, J. R., Simmons, A., Frangou, S., & Williams, S. S. R. (2011). Structural Neuroimaging Studies in Major Depressive Disorder. Meta analysis and comparison with bipolar disorder. *Archives of General Psychiatry*, *68*(7), 675-690. https://doi.org/10.1001/archgenpsychiatry.2011.60

Kersten, H., Molden, E., Tolo, I. K., Skovlund, E., Engedal, K., & Wyller, T. B. (2013). Cognitive effects of reducing anticholinergic drug burden in a frail elderly population: a randomized controlled trial. *J Gerontol A Biol Sci Med Sci*, *68*(3), 271-278. https://doi.org/10.1093/gerona/gls176

Kersten, H., & Wyller, T. B. (2014). Anticholinergic drug burden in older people's brain - how well is it measured? *Basic Clin Pharmacol Toxicol*, *114*(2), 151-159. https://doi.org/10.1111/bcpt.12140

Ketai, L. H., Komesu, Y. M., Schrader, R. M., Rogers, R. G., Sapien, R. E., Dodd, A. B., & Mayer, A. R. (2021). Mind-body (hypnotherapy) treatment of women with urgency urinary incontinence: changes in brain attentional networks. *Am J Obstet Gynecol*, *224*(5), 498 e491-498 e410. https://doi.org/10.1016/j.ajog.2020.10.041

Keyes, K. M., & Westreich, D. (2019). UK Biobank, big data, and the consequences of non-representativeness. *The Lancet*, *393*(10178). https://doi.org/10.1016/s0140-6736(18)33067-8

Khakpai, F., Nasehi, M., Haeri-Rohani, A., Eidi, A., & Zarrindast, M. R. (2012). Scopolamine induced memory impairment; possible involvement of NMDA receptor mechanisms of dorsal

hippocampus and/or septum. *Behav Brain Res*, *231*(1), 1-10. https://doi.org/10.1016/j.bbr.2012.02.049

Kiesel, E. K., Hopf, Y. M., & Drey, M. (2018). An anticholinergic burden score for German prescribers: score development. *BMC Geriatr*, *18*(1), 239. https://doi.org/10.1186/s12877-018-0929-6

Kilimann, I., Grothe, M., Heinsen, H., Alho, E. J., Grinberg, L., Amaro, E., Jr., Dos Santos, G. A., da Silva, R. E., Mitchell, A. J., Frisoni, G. B., Bokde, A. L., Fellgiebel, A., Filippi, M., Hampel, H., Klöppel, S., & Teipel, S. J. (2014). Subregional basal forebrain atrophy in Alzheimer's disease: a multicenter study. *J Alzheimers Dis*, *40*(3), 687-700. https://doi.org/10.3233/JAD-132345

Kilimann, I., Wucherer, D., Ittermann, T., Volzke, H., Bulow, R., Hoffmann, W., Grabe, H. J., Wittfeld, K., & Teipel, S. J. (2021). Inverse association between the anticholinergic burden and hippocampus volume in a population-based cohort across the entire adult age range. *Geroscience*. https://doi.org/10.1007/s11357-021-00497-w

Kilmer, F. B. (1894). *Belladonna: a study of its history, action and uses in medicine*. Johnson & Johnson.

Kim, J.-H., Jung, A. H., Jeong, D., Choi, I., Kim, K., Shin, S., Kim, S. J., & Lee, S.-H. (2016). Selectivity of Neuromodulatory Projections from the Basal Forebrain and Locus Ceruleus to Primary Sensory Cortices. *The Journal of Neuroscience*, *36*(19), 5314-5327. https://doi.org/10.1523/jneurosci.4333-15.2016

Kinirons, M. T., & O'Mahony, M. S. (2004). Drug metabolism and ageing. *Br J Clin Pharmacol*, *57*(5), 540-544. https://doi.org/10.1111/j.1365-2125.2004.02096.x

Kirkman, M. A., Mahattanakul, W., Gregson, B. A., & Mendelow, A. D. (2009). The accuracy of hospital discharge coding for hemorrhagic stroke. *Acta Neurol Belg*, *109*(2), 114-119. https://www.ncbi.nlm.nih.gov/pubmed/19681442

Kirrane, R. M., Mitropoulou, V., Nunn, M., Silverman, J., & Siever, L. J. (2001). Physostigmine and cognition in schizotypal personality disorder. *Schizophr Res*, *48*(1), 1-5. https://doi.org/10.1016/s0920-9964(00)00059-1

Klamer, T. T., Wauters, M., Azermai, M., Duran, C., Christiaens, T., Elseviers, M., & Vander Stichele, R. (2017). A Novel Scale Linking Potency and Dosage to Estimate Anticholinergic Exposure in Older Adults: the Muscarinic Acetylcholinergic Receptor ANTagonist Exposure Scale. *Basic Clin Pharmacol Toxicol*, *120*(6), 582-590. https://doi.org/10.1111/bcpt.12699

Klapproth, H., Racké, K., & Wessler, I. (1998). Acetylcholine and nicotine stimulate the release of granulocyte-macrophage colony stimulating factor from cultured human bronchial epithelial cells. *Naunyn-Schmiedeberg's Archives of Pharmacology*, *367*(4), 472-475. https://doi.org/10.1007/PL00005195

Knox, C., Law, V., Jewison, T., Liu, P., Ly, S., Frolkis, A., Pon, A., Banco, K., Mak, C., Neveu, V., Djoumbou, Y., Eisner, R., Guo, A. C., & Wishart, D. S. (2011). DrugBank 3.0: a comprehensive resource for 'omics' research on drugs. *Nucleic Acids Res*, *39*(Database issue), D1035-1041. https://doi.org/10.1093/nar/gkq1126

Knox, D. (2016). The role of basal forebrain cholinergic neurons in fear and extinction memory. *Neurobiol Learn Mem*, *133*, 39-52. https://doi.org/10.1016/j.nlm.2016.06.001

Kobayashi, M., Ohno, M., Yamamoto, T., & Watanabe, S. (1995). Concurrent blockade of β-adrenergic and muscarinic receptors disrupts working memory but not reference memory in rats. *Physiology and Behavior*, *58*(2), 307-314. https://doi.org/10.1016/0031-9384(95)00052-K

Kolanowski, A., Fick, D. M., Campbell, J., Litaker, M., & Boustani, M. (2009). A preliminary study of anticholinergic burden and relationship to a quality of life indicator, engagement in activities, in nursing home residents with dementia. *J Am Med Dir Assoc*, *10*(4), 252-257. https://doi.org/10.1016/j.jamda.2008.11.005

Koller, G., Satzger, W., Adam, M., Wagner, M., Kathmann, N., Soyka, M., & Engel, R. (2003). Effects of scopolamine on matching to sample paradigm and related tests in human subjects. *Neuropsychobiology*, *48*(2), 87-94. https://doi.org/10.1159/000072883

Kose, E., Hirai, T., Seki, T., Okudaira, M., & Yasuno, N. (2022). Anticholinergic Load Is Associated with Swallowing Dysfunction in Convalescent Older Patients after a Stroke. *Nutrients*, *14*(10). https://doi.org/10.3390/nu14102121

Kotsias, B. A. (2002). Scopolamine and the murder of King Hamlet. *Arch Otolaryngol Head Neck Surg*, *128*(7), 847-849. https://doi.org/10.1001/archotol.128.7.847

Koyama, A., Steinman, M., Ensrud, K., Hillier, T. A., & Yaffe, K. (2014). Long-term cognitive and functional effects of potentially inappropriate medications in older women. *J Gerontol A Biol Sci Med Sci*, *69*(4), 423-429. https://doi.org/10.1093/gerona/glt192

Koyama, S., Rennard, S. I., & Robbins, R. A. (1992). Acetylcholine stimulates bronchial epithelial cells to release neutrophil and monocyte chemotactic activity. *American Journal of Physiology - Lung Cellular and Molecular Physiology*, *262*(4), 455-471. https://doi.org/10.1152/ajplung.1992.262.4.l466

Kraemer, H. (1894). Belladonna Pharmacognosy. In F. B. Kilmer (Ed.), *Belladonna: a study of its history, action and uses in medicine* (pp. 5-6). Johnson & Johnson.

Kristensson, J. H., Zahirovic, I., Londos, E., & Modig, S. (2021). Medications causing potential cognitive impairment are common in nursing home dementia units – A cross-sectional study. *Exploratory Research in Clinical and Social Pharmacy*, *3*. https://doi.org/10.1016/j.rcsop.2021.100054

Kukolja, J., Thiel, C. M., & Fink, G. R. (2009). Cholinergic stimulation enhances neural activity associated with encoding but reduces neural activity associated with retrieval in humans. *J Neurosci*, *29*(25), 8119-8128. https://doi.org/10.1523/JNEUROSCI.0203-09.2009

Kumpula, E. K., Bell, J. S., Soini, H., & Pitkala, K. H. (2011). Anticholinergic drug use and mortality among residents of long-term care facilities: a prospective cohort study. *J Clin Pharmacol*, *51*(2), 256-263. https://doi.org/10.1177/0091270010368410

Kurzen, H., Berger, H., Jäger, C., Hartschuh, W., Näher, H., Gratchev, A., Goerdt, S., & Deichmann, M. (2004). Phenotypical and molecular profiling of the extraneuronal cholinergic system of the skin. *J Invest Dermatol*, *123*(5), 937-949. https://doi.org/10.1111/j.0022-202X.2004.23425.x

Ladenburg, A. (1881). Die natürlich vorkommenden mydriatisch wirkenden Alkaloïde. *Justus Liebig's Annalen der Chemie*, *206*(3), 274-307. https://doi.org/10.1002/jlac.18812060303

Langley, J. (1878). On the physiology of salivary secretion. Part II. On the mutual antagonism of atropin and pilocarpin, having especial reference to their relations in the sub-maxillary gland of the cat. *Journal of Physiology*, *1*, 339-369.

Langley, J. N. (1905). On the reaction of cells and of nerve-endings to certain poisons, chiefly as regards the reaction of striated muscle to nicotine and to curari. *The Journal of Physiology*, *33*(4-5), 374-413. https://doi.org/10.1113/jphysiol.1905.sp001128

Langmead, C. J., Watson, J., & Reavill, C. (2008). Muscarinic acetylcholine receptors as CNS drug targets. *Pharmacol Ther*, *117*(2), 232-243. https://doi.org/10.1016/j.pharmthera.2007.09.009

Lebois, E. P., Thorn, C., Edgerton, J. R., Popiolek, M., & Xi, S. (2018). Muscarinic receptor subtype distribution in the central nervous system and relevance to aging and Alzheimer's disease. *Neuropharmacology*, *136*(Pt C), 362-373. https://doi.org/10.1016/j.neuropharm.2017.11.018

Lee, M. R. (1999). The snowdrop (Galanthus nivalis): from Odysseus to Alzheimer. *Proc R Coll Physicians Edinb*, *29*(4), 349-352.

Lee, M. R. (2006a). The Solanaceae II: The mandrake (Mandragora officinarum); in league with the Devil. *J R Coll Physicians Edinb*, *36*(3), 278-285. https://www.rcpe.ac.uk/college/journal/solanaceae-ii-mandrake-mandragora-officinarum-league-devil

Lee, M. R. (2006b). Solanaceae III: henbane, hags and Hawley Harvey Crippen. *J R Coll Physicians Edinb*, *36*(4), 366-373. https://www.ncbi.nlm.nih.gov/pubmed/17526134

Lee, M. R. (2006c). The Solanaceae: foods and poisons. *J R Coll Physicians Edinb*, *36*(2), 162-169. https://www.ncbi.nlm.nih.gov/pubmed/17153152

Lee, M. R. (2007). Solanaceae IV: Atropa belladonna, deadly nightshade. *J R Coll Physicians Edinb*, *37*(1), 77-84. https://www.ncbi.nlm.nih.gov/pubmed/17575737

Lee, S. T., Chu, K., Jung, K. H., Kang, K. M., Kim, J. H., Bahn, J. J., Jeon, D., Kim, M., Lee, S. K., & Roh, J. K. (2010). Cholinergic anti-inflammatory pathway in intracerebral hemorrhage. *Brain Res*, *1309*, 164-171. https://doi.org/10.1016/j.brainres.2009.10.076

Leelakanok, N., & D'Cunha, R. R. (2019). Association between polypharmacy and dementia - A systematic review and metaanalysis. *Aging Ment Health*, *23*(8), 932-941. https://doi.org/10.1080/13607863.2018.1468411

Lehner, K. R., Silverman, H. A., Addorisio, M. E., Roy, A., Al-Onaizi, M. A., Levine, Y., Olofsson, P. S., Chavan, S. S., Gros, R., Nathanson, N. M., Al-Abed, Y., Metz, C. N., Prado, V. F., Prado, M. A. M., Tracey, K. J., & Pavlov, V. A. (2019). Forebrain Cholinergic Signaling Regulates Innate Immune Responses and Inflammation. *Front Immunol*, *10*, 585. https://doi.org/10.3389/fimmu.2019.00585

Lertxundi, U., Domingo-Echaburu, S., Hernandez, R., Peral, J., & Medrano, J. (2013). Expert-based drug lists to measure anticholinergic burden: similar names, different results. *Psychogeriatrics*, *13*(1), 17-24. https://doi.org/10.1111/j.1479-8301.2012.00418.x

Levey, A. I., Edmunds, S. M., Heilman, C. J., Desmond, T. J., & Frey, K. A. (1994). Localization of muscarinic m3 receptor protein and M3 receptor binding in rat brain. *Neuroscience*, *63*(1), 207-221. https://doi.org/10.1016/0306-4522(94)90017-5

Levey, A. I., Kitt, C. A., Simonds, W. F., Price, D. L., & Brann, M. R. (1991). Identification and localization of muscarinic acetylcholine receptor proteins in brain with subtype-specific antibodies. *J Neurosci*, *11*(10), 3218-3226. https://www.ncbi.nlm.nih.gov/pubmed/1941081

Liang, C. K., Chou, M. Y., Hsu, Y. H., Wang, Y. C., Liao, M. C., Chen, M. T., Hsiao, P. Y., Chen, L. K., & Lin, Y. T. (2022). The association of potentially inappropriate medications, polypharmacy, and anticholinergic burden with readmission and emergency room revisit after discharge: a hospital-based retrospective cohort study. *Br J Clin Pharmacol*. https://doi.org/10.1111/bcp.15457

Lisibach, A., Benelli, V., Ceppi, M. G., Waldner-Knogler, K., Csajka, C., & Lutters, M. (2021). Quality of anticholinergic burden scales and their impact on clinical outcomes: a systematic review. *Eur J Clin Pharmacol*, *77*(2), 147-162. https://doi.org/10.1007/s00228-020-02994-x

Lisibach, A., Gallucci, G., Beeler, P. E., Csajka, C., & Lutters, M. (2022). High anticholinergic burden at admission associated with in-hospital mortality in older patients: A comparison of 19 different anticholinergic burden scales. *Basic Clin Pharmacol Toxicol*, *130*(2), 288-300. https://doi.org/10.1111/bcpt.13692

Lisibach, A., Gallucci, G., Benelli, V., Kalin, R., Schulthess, S., Beeler, P. E., Csajka, C., & Lutters, M. (2022). Evaluation of the association of anticholinergic burden and delirium in older hospitalised patients - a cohort study comparing 19 anticholinergic burden scales. *Br J Clin Pharmacol*. https://doi.org/10.1111/bcp.15432

Liskowsky, W., & Schliebs, R. (2006). Muscarinic acetylcholine receptor inhibition in transgenic Alzheimer-like Tg2576 mice by scopolamine favours the amyloidogenic route of processing

of amyloid precursor protein. *Int J Dev Neurosci*, *24*(2-3), 149-156. https://doi.org/10.1016/j.ijdevneu.2005.11.010

Liu, A. K., Chang, R. C., Pearce, R. K., & Gentleman, S. M. (2015). Nucleus basalis of Meynert revisited: anatomy, history and differential involvement in Alzheimer's and Parkinson's disease. *Acta Neuropathol*, *129*(4), 527-540. https://doi.org/10.1007/s00401-015-1392-5

Livingston, G., Huntley, J., Sommerlad, A., Ames, D., Ballard, C., Banerjee, S., Brayne, C., Burns, A., Cohen-Mansfield, J., Cooper, C., Costafreda, S. G., Dias, A., Fox, N., Gitlin, L. N., Howard, R., Kales, H. C., Kivimäki, M., Larson, E. B., Ogunniyi, A., . . . Mukadam, N. (2020). Dementia prevention, intervention, and care: 2020 report of the Lancet Commission. *The Lancet*, *396*(10248), 413-446. https://doi.org/10.1016/s0140-6736(20)30367-6

Lockery, J. E., Broder, J. C., Ryan, J., Stewart, A. C., Woods, R. L., Chong, T. T., Cloud, G. C., Murray, A., Rigby, J. D., Shah, R., Storey, E., Ward, S. A., Wolfe, R., Reid, C. M., Collyer, T. A., Ernst, M. E., & Aspree Investigator Group, A. I. G. l. o. w. a. o. (2021). A Cohort Study of Anticholinergic Medication Burden and Incident Dementia and Stroke in Older Adults. *J Gen Intern Med*, *36*(6), 1629-1637. https://doi.org/10.1007/s11606-020-06550-2

Loewi, O. (1921). Über humorale Übertragbarkeit der Herznervenwirkung. *Pflüger's Archiv für die gesamte Physiologie des Menschen und der Tiere*, *189*, 239-242. https://doi.org/10.1007/BF01738910

Loewi, O. (1935). On Problems Connected with the Principle of Humoral Transmission of Nervous Impulses. *Proceedings of the Royal Society of London*, *118*(809), 299-316.

Longo, V. G. (1966). Behavioral and electroencephalographic effects of atropine and related compounds. *Pharmacological Reviews*, *19*(2), 965-996.

López-Muñoz, F., & Alamo, C. (2009). Historical evolution of the neurotransmission concept. *J Neural Transm (Vienna)*, *116*(5), 515-533. https://doi.org/10.1007/s00702-009-0213-1

Low, L. F., Anstey, K. J., & Sachdev, P. (2009). Use of medications with anticholinergic properties and cognitive function in a young-old community sample. *Int J Geriatr Psychiatry*, *24*(6), 578-584. https://doi.org/10.1002/gps.2157

Lowry, E., Woodman, R. J., Soiza, R. L., & Mangoni, A. A. (2011). Associations between the anticholinergic risk scale score and physical function: potential implications for adverse outcomes in older hospitalized patients. *J Am Med Dir Assoc*, *12*(8), 565-572. https://doi.org/10.1016/j.jamda.2011.03.006

Lozano-Ortega, G., Johnston, K. M., Cheung, A., Wagg, A., Campbell, N. L., Dmochowski, R. R., & Ng, D. B. (2020). A review of published anticholinergic scales and measures and their applicability in database analyses. *Arch Gerontol Geriatr*, *87*, 103885. https://doi.org/10.1016/j.archger.2019.05.010

Lund, J. L., Richardson, D. B., & Sturmer, T. (2015). The active comparator, new user study design in pharmacoepidemiology: historical foundations and contemporary application. *Curr Epidemiol Rep*, *2*(4), 221-228. https://doi.org/10.1007/s40471-015-0053-5

Lyall, D. M., Cullen, B., Allerhand, M., Smith, D. J., Mackay, D., Evans, J., Anderson, J., Fawns-Ritchie, C., McIntosh, A. M., Deary, I. J., & Pell, J. P. (2016). Cognitive Test Scores in UK Biobank: Data Reduction in 480,416 Participants and Longitudinal Stability in 20,346 Participants. *PLoS One*, *11*(4), e0154222. https://doi.org/10.1371/journal.pone.0154222

MacDonald, C. (1887). Therapeutics of Nervous System. The Effects of Hyoscine Hydrobromate and of Hyoscyamine in Insanity. *The Journal of Nervous and Mental Disease*, *14*(2), 117-118. https://doi.org/10.1097/00005053-188702000-00008

Machado-Alba, J. E., Castro-Rodriguez, A., Alzate-Piedrahita, J. A., Hoyos-Pulgarin, J. A., & Medina-Morales, D. A. (2016). Anticholinergic Risk and Frequency of Anticholinergic Drug

Prescriptions in a Population Older Than 65. *J Am Med Dir Assoc*, *17*(3), 275 e271-274. https://doi.org/10.1016/j.jamda.2015.12.003
Maehle, A.-H., Prüll, C.-R., & Halliwell, R. F. (2002). The emergence of the drug receptor theory. *Nature Reviews Drug Discovery*, *1*(August), 637-641. https://doi.org/10.1038/nrd875
Magoun, C. (1839). New application of belladonna. *The Boston Medical and Surgical Journal (1828-1851)*, *21*(10), 160-161.
Mahaffey, S., & Garcia-Rill, E. (2015). Wiring Diagram of the RAS. In *Waking and the Reticular Activating System in Health and Disease* (pp. 49-80). https://doi.org/10.1016/b978-0-12-801385-4.00004-5
Malagaris, I., Mehta, H. B., Li, S., & Goodwin, J. S. (2020). Decrease of Anticholinergic Drug Use in Nursing Home Residents in the United States, 2009 to 2017. *J Am Geriatr Soc*, *68*(12), 2797-2804. https://doi.org/10.1111/jgs.16776
Malcher, M. F., Droupy, S., Berr, C., Ziad, A., Huguet, H., Faillie, J. L., Serrand, C., & Mura, T. (2022). Dementia Associated with Anticholinergic Drugs Used for Overactive Bladder: A Nested-Case Control Study Using the French National Medical-Administrative Database. *J Urol*, 101097JU0000000000002804. https://doi.org/10.1097/JU.0000000000002804
Mangoni, A. A., van Munster, B. C., Woodman, R. J., & de Rooij, S. E. (2013). Measures of anticholinergic drug exposure, serum anticholinergic activity, and all-cause postdischarge mortality in older hospitalized patients with hip fractures. *Am J Geriatr Psychiatry*, *21*(8), 785-793. https://doi.org/10.1016/j.jagp.2013.01.012
Marek, S., Tervo-Clemmens, B., Calabro, F. J., Montez, D. F., Kay, B. P., Hatoum, A. S., Donohue, M. R., Foran, W., Miller, R. L., Hendrickson, T. J., Malone, S. M., Kandala, S., Feczko, E., Miranda-Dominguez, O., Graham, A. M., Earl, E. A., Perrone, A. J., Cordova, M., Doyle, O., . . . Dosenbach, N. U. F. (2022). Reproducible brain-wide association studies require thousands of individuals. *Nature*, *603*(7902), 654-660. https://doi.org/10.1038/s41586-022-04492-9
Mark, G. P., Rada, P. V., & Shors, T. J. (1996). Inescapable stress enhances extracellular acetylcholine in the rat hippocampus and prefrontal cortex but not the nucleus accumbens or amygdala. *Neuroscience*, *74*(3), 767-774. https://doi.org/10.1016/0306-4522(96)00211-4
Martinello, K., Huang, Z., Lujan, R., Tran, B., Watanabe, M., Cooper, E. C., Brown, D. A., & Shah, M. M. (2015). Cholinergic afferent stimulation induces axonal function plasticity in adult hippocampal granule cells. *Neuron*, *85*(2), 346-363. https://doi.org/10.1016/j.neuron.2014.12.030
Marucci, G., Buccioni, M., Ben, D. D., Lambertucci, C., Volpini, R., & Amenta, F. (2021). Efficacy of acetylcholinesterase inhibitors in Alzheimer's disease. *Neuropharmacology*, *190*, 108352. https://doi.org/10.1016/j.neuropharm.2020.108352
Maruyama, S., Tsukada, H., Nishiyama, S., Kakiuchi, T., Fukumoto, D., Oku, N., & Yamada, S. (2008). In vivo quantitative autoradiographic analysis of brain muscarinic receptor occupancy by antimuscarinic agents for overactive bladder treatment. *J Pharmacol Exp Ther*, *325*(3), 774-781. https://doi.org/10.1124/jpet.108.136390
Mash, D. C., Flynn, D. D., & Potter, L. T. (1985). Loss of M2 Muscarine Receptors in the Cerebral Cortex in Alzheimer's Disease and Experimental Cholinergic Denervation. *Science*, *228*(4703), 1115-1118. https://doi.org/10.1126/science.3992249
Massoud, F., & Gauthier, S. (2010). Update on the Pharmacological Treatment of Alzheimers Disease. *Current Neuropharmacology*, *8*(1), 69-80. https://doi.org/10.2174/157015910790909520
Masters, C. L., Bateman, R., Blennow, K., Rowe, C. C., Sperling, R. A., & Cummings, J. L. (2015). Alzheimer's disease. *Nat Rev Dis Primers*, *1*, 15056. https://doi.org/10.1038/nrdp.2015.56

Mayer, T., Haefeli, W. E., & Seidling, H. M. (2015). Different methods, different results--how do available methods link a patient's anticholinergic load with adverse outcomes? *Eur J Clin Pharmacol*, *71*(11), 1299-1314. https://doi.org/10.1007/s00228-015-1932-x

McCullough, J. (1901). Belladonna in obstruction of the bowels. *Medical Record (1866-1922)*, *60*(12), 475.

McLachlan, A. J., Hilmer, S. N., & Le Couteur, D. G. (2009). Variability in response to medicines in older people: phenotypic and genotypic factors. *Clin Pharmacol Ther*, *85*(4), 431-433. https://doi.org/10.1038/clpt.2009.1

McLaughlin, I., Dani, J. A., & De Biasi, M. (2017). The medial habenula and interpeduncular nucleus circuitry is critical in addiction, anxiety, and mood regulation. *J Neurochem*, *142 Suppl 2*, 130-143. https://doi.org/10.1111/jnc.14008

McLean, A. J., & Le Couteur, D. G. (2004). Aging biology and geriatric clinical pharmacology. *Pharmacol Rev*, *56*(2), 163-184. https://doi.org/10.1124/pr.56.2.4

McMichael, A. J., Zafeiridi, E., Ryan, M., Cunningham, E. L., Passmore, A. P., & McGuinness, B. (2021). Anticholinergic drug use and risk of mortality for people with dementia in Northern Ireland. *Aging Ment Health*, *25*(8), 1475-1482. https://doi.org/10.1080/13607863.2020.1830028

Mehdizadeh, D., Hale, M., Todd, O., Zaman, H., Marques, I., Petty, D., Alldred, D. P., Johnson, O., Faisal, M., Gardner, P., & Clegg, A. (2021). Associations Between Anticholinergic Medication Exposure and Adverse Health Outcomes in Older People with Frailty: A Systematic Review and Meta-analysis. *Drugs Real World Outcomes*, *8*(4), 431-458. https://doi.org/10.1007/s40801-021-00256-5

Mein, F. G. (1833). Darstellung des Atropins in weissen Krystallen. *Annalen der Pharmacie*, *6*(1), 67-72. https://doi.org/10.1002/jlac.18330060109

Meng, D., Mohammadi-Nejad, A. R., Sotiropoulos, S. N., Auer, D. P., & Alzheimer's Disease Neuroimaging, I. (2022). Anticholinergic drugs and forebrain magnetic resonance imaging changes in cognitively normal people and those with mild cognitive impairment. *Eur J Neurol*. https://doi.org/10.1111/ene.15251

Menhaji, K., Cardenas-Trowers, O. O., Chang, O. H., Hall, E. F., Ringel, N. E., & Falk, K. N. (2021). Anticholinergic prescribing pattern changes of urogynecology providers in response to evidence of potential dementia risk. *Int Urogynecol J*, *32*(10), 2819-2826. https://doi.org/10.1007/s00192-021-04736-8

Mesulam, M. (2004). The cholinergic lesion of Alzheimer's disease: pivotal factor or side show? *Learn Mem*, *11*(1), 43-49. https://doi.org/10.1101/lm.69204

Mesulam, M. M. (2013). Cholinergic circuitry of the human nucleus basalis and its fate in Alzheimer's disease. *J Comp Neurol*, *521*(18), 4124-4144. https://doi.org/10.1002/cne.23415

Mesulam, M. M., Mufson, E. J., Levey, A. I., & Wainer, B. H. (1983). Cholinergic innervation of cortex by the basal forebrain: cytochemistry and cortical connections of the septal area, diagonal band nuclei, nucleus basalis (substantia innominata), and hypothalamus in the rhesus monkey. *J Comp Neurol*, *214*(2), 170-197. https://doi.org/10.1002/cne.902140206

Miller, E. K., Lundqvist, M., & Bastos, A. M. (2018). Working Memory 2.0. *Neuron*, *100*(2), 463-475. https://doi.org/10.1016/j.neuron.2018.09.023

Miller, K. L., Alfaro-Almagro, F., Bangerter, N. K., Thomas, D. L., Yacoub, E., Xu, J., Bartsch, A. J., Jbabdi, S., Sotiropoulos, S. N., Andersson, J. L., Griffanti, L., Douaud, G., Okell, T. W., Weale, P., Dragonu, I., Garratt, S., Hudson, S., Collins, R., Jenkinson, M., . . . Smith, S. M. (2016). Multimodal population brain imaging in the UK Biobank prospective epidemiological study. *Nat Neurosci*, *19*(11), 1523-1536. https://doi.org/10.1038/nn.4393

Miller, P. S., Richardson, J. S., Jyu, C. A., Lemay, J. S., Hiscock, M., & Keegan, D. L. (1988). Association of low serum anticholinergic levels and cognitive impairment in elderly presurgical patients. *Am J Psychiatry*, *145*(3), 342-345. https://doi.org/10.1176/ajp.145.3.342

Milner, B., Squire, L. R., & Kandel, E. R. (1998). Cognitive neuroscience and the study of memory. *Neuron*, *20*(3), 445-468. https://doi.org/10.1016/S0896-6273(00)80987-3

Minzenberg, M. J., Poole, J. H., Benton, C., & Vinogradov, S. (2004). Association of anticholinergic load with impairment of complex attention and memory in schizophrenia. *Am J Psychiatry*, *161*(1), 116-124. https://doi.org/10.1176/appi.ajp.161.1.116

Mitsushima, D., Sano, A., & Takahashi, T. (2013). A cholinergic trigger drives learning-induced plasticity at hippocampal synapses. *Nat Commun*, *4*, 2760. https://doi.org/10.1038/ncomms3760

Morán, M. A., Mufson, E. J., & Gómez-Ramos, P. (1993). Colocalization of cholinesterases with beta amyloid protein in aged and Alzheimer's brains. *Acta Neuropathol*, *85*(4), 362-369. https://doi.org/10.1007/BF00334445

Mori, F., Lai, C. C., Fusi, F., & Giacobini, E. (1995). Cholinesterase inhibitors increase secretion of APPs in rat brain cortex. *Neuroreport*, *6*(4), 633-636. https://doi.org/10.1097/00001756-199503000-00012

Moriarty, F., Savva, G. M., Grossi, C. M., Bennett, K., Fox, C., Maidment, I., Loke, Y. K., Steel, N., Kenny, R. A., & Richardson, K. (2021). Cognitive decline associated with anticholinergics, benzodiazepines and Z-drugs: Findings from The Irish Longitudinal Study on Ageing (TILDA). *Br J Clin Pharmacol*, *87*(7), 2818-2829. https://doi.org/10.1111/bcp.14687

Morris, R. G., Garrud, P., Rawlins, J. N., & O'Keefe, J. (1982). Place navigation impaired in rats with hippocampal lesions. *Nature*, *297*(5868), 681-683. https://doi.org/10.1038/297681a0

Mufson, E. J., Martin, T. L., Mash, D. C., Wainer, B. H., & Mesulam, M. M. (1986). Cholinergic projections from the parabigeminal nucleus (Ch8) to the superior colliculus in the mouse: a combined analysis of horseradish peroxidase transport and choline acetyltransferase immunohistochemistry. *Brain Res*, *370*(1), 144-148. https://doi.org/10.1016/0006-8993(86)91114-5

Müller, D. M., Mendla, K., Farber, S. A., & Nitsch, R. M. (1997). Muscarinic M1 receptor agonists increase the secretion of the amyloid precursor protein ectodomain. *Life Sci*, *60*(13-14), 985-991. https://doi.org/10.1016/s0024-3205(97)00038-6

Muñoz, W., & Rudy, B. (2014). Spatiotemporal specificity in cholinergic control of neocortical function. *Curr Opin Neurobiol*, *26*, 149-160. https://doi.org/10.1016/j.conb.2014.02.015

Munyaka, P., Rabbi, M. F., Pavlov, V. A., Tracey, K. J., Khafipour, E., & Ghia, J. E. (2014). Central muscarinic cholinergic activation alters interaction between splenic dendritic cell and CD4+CD25- T cells in experimental colitis. *PLoS One*, *9*(10), e109272. https://doi.org/10.1371/journal.pone.0109272

Mur, J., Cox, S. R., Marioni, R. E., Muniz-Terrera, G., & Russ, T. C. (2021). Increase in anticholinergic burden from 1990 to 2015: Age-period-cohort analysis in UK biobank. *Br J Clin Pharmacol*. https://doi.org/10.1111/bcp.15045

Mur, J., Russ, T. C., Cox, S. R., Marioni, R. E., & Muniz-Terrera, G. (2022). Association between anticholinergic burden and dementia in UK Biobank. *Alzheimers Dement (N Y)*, *8*(1), e12290. https://doi.org/10.1002/trc2.12290

Myint, P. K., Fox, C., Kwok, C. S., Luben, R. N., Wareham, N. J., & Khaw, K. T. (2015). Total anticholinergic burden and risk of mortality and cardiovascular disease over 10 years in 21,636 middle-aged and older men and women of EPIC-Norfolk prospective population study. *Age Ageing*, *44*(2), 219-225. https://doi.org/10.1093/ageing/afu185

Naharci, M. I., Katipoglu, B., & Tasci, I. (2022). Association of anticholinergic burden with undernutrition in older adults: A cross-sectional study. *Nutr Clin Pract*. https://doi.org/10.1002/ncp.10821

Naharci, M. I., & Tasci, I. (2020). Frailty status and increased risk for falls: The role of anticholinergic burden. *Arch Gerontol Geriatr*, *90*, 104136. https://doi.org/10.1016/j.archger.2020.104136

Nail-Boucherie, K., Dourmap, N., Jaffard, R., & Costentin, J. (1998). The specific dopamine uptake inhibitor GBR 12783 improves learning of inhibitory avoidance and increases hippocampal acetylcholine release. *Brain Res Cogn Brain Res*, *7*(2), 203-205. https://doi.org/10.1016/s0926-6410(98)00023-8

Nakahara, N., Iga, Y., Saito, Y., Mizobe, F., & Kawanishi, G. (1989). Beneficial effects of FKS-508 (AF102B), a selective M1 agonist, on the impaired working memory in AF64A-treated rats. *Jpn J Pharmacol*, *51*(4), 539-547. https://doi.org/10.1254/jjp.51.539

Nakham, A., Myint, P. K., Bond, C. M., Newlands, R., Loke, Y. K., & Cruickshank, M. (2020). Interventions to Reduce Anticholinergic Burden in Adults Aged 65 and Older: A Systematic Review. *J Am Med Dir Assoc*, *21*(2), 172-180 e175. https://doi.org/10.1016/j.jamda.2019.06.001

Naples, J. G., Marcum, Z. A., Perera, S., Gray, S. L., Newman, A. B., Simonsick, E. M., Yaffe, K., Shorr, R. I., Hanlon, J. T., Health, A., & Body Composition, S. (2015). Concordance Between Anticholinergic Burden Scales. *J Am Geriatr Soc*, *63*(10), 2120-2124. https://doi.org/10.1111/jgs.13647

Nathanson, N. M. (2008). Synthesis, trafficking, and localization of muscarinic acetylcholine receptors. *Pharmacol Ther*, *119*(1), 33-43. https://doi.org/10.1016/j.pharmthera.2008.04.006

Navickas, R., Petric, V. K., Feigl, A. B., & Seychell, M. (2016). Multimorbidity: What do we know? What should we do? *J Comorb*, *6*(1), 4-11. https://doi.org/10.15256/joc.2016.6.72

Neal, S. R., Wood, A. D., Ablett, A. D., Gregory, J. S., Guillot, J., Macdonald, H. M., Reid, D. M., & Myint, P. K. (2020). Anticholinergic burden in middle-aged women and recurrent falls in later life: findings from the Aberdeen prospective osteoporosis screening study (APOSS). *Ther Adv Drug Saf*, *11*, 2042098620929852. https://doi.org/10.1177/2042098620929852

Neelamegam, M., Zgibor, J., Chen, H., O'Rourke, K., Bakour, C., Rajaram, L., & Anstey, K. J. (2020). The Effect of Cumulative Anticholinergic Use on the Cognitive Function of Older Adults: Results from the Personality and Total Health (PATH) Through Life Study. *J Gerontol A Biol Sci Med Sci*, *75*(9), 1706-1714. https://doi.org/10.1093/gerona/glaa145

Nery, R. T., & Reis, A. M. M. (2019). Development of a Brazilian anticholinergic activity drug scale. *Einstein (Sao Paulo)*, *17*(2), eAO4435. https://doi.org/10.31744/einstein_journal/2019AO4435

Newman, E. L., Climer, J. R., & Hasselmo, M. E. (2014). Grid cell spatial tuning reduced following systemic muscarinic receptor blockade. *Hippocampus*, *24*(6), 643-655. https://doi.org/10.1002/hipo.22253

Nguyen, P. V., Pelletier, L., Payot, I., & Latour, J. (2018). The Delirium Drug Scale is associated to delirium incidence in the emergency department. *Int Psychogeriatr*, *30*(4), 503-510. https://doi.org/10.1017/S1041610217002538

NICE. (2018). *Dementia: assessment, management and support for people living with dementia and their carers*.

Nilsson, L., Nordberg, A., Hardy, J., Wester, P., & Winblad, B. (1986). Physostigmine restores 3H-acetylcholine efflux from Alzheimer brain slices to normal level. *J Neural Transm*, *67*(3-4), 275-285. https://doi.org/10.1007/BF01243353

Nishtala, P. S., & Chyou, T. Y. (2022). Risk of delirium associated with antimuscarinics in older adults: A case-time-control study. *Pharmacoepidemiol Drug Saf*. https://doi.org/10.1002/pds.5480

Nishtala, P. S., Narayan, S. W., Wang, T., & Hilmer, S. N. (2014). Associations of drug burden index with falls, general practitioner visits, and mortality in older people. *Pharmacoepidemiol Drug Saf*, *23*(7), 753-758. https://doi.org/10.1002/pds.3624

Nitsch, R. M., Deng, M., Tennis, M., Schoenfeld, D., & Growdon, J. H. (2000). The selective muscarinic M1 agonist AF102B decreases levels of total Abeta in cerebrospinal fluid of patients with Alzheimer's disease. *Ann Neurol*, *48*(6), 913-918. https://www.ncbi.nlm.nih.gov/pubmed/11117548

Niznik, J., Zhao, X., Jiang, T., Hanlon, J. T., Aspinall, S. L., Thorpe, J., & Thorpe, C. (2017). Anticholinergic Prescribing in Medicare Part D Beneficiaries Residing in Nursing Homes: Results from a Retrospective Cross-Sectional Analysis of Medicare Data. *Drugs Aging*, *34*(12), 925-939. https://doi.org/10.1007/s40266-017-0502-6

O'Mahony, D., O'Sullivan, D., Byrne, S., O'Connor, M. N., Ryan, C., & Gallagher, P. (2015). STOPP/START criteria for potentially inappropriate prescribing in older people: version 2. *Age Ageing*, *44*(2), 213-218. https://doi.org/10.1093/ageing/afu145

Oda, Y., & Nakanishi, I. (2000). The distribution of cholinergic neurons in the human central nervous system. *Histol Histopathol*, *15*(3), 825-834. https://doi.org/10.14670/HH-15.825

Ogawa, Y., Hirai, T., & Mihara, K. (2021). A meta-analysis of observational studies on anticholinergic burden and fracture risk: evaluation of conventional burden scales. *J Pharm Health Care Sci*, *7*(1), 30. https://doi.org/10.1186/s40780-021-00213-y

Ohno, M., Yamamoto, T., & Watanabe, S. (1994). Blockade of hippocampal M1 muscarinic receptors impairs working memory performance of rats. *Brain Research*, *650*(2), 260-266. https://doi.org/10.1016/0006-8993(94)91790-6

Ollier, W., Sprosen, T., & Peakman, T. (2005). UK Biobank: from concept to reality. *Pharmacogenomics*, *6*(6), 639-646. https://doi.org/10.2217/14622416.6.6.639

Oorschot, D. E. (2013). The percentage of interneurons in the dorsal striatum of the rat, cat, monkey and human: A critique of the evidence. *Basal Ganglia*, *3*(1), 19-24. https://doi.org/10.1016/j.baga.2012.11.001

Pagoria, D., O'Connor, R. C., & Guralnick, M. L. (2011). Antimuscarinic drugs: review of the cognitive impact when used to treat overactive bladder in elderly patients. *Curr Urol Rep*, *12*(5), 351-357. https://doi.org/10.1007/s11934-011-0198-9

Pahlman, I., d'Argy, R., & Nilvebrant, L. (2001). Tissue distribution of tolterodine, a muscarinic receptor antagonist, and transfer into fetus and milk in mice. *Arzneimittelforschung*, *51*(2), 125-133. https://doi.org/10.1055/s-0031-1300014

Pak, R. W., Petrou, S. P., & Staskin, D. R. (2003). Trospium chloride: a quaternary amine with unique pharmacologic properties. *Curr Urol Rep*, *4*(6), 436-440. https://doi.org/10.1007/s11934-003-0023-1

Pakulski, C., Drobnik, L., & Millo, B. (2000). Age and sex as factors modifying the function of the blood-cerebrospinal fluid barrier. *Med Sci Monit*, *6*(2), 314-318.

Palmer, J. B., Albrecht, J. S., Park, Y., Dutcher, S., Rattinger, G. B., Simoni-Wastila, L., Walker, L. D., & Zuckerman, I. H. (2015). Use of drugs with anticholinergic properties among nursing home residents with dementia: a national analysis of Medicare beneficiaries from 2007 to 2008. *Drugs Aging*, *32*(1), 79-86. https://doi.org/10.1007/s40266-014-0227-8

Papenberg, G., Bäckman, L., Fratiglioni, L., Laukka, E. J., Fastbom, J., & Johnell, K. (2017). Anticholinergic drug use is associated with episodic memory decline in older adults without dementia. *Neurobiol Aging*, *55*, 27-32. https://doi.org/10.1016/j.neurobiolaging.2017.03.009

Parent, M. B., & Baxter, M. G. (2004). Septohippocampal Acetylcholine: Involved in but not Necessary for Learning and Memory? *Learn Mem*, *11*(1), 9-20. https://doi.org/10.1101/lm.69104

Parfitt, G. M., Campos, R. C., Barbosa, A. K., Koth, A. P., & Barros, D. M. (2012). Participation of hippocampal cholinergic system in memory persistence for inhibitory avoidance in rats. *Neurobiol Learn Mem*, *97*(2), 183-188. https://doi.org/10.1016/j.nlm.2011.12.001

Parikh, V., Kozak, R., Martinez, V., & Sarter, M. (2007). Prefrontal acetylcholine release controls cue detection on multiple timescales. *Neuron*, *56*(1), 141-154. https://doi.org/10.1016/j.neuron.2007.08.025

Paul, S., Jeon, W. K., Bizon, J. L., & Han, J. S. (2015). Interaction of basal forebrain cholinergic neurons with the glucocorticoid system in stress regulation and cognitive impairment. *Front Aging Neurosci*, *7*, 43. https://doi.org/10.3389/fnagi.2015.00043

Pavlov, V. A., Ochani, M., Gallowitsch-Puerta, M., Ochani, K., Huston, J. M., Czura, C. J., Al-Abed, Y., & Tracey, K. J. (2006). Central muscarinic cholinergic regulation of the systemic inflammatory response during endotoxemia. *Proc Natl Acad Sci U S A*, *103*(13), 5219-5223. https://doi.org/10.1073/pnas.0600506103

Pavlov, V. A., Parrish, W. R., Rosas-Ballina, M., Ochani, M., Puerta, M., Ochani, K., Chavan, S., Al-Abed, Y., & Tracey, K. J. (2009). Brain acetylcholinesterase activity controls systemic cytokine levels through the cholinergic anti-inflammatory pathway. *Brain Behav Immun*, *23*(1), 41-45. https://doi.org/10.1016/j.bbi.2008.06.011

Pavlov, V. A., Wang, H., Czura, C. J., Friedman, S. G., & Tracey, K. J. (2003). The cholinergic anti-inflammatory pathway: a missing link in neuroimmunomodulation. *Mol Med*, *9*(5-8), 125-134. https://www.ncbi.nlm.nih.gov/pubmed/14571320

Pennanen, C., Kivipelto, M., Tuomainen, S., Hartikainen, P., Hänninen, T., Laakso, M. P., Hallikainen, M., Vanhanen, M., Nissinen, A., Helkala, E.-L., Vainio, P., Vanninen, R., Partanen, K., & Soininen, H. (2004). Hippocampus and entorhinal cortex in mild cognitive impairment and early AD. *Neurobiology of Aging*, *25*(3), 303-310. https://doi.org/10.1016/s0197-4580(03)00084-8

Perry, E. K., Kilford, L., Lees, A. J., Burn, D. J., & Perry, R. H. (2003). Increased Alzheimer pathology in Parkinson's disease related to antimuscarinic drugs. *Ann Neurol*, *54*(2), 235-238. https://doi.org/10.1002/ana.10639

Perry, R. H., Bergman, K., Tomlinson, B. E., Blessed, G., & Gibson, P. H. (1978). Correlation of cholinergic abnormalities with senile plaques and mental test scores in senile dementia. *British Medical Journal*, *2*(6150), 1457-1457. https://doi.org/10.1136/bmj.2.6150.1457

Peters, N. L. (1989). Snipping the thread of life. Antimuscarinic side effects of medications in the elderly. *Archives of Internal Medicine*, *149*(11), 2414-2420. https://doi.org/10.1001/archinte.149.11.2414

Phillips, A., Heier, M., Strobl, R., Linkohr, B., Holle, R., Peters, A., & Grill, E. (2019). Exposure to anticholinergic and sedative medications using the Drug Burden Index and its association with vertigo, dizziness and balance problems in older people - Results from the KORA-FF4 Study. *Exp Gerontol*, *124*, 110644. https://doi.org/10.1016/j.exger.2019.110644

Phillips, R. G., & LeDoux, J. E. (1992). Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. *Behav Neurosci*, *106*(2), 274-285. https://doi.org/10.1037//0735-7044.106.2.274

Pieper, N. T., Grossi, C. M., Chan, W. Y., Loke, Y. K., Savva, G. M., Haroulis, C., Steel, N., Fox, C., Maidment, I. D., Arthur, A. J., Myint, P. K., Smith, T. O., Robinson, L., Matthews, F. E., Brayne, C., & Richardson, K. (2020). Anticholinergic drugs and incident dementia, mild cognitive

impairment and cognitive decline: a meta-analysis. *Age Ageing*, *49*(6), 939-947. https://doi.org/10.1093/ageing/afaa090

Pinto, L., Goard, M. J., Estandian, D., Xu, M., Kwan, A. C., Lee, S. H., Harrison, T. C., Feng, G., & Dan, Y. (2013). Fast modulation of visual perception by basal forebrain cholinergic neurons. *Nat Neurosci*, *16*(12), 1857-1863. https://doi.org/10.1038/nn.3552

Plaschke, K., Thomas, C., Engelhardt, R., Teschendorf, P., Hestermann, U., Weigand, M. A., Martin, E., & Kopitz, J. (2007). Significant correlation between plasma and CSF anticholinergic activity in presurgical patients. *Neurosci Lett*, *417*(1), 16-20. https://doi.org/10.1016/j.neulet.2007.02.015

Plutarch, & Pelling, C. B. R. (1988). *Plutarch : life of Antony*. Cambridge University Press.

Pomara, N., Willoughby, L. M., Wesnes, K., & Sidtis, J. J. (2003). Increased Anticholinergic Challenge-Induced Memory Impairment Associated with the APOE-ε4 Allele in the Elderly: A Controlled Pilot Study. *Neuropsychopharmacology*, *29*(2), 403-409. https://doi.org/10.1038/sj.npp.1300305

Popelikova, A., Bahnik, S., Lobellova, V., Svoboda, J., & Stuchlik, A. (2018). Mnemonic and behavioral effects of biperiden, an M1-selective antagonist, in the rat. *Psychopharmacology (Berl)*, *235*(7), 2013-2025. https://doi.org/10.1007/s00213-018-4899-3

Poppi, L. A., Ho-Nguyen, K. T., Shi, A., Daut, C. T., & Tischfield, M. A. (2021). Recurrent Implication of Striatal Cholinergic Interneurons in a Range of Neurodevelopmental, Neurodegenerative, and Neuropsychiatric Disorders. *Cells*, *10*(4). https://doi.org/10.3390/cells10040907

Posis, A. I. B., Tarraf, W., Gonzalez, K. A., Soria-Lopez, J. A., Léger, G. C., Stickel, A. M., Daviglus, M. L., Lamar, M., Zeng, D., & González, H. M. (2022). Anticholinergic Drug Burden and Neurocognitive Performance in the Study of Latinos-Investigation of Neurocognitive Aging. *J Alzheimers Dis*. https://doi.org/10.3233/JAD-215247

Potter, P. E., Rauschkolb, P. K., Pandya, Y., Sue, L. I., Sabbagh, M. N., Walker, D. G., & Beach, T. G. (2011). Pre- and post-synaptic cortical cholinergic deficits are proportional to amyloid plaque presence and density at preclinical stages of Alzheimer's disease. *Acta Neuropathol*, *122*(1), 49-60. https://doi.org/10.1007/s00401-011-0831-1

Power, A. E., & McGaugh, J. L. (2002a). Cholinergic activation of the basolateral amygdala regulates unlearned freezing behavior in rats. *Behav Brain Res*, *134*(1-2), 307-315. https://doi.org/10.1016/s0166-4328(02)00046-3

Power, A. E., & McGaugh, J. L. (2002b). Phthalic acid amygdalopetal lesion of the nucleus basalis magnocellularis induces reversible memory deficits in rats. *Neurobiol Learn Mem*, *77*(3), 372-388. https://doi.org/10.1006/nlme.2001.4030

Price, D. L., Whitehouse, P. J., & Struble, R. G. (1985). Alzheimer's disease. *Annual Review of Medicine*, *36*, 349-356. https://doi.org/10.1146/annurev.me.36.020185.002025

Racchi, M., Sironi, M., Caprera, A., König, G., & Govoni, S. (2001). Short- and long-term effect of acetylcholinesterase inhibition on the expression and metabolism of the amyloid precursor protein. *Mol Psychiatry*, *6*(5), 520-528. https://doi.org/10.1038/sj.mp.4000878

Ramos, H., Moreno, L., Pérez-Tur, J., Cháfer-Pericás, C., García-Lluch, G., & Pardo, J. (2022). CRIDECO Anticholinergic Load Scale: An Updated Anticholinergic Burden Scale. Comparison with the ACB Scale in Spanish Individuals with Subjective Memory Complaints. *J Pers Med*, *12*(2). https://doi.org/10.3390/jpm12020207

Ramskill, J. (1863). The treatment of epilepsy by belladonna. *Chicago Medical Examiner (1860-1871)*, *4*(8), 405-409.

Rasch, B., Gais, S., & Born, J. (2009). Impaired off-line consolidation of motor memories after combined blockade of cholinergic receptors during REM sleep-rich sleep. *Neuropsychopharmacology*, *34*(7), 1843-1853. https://doi.org/10.1038/npp.2009.6

Rees, T., Hammond, P. I., Soreq, H., Younkin, S., & Brimijoin, S. (2003). Acetylcholinesterase promotes beta-amyloid plaques in cerebral cortex. *Neurobiology of Aging*, *24*(6), 777-787. https://doi.org/10.1016/s0197-4580(02)00230-0

Reidenberg, M. M. (1982). Drugs in the elderly. *Medical Clinics of North America*, *66*(5), 1073-1078. https://doi.org/10.1016/S0025-7125(16)31382-7

Reinold, J., Braitmaier, M., Riedel, O., & Haug, U. (2021). Anticholinergic burden: First comprehensive analysis using claims data shows large variation by age and sex. *PLoS One*, *16*(6), e0253336. https://doi.org/10.1371/journal.pone.0253336

Reinold, J., Schafer, W., Christianson, L., Barone-Adesi, F., Riedel, O., & Pisa, F. E. (2020). Anticholinergic Burden and Fractures: A Systematic Review with Methodological Appraisal. *Drugs Aging*, *37*(12), 885-897. https://doi.org/10.1007/s40266-020-00806-6

Reisinger, M., Reininghaus, E. Z., De Biasi, J., Fellendorf, F. T., & Schoberer, D. (2022). Delirium-associated medication in people at risk. A systematic update review, meta-analyses and GRADE-profiles. *Acta Psychiatr Scand*. https://doi.org/10.1111/acps.13505

Rémillard, A. J. (1996). A pharmacoepidemiological evaluation of anticholinergic prescribing patterns in the elderly. *Pharmacoepidemiology and Drug Safety*, *5*(3), 155-164.

Reyes, A. E., Chacón, M. A., Dinamarca, M. C., Cerpa, W., Morgan, C., & Inestrosa, N. C. (2004). Acetylcholinesterase-Aβ Complexes Are More Toxic than Aβ Fibrils in Rat Hippocampus. *The American Journal of Pathology*, *164*(6), 2163-2174. https://doi.org/10.1016/s0002-9440(10)63774-1

Reynolds, W. S., McPheeters, M., Blume, J., Surawicz, T., Worley, K., Wang, L., & Hartmann, K. (2015). Comparative Effectiveness of Anticholinergic Therapy for Overactive Bladder in Women: A Systematic Review and Meta-analysis. *Obstet Gynecol*, *125*(6), 1423-1432. https://doi.org/10.1097/AOG.0000000000000851

Rhee, T. G., Choi, Y. C., Ouellet, G. M., & Ross, J. S. (2018). National Prescribing Trends for High-Risk Anticholinergic Medications in Older Adults. *J Am Geriatr Soc*, *66*(7), 1382-1387. https://doi.org/10.1111/jgs.15357

Rice, C. (1894). Derivation of the Name Belladonna. *Belladonna: a study of its history, action and uses in medicine*, 7.

Richardson, K., Bennett, K., Maidment, I. D., Fox, C., Smithard, D., & Kenny, R. A. (2015). Use of Medications with Anticholinergic Activity and Self-Reported Injurious Falls in Older Community-Dwelling Adults. *J Am Geriatr Soc*, *63*(8), 1561-1569. https://doi.org/10.1111/jgs.13543

Richardson, K., Fox, C., Maidment, I., Steel, N., Loke, Y. K., Arthur, A., Myint, P. K., Grossi, C. M., Mattishent, K., Bennett, K., Campbell, N. L., Boustani, M., Robinson, L., Brayne, C., Matthews, F. E., & Savva, G. M. (2018). Anticholinergic drugs and risk of dementia: case-control study. *BMJ*, *361*, k1315. https://doi.org/10.1136/bmj.k1315

Richardson, K., Wharton, S. B., Grossi, C. M., Matthews, F. E., Fox, C., Maidment, I., Loke, Y. K., Steel, N., Arthur, A., Myint, P. K., Boustani, M., Campbell, N., Robinson, L., Brayne, C., & Savva, G. M. (2020). Neuropathological Correlates of Cumulative Benzodiazepine and Anticholinergic Drug Use. *J Alzheimers Dis*, *74*(3), 999-1009. https://doi.org/10.3233/JAD-191199

Richelson, E. (1996). Synaptic effects of antidepressants. *J Clin Psychopharmacol*, *16*(3 Suppl 2), 1S-7S; discussion 7S-9S. https://doi.org/10.1097/00004714-199606002-00001

Richerson, G. B., Aston-Jones, G., & Saper, C. B. (2013). The modulatory functions of the brain stem. In E. R. Kandel, J. H. Schwartz, T. M. Jessel, S. A. Siegelbaum, & A. J. Hudspeth (Eds.), *Principles of Neural Science* (5 ed., pp. 1038-1055). McGraw-Hill.

Rigor, J., Rueff Rato, I., Ferreira, P. M., Pereira, R., Ribeiro, C., Teixeira, D., Mesquita Oliveira, P., Silva, A. M., Pereira, S., Costa, I., Ferreira, P., Sequeira, M., Monteiro-Soares, M., & Martins-

Mendes, D. (2020). Prehospital Anticholinergic Burden Is Associated With Delirium but Not With Mortality in a Population of Acutely Ill Medical Patients. *J Am Med Dir Assoc*, *21*(4), 481-485. https://doi.org/10.1016/j.jamda.2019.12.018

Rinkinen, M. O., Roitto, H. M., Öhman, H. R., Kautiainen, H. J., Tilvis, R. S., Strandberg, T. E., Pitkala, K. H., & Aalto, U. L. (2022). Temporal Trends Over Two Decades in the Use of Anticholinergic Drugs Among Older Community-Dwelling People in Helsinki, Finland. *Drugs Aging*. https://doi.org/10.1007/s40266-022-00968-5

Risacher, S. L., McDonald, B. C., Tallman, E. F., West, J. D., Farlow, M. R., Unverzagt, F. W., Gao, S., Boustani, M., Crane, P. K., Petersen, R. C., Jack, C. R., Jr., Jagust, W. J., Aisen, P. S., Weiner, M. W., Saykin, A. J., & Alzheimer's Disease Neuroimaging, I. (2016). Association Between Anticholinergic Medication Use and Cognition, Brain Metabolism, and Brain Atrophy in Cognitively Normal Older Adults. *JAMA Neurol*, *73*(6), 721-732. https://doi.org/10.1001/jamaneurol.2016.0580

Roberts, L. M., Black, D. S., Raman, C., Woodford, K., Zhou, M., Haggerty, J. E., Yan, A. T., Cwirla, S. E., & Grindstaff, K. K. (2008). Subcellular localization of transporters along the rat blood-brain barrier and blood-cerebral-spinal fluid barrier by in vivo biotinylation. *Neuroscience*, *155*(2), 423-438. https://doi.org/10.1016/j.neuroscience.2008.06.015

Robins, J. M. (1986). A new approach to causal inference in mortality studies with a sustained exposure period—application to control of the healthy worker survivor effect. *Mathematical Modelling*, *7*(9-12), 1393-1512. https://doi.org/10.1016/0270-0255(86)90088-6

Robins, J. M. (1993). Analytic Methods for Estimating HIV-Treatment and Cofactor Effects. In D. G. Ostrow & R. C. Kessler (Eds.), *Methodological Issues in AIDS Behavioral Research* (1 ed., pp. 213-288). Springer. https://doi.org/10.1007/b112228

Robins, J. M., Hernán, M. A., & Brumback, B. (2000). Marginal structural models and causal inference in epidemiology. *Epidemiology*, *11*(5), 550-560. https://doi.org/10.1097/00001648-200009000-00011

Rodríguez-Ramallo, H., Báez-Gutiérrez, N., Prado-Mel, E., Alfaro-Lara, E. R., Santos-Ramos, B., & Sánchez-Fidalgo, S. (2021). Association between Anticholinergic Burden and Constipation: A Systematic Review. *Healthcare (Basel)*, *9*(5). https://doi.org/10.3390/healthcare9050581

Rogers, J. L., & Kesner, R. P. (2004). Cholinergic modulation of the hippocampus during encoding and retrieval of tone/shock-induced fear conditioning. *Learn Mem*, *11*(1), 102-107. https://doi.org/10.1101/lm.64604

Rosso, A. L., Marcum, Z. A., Zhu, X., Bohnen, N., & Rosano, C. (2021). Anticholinergic Medication Use, Dopaminergic Genotype, and Recurrent Falls. *J Gerontol A Biol Sci Med Sci*. https://doi.org/10.1093/gerona/glab258

Roy, R., Niccolini, F., Pagano, G., & Politis, M. (2016). Cholinergic imaging in dementia spectrum disorders. *European Journal of Nuclear Medicine and Molecular Imaging*, *43*(7), 1376-1386. https://doi.org/10.1007/s00259-016-3349-x

Royal Institute of France. (1824). *The Lancet*, *1*(26), 424-425. https://doi.org/10.1016/S0140-6736(02)95789-2

Rubin, D. B. (1974). Estimating causal effects of treatments in randomized and nonrandomized studies. *Journal of Educational Psychology*, *66*(5), 688-701. https://doi.org/10.1037/h0037350

Rubin, D. B. (1978). Bayesian Inference for Causal Effects: The Role of Randomization. *The Annals of Statistics*, *6*(1). https://doi.org/10.1214/aos/1176344064

Rudd, K. M., Raehl, C. L., Bond, C. A., Abbruscato, T. J., & Stenhouse, A. C. (2005). Methods for Assessing Drug-Related Anticholinergic Activity. *Pharmacotherapy*, *25*(11), 1592-1601. https://doi.org/10.1592/phco.2005.25.11.1592

Rudolph, J. L., Salow, M. J., Angelini, M. C., & McGlinchey, R. E. (2008). The anticholinergic risk scale and anticholinergic adverse effects in older persons. *Archives of Internal Medicine*, *168*(5), 508-513. https://doi.org/10.1001/archinternmed.2007.106

Ruiz, S. J., Cevallos, V., Baskaran, D., Mintzer, M. J., & Ruiz, J. G. (2021). The cross-sectional association of frailty with past and current exposure to strong anticholinergic drugs. *Aging Clin Exp Res*, *33*(8), 2283-2289. https://doi.org/10.1007/s40520-020-01742-6

Runfeldt, M. J., Sadovsky, A. J., & MacLean, J. N. (2014). Acetylcholine functionally reorganizes neocortical microcircuits. *J Neurophysiol*, *112*(5), 1205-1216. https://doi.org/10.1152/jn.00071.2014

Rupniak, N. M., Samson, N. A., Tye, S. J., Field, M. J., & Iversen, S. D. (1991). Evidence against a specific effect of cholinergic drugs on spatial memory in primates. *Behav Brain Res*, *43*(1), 1-6. https://doi.org/10.1016/s0166-4328(05)80047-6

Ruxton, K., Woodman, R. J., & Mangoni, A. A. (2015). Drugs with anticholinergic effects and cognitive impairment, falls and all-cause mortality in older adults: A systematic review and meta-analysis. *Br J Clin Pharmacol*, *80*(2), 209-220. https://doi.org/10.1111/bcp.12617

Rylett, R. J., Ball, M. J., & Colhoun, E. H. (1983). Evidence for high affinity choline transport in synaptosomes prepared from hippocampus and neocortex of patients with Alzheimer's disease. *Brain Research*, *289*(1-2), 169-175. https://doi.org/10.1016/0006-8993(83)90017-3

Sadot, E., Gurwitz, D., Barg, J., Behar, L., Ginzburg, I., & Fisher, A. (1996). Activation of m1 muscarinic acetylcholine receptor regulates tau phosphorylation in transfected PC12 cells. *J Neurochem*, *66*(2), 877-880. https://doi.org/10.1046/j.1471-4159.1996.66020877.x

Saladinus, d. A. (1489). *Aromatariorum compendium*. Pasqualibus, Peregrinus de, Bononiensis, active 15th century.

Salahudeen, M. S., Alfahmi, A., Farooq, A., Akhtar, M., Ajaz, S., Alotaibi, S., Faiz, M., & Ali, S. (2022). Effectiveness of Interventions to Improve the Anticholinergic Prescribing Practice in Older Adults: A Systematic Review. *J Clin Med*, *11*(3). https://doi.org/10.3390/jcm11030714

Salahudeen, M. S., Chyou, T. Y., & Nishtala, P. S. (2016). Serum Anticholinergic Activity and Cognitive and Functional Adverse Outcomes in Older People: A Systematic Review and Meta-Analysis of the Literature. *PLoS One*, *11*(3), e0151084. https://doi.org/10.1371/journal.pone.0151084

Salahudeen, M. S., Duffull, S. B., & Nishtala, P. S. (2014). Impact of anticholinergic discontinuation on cognitive outcomes in older people: a systematic review. *Drugs Aging*, *31*(3), 185-192. https://doi.org/10.1007/s40266-014-0158-4

Salahudeen, M. S., Duffull, S. B., & Nishtala, P. S. (2015). Anticholinergic burden quantified by anticholinergic risk scales and adverse outcomes in older people: a systematic review. *BMC Geriatr*, *15*, 31. https://doi.org/10.1186/s12877-015-0029-9

Salahudeen, M. S., Hilmer, S. N., & Nishtala, P. S. (2015). Comparison of anticholinergic risk scales and associations with adverse health outcomes in older people. *J Am Geriatr Soc*, *63*(1), 85-90. https://doi.org/10.1111/jgs.13206

Salahudeen, M. S., & Nishtala, P. S. (2016). Examination and Estimation of Anticholinergic Burden: Current Trends and Implications for Future Research. *Drugs Aging*, *33*(5), 305-313. https://doi.org/10.1007/s40266-016-0362-5

Salas, M., Hofman, A., & Stricker, B. H. (1999). Confounding by indication: an example of variation in the use of epidemiologic terminology. *Am J Epidemiol*, *149*(11), 981-983. https://doi.org/10.1093/oxfordjournals.aje.a009758

Salmond, C. H., Chatfield, D. A., Menon, D. K., Pickard, J. D., & Sahakian, B. J. (2005). Cognitive sequelae of head injury: involvement of basal forebrain and associated structures. *Brain*, *128*(Pt 1), 189-200. https://doi.org/10.1093/brain/awh352

Salter, H. (1869). On the treatment of asthma by belladonna. *The Lancet*, *93*(2370), 151-153.

Samuelsson, E., Odeberg, J., Stenzelius, K., Molander, U., Hammarstrom, M., Franzen, K., Andersson, G., & Midlov, P. (2015). Effect of pharmacological treatment for urinary incontinence in the elderly and frail elderly: A systematic review. *Geriatr Gerontol Int*, *15*(5), 521-534. https://doi.org/10.1111/ggi.12451

Sanghavi, R., Pana, T. A., Mamayusupova, H., Maidment, I., Fox, C., Boekholdt, S. M., Mamas, M. A., Wareham, N. J., Khaw, K. T., & Myint, P. K. (2022). Higher anticholinergic burden from medications is associated with significant increase in markers of inflammation in the EPIC-Norfolk prospective population-based cohort study. *Br J Clin Pharmacol*, *88*(7), 3297-3306. https://doi.org/10.1111/bcp.15261

Schaaf, C. P. (2014). Nicotinic acetylcholine receptors in human genetic disease. *Genet Med*, *16*(9), 649-656. https://doi.org/10.1038/gim.2014.9

Scheife, R., & Takeda, M. (2005). Central nervous system safety of anticholinergic drugs for the treatment of overactive bladder in the elderly. *Clin Ther*, *27*(2), 144-153. https://doi.org/10.1016/j.clinthera.2005.02.014

Scheinman, R. I., Cogswell, P. C., Lofquist, A. K., & Baldwin, A. S., Jr. (1995). Role of transcriptional activation of I kappa B alpha in mediation of immunosuppression by glucocorticoids. *Science*, *270*(5234), 283-286. https://doi.org/10.1126/science.270.5234.283

Scheltens, P., Blennow, K., Breteler, M. M. B., de Strooper, B., Frisoni, G. B., Salloway, S., & Van der Flier, W. M. (2016). Alzheimer's disease. *The Lancet*, *388*(10043), 505-517. https://doi.org/10.1016/s0140-6736(15)01124-1

Schliebs, R., & Arendt, T. (2006). The significance of the cholinergic system in the brain during aging and in Alzheimer's disease. *J Neural Transm (Vienna)*, *113*(11), 1625-1644. https://doi.org/10.1007/s00702-006-0579-2

Schmiederberg, O., & Kopper, R. (1869). *Das Muscarin: das giftige Alkaloid des Fliegenpilzes (Agaricus muscarius L.) : seine Darstellung, chemischen Eigenschaften, physiologischen Wirkungen, toxicologische Bedeutung und sein Verhältniss zur Pilzvergiftung im allgemeinen*. F. C. W. Vogel.

Schmitz, T. W., Nathan Spreng, R., & Alzheimer's Disease Neuroimaging, I. (2016). Basal forebrain degeneration precedes and predicts the cortical spread of Alzheimer's pathology. *Nat Commun*, *7*, 13249. https://doi.org/10.1038/ncomms13249

Schon, K., Atri, A., Hasselmo, M. E., Tricarico, M. D., LoPresti, M. L., & Stern, C. E. (2005). Scopolamine reduces persistent activity related to long-term encoding in the parahippocampal gyrus during delayed matching in humans. *J Neurosci*, *25*(40), 9112-9123. https://doi.org/10.1523/JNEUROSCI.1982-05.2005

Schulz, J., Pagano, G., Fernandez Bonfante, J. A., Wilson, H., & Politis, M. (2018). Nucleus basalis of Meynert degeneration precedes and predicts cognitive impairment in Parkinson's disease. *Brain*, *141*(5), 1501-1516. https://doi.org/10.1093/brain/awy072

Secchi, A., Mamayusupova, H., Sami, S., Maidment, I., Coulton, S., Myint, P. K., & Fox, C. (2022). A novel Artificial Intelligence-based tool to assess anticholinergic burden: a survey. *Age Ageing*, *51*(8). https://doi.org/10.1093/ageing/afac196

Seeger, T., Fedorova, I., Zheng, F., Miyakawa, T., Koustova, E., Gomeza, J., Basile, A. S., Alzheimer, C., & Wess, J. (2004). M2 muscarinic acetylcholine receptor knock-out mice show deficits in behavioral flexibility, working memory, and hippocampal plasticity. *J Neurosci*, *24*(45), 10117-10127. https://doi.org/10.1523/JNEUROSCI.3581-04.2004

Selden, N. R., Gitelman, D. R., Salamon-Murayama, N., Parrish, T. B., & Mesulam, M. M. (1998). Trajectories of cholinergic pathways within the cerebral hemispheres of the human brain. *Brain*, *121*(12), 2249-2257. https://doi.org/10.1093/brain/121.12.2249

Sen, A., Capelli, V., & Husain, M. (2018). Cognition and dementia in older patients with epilepsy. *Brain*, *141*(6), 1592-1608. https://doi.org/10.1093/brain/awy022

Sendor, R., & Stürmer, T. (2022). Core concepts in pharmacoepidemiology: Confounding by indication and the role of active comparators. *Pharmacoepidemiol Drug Saf*, *31*(3), 261-269. https://doi.org/10.1002/pds.5407

Senior, K. (2006). UK Biobank launched to mixed reception. *The Lancet Neurology*, *5*(5). https://doi.org/10.1016/s1474-4422(06)70436-7

Serrano-Pozo, A., Frosch, M. P., Masliah, E., & Hyman, B. T. (2011). Neuropathological alterations in Alzheimer disease. *Cold Spring Harb Perspect Med*, *1*(1), a006189. https://doi.org/10.1101/cshperspect.a006189

Shah, R. C., Janos, A. L., Kline, J. E., Yu, L., Leurgans, S. E., Wilson, R. S., Wei, P., Bennett, D. A., Heilman, K. M., & Tsao, J. W. (2013). Cognitive decline in older persons initiating anticholinergic medications. *PLoS One*, *8*(5), e64111. https://doi.org/10.1371/journal.pone.0064111

Sheffield, E. B., Quick, M. W., & Lester, R. A. (2000). Nicotinic acetylcholine receptor subunit mRNA expression and channel function in medial habenula neurons. *Neuropharmacology*, *39*(13), 2591-2603. https://doi.org/10.1016/s0028-3908(00)00138-6

Shekhar, A., Potter, W. Z., Lightfoot, J., Lienemann, J., Dubé, S., Mallinckrodt, C., Bymaster, F. P., McKinzie, D. L., & Felder, C. C. (2008). Selective muscarinic receptor agonist xanomeline as a novel treatment approach for schizophrenia. *American Journal of Psychiatry*, *165*(8), 1033-1039. https://doi.org/10.1176/appi.ajp.2008.06091591

Sherman, S. J., Atri, A., Hasselmo, M. E., Stern, C. E., & Howard, M. W. (2003). Scopolamine impairs human recognition memory: data and modeling. *Behav Neurosci*, *117*(3), 526-539. https://doi.org/10.1037/0735-7044.117.3.526

Shmuel, S., Pate, V., Pepin, M. J., Bailey, J. C., Hanson, L. C., Stürmer, T., Naumann, R. B., Golightly, Y. M., Gnjidic, D., & Lund, J. L. (2021). Quantifying cumulative anticholinergic and sedative drug load among US Medicare Beneficiaries. *Pharmacoepidemiol Drug Saf*, *30*(2), 144-156. https://doi.org/10.1002/pds.5144

Sittironnarit, G., Ames, D., Bush, A. I., Faux, N., Flicker, L., Foster, J., Hilmer, S., Lautenschlager, N. T., Maruff, P., Masters, C. L., Martins, R. N., Rowe, C., Szoeke, C., Ellis, K. A., & group, A. r. (2011). Effects of anticholinergic drugs on cognitive function in older Australians: results from the AIBL study. *Dement Geriatr Cogn Disord*, *31*(3), 173-178. https://doi.org/10.1159/000325171

Slovis, T. L., Ott, J. E., Teitelbaum, D. T., & Lipscomb, W. (1971). Physostigmine therapy in acute tricyclic antidepressant poisoning. *Clinical Toxicology*, *4*(3), 451-459. https://doi.org/10.3109/15563657108990496

Smith, S., Alfaro-Almagro, F., & Miller, K. (2020). UK Biobank Brain Imaging Documentation Version 1.8. In. Oxford University on behalf of UK Biobank: Wellcome Centre for Integrative Neuroimaging

Smith, V. M. (1964). Newer Anticholinergic Drugs. *Medical Clinics of North America*, *48*(2), 399-409. https://doi.org/10.1016/S0025-7125(16)33472-1

Smulyan, H. (2018). The Beat Goes On: The Story of Five Ageless Cardiac Drugs. *Am J Med Sci*, *356*(5), 441-450. https://doi.org/10.1016/j.amjms.2018.04.011

Solari, N., & Hangya, B. (2018). Cholinergic modulation of spatial learning, memory and navigation. *Eur J Neurosci*, *48*(5), 2199-2230. https://doi.org/10.1111/ejn.14089

Sørensen, H. T., Lash, T. L., & Rothman, K. J. (2006). Beyond randomized controlled trials: a critical comparison of trials with nonrandomized studies. *Hepatology*, *44*(5), 1075-1082. https://doi.org/10.1002/hep.21404

Sorensen, S. R., Frederiksen, J. D., Anru, P. L., Masud, T., Petrovic, M., Rosholm, J. U., & Ryg, J. (2021). Use of Drugs with Anticholinergic Properties at Hospital Admission Associated with Mortality in Older Patients: A Danish Nationwide Register-Based Cohort Study. *Drugs Real World Outcomes*. https://doi.org/10.1007/s40801-021-00270-7

Sperling, R., Greve, D., Dale, A., Killiany, R., Holmes, J., Rosas, H. D., Cocchiarella, A., Firth, P., Rosen, B., Lake, S., Lange, N., Routledge, C., & Albert, M. (2002). Functional MRI detection of pharmacologically induced memory impairment. *Proc Natl Acad Sci U S A*, *99*(1), 455-460. https://doi.org/10.1073/pnas.012467899

Spinelli, S., Ballard, T., Feldon, J., Higgins, G. A., & Pryce, C. R. (2006). Enhancing effects of nicotine and impairing effects of scopolamine on distinct aspects of performance in computerized attention and working memory tasks in marmoset monkeys. *Neuropharmacology*, *51*(2), 238-250. https://doi.org/10.1016/j.neuropharm.2006.03.012

Splawa-Neyman, J., Dabrowska, D. M., & Speed, T. P. (1990). On the Application of Probability Theory to Agricultural Experiments. Essay on Principles (1923). *Statistical Science*, *5*(4), 465–572. https://doi.org/10.1214/ss/1177012031

St Germaine-Smith, C., Metcalfe, A., Pringsheim, T., Roberts, J. I., Beck, C. A., Hemmelgarn, B. R., McChesney, J., Quan, H., & Jette, N. (2012). Recommendations for optimal ICD codes to study neurologic conditions: a systematic review. *Neurology*, *79*(10), 1049-1055. https://doi.org/10.1212/WNL.0b013e3182684707

Staley, K. (2015). 'Is it worth doing?' Measuring the impact of patient and public involvement in research. *Res Involv Engagem*, *1*, 6. https://doi.org/10.1186/s40900-015-0008-5

Stamatakis, E., Owen, K. B., Shepherd, L., Drayton, B., Hamer, M., & Bauman, A. E. (2021). Is Cohort Representativeness Passe? Poststratified Associations of Lifestyle Risk Factors with Mortality in the UK Biobank. *Epidemiology*, *32*(2), 179-188. https://doi.org/10.1097/EDE.0000000000001316

Starr, J. M., Wardlaw, J., Ferguson, K., MacLullich, A., Deary, I. J., & Marshall, I. (2003). Increased blood-brain barrier permeability in type II diabetes demonstrated by gadolinium magnetic resonance imaging. *J Neurol Neurosurg Psychiatry*, *74*(1), 70-76. https://doi.org/10.1136/jnnp.74.1.70

Staskin, D., Kay, G., Tannenbaum, C., Goldman, H. B., Bhashi, K., Ling, J., & Oefelein, M. G. (2010). Trospium chloride has no effect on memory testing and is assay undetectable in the central nervous system of older patients with overactive bladder. *Int J Clin Pract*, *64*(9), 1294-1300. https://doi.org/10.1111/j.1742-1241.2010.02433.x

Sternberg, E. M. (1997). Neural-immune interactions in health and disease. *J Clin Invest*, *100*(11), 2641-2647. https://doi.org/10.1172/JCI119807

Stewart, C., Gallacher, K., Nakham, A., Cruickshank, M., Newlands, R., Bond, C., Myint, P. K., Bhattacharya, D., & Mair, F. S. (2021). Barriers and facilitators to reducing anticholinergic burden: a qualitative systematic review. *Int J Clin Pharm*, *43*(6), 1451-1460. https://doi.org/10.1007/s11096-021-01293-4

Stewart, C., Taylor-Rowan, M., Soiza, R. L., Quinn, T. J., Loke, Y. K., & Myint, P. K. (2021). Anticholinergic burden measures and older people's falls risk: a systematic prognostic review. *Ther Adv Drug Saf*, *12*, 20420986211016645. https://doi.org/10.1177/20420986211016645

Stewart, C., Yrjana, K., Kishor, M., Soiza, R. L., Taylor-Rowan, M., Quinn, T. J., Loke, Y. K., & Myint, P. K. (2021). Anticholinergic Burden Measures Predict Older People's Physical Function and Quality of Life: A Systematic Review. *J Am Med Dir Assoc*, *22*(1), 56-64. https://doi.org/10.1016/j.jamda.2020.05.065

Sudlow, C., Gallacher, J., Allen, N., Beral, V., Burton, P., Danesh, J., Downey, P., Elliott, P., Green, J., Landray, M., Liu, B., Matthews, P., Ong, G., Pell, J., Silman, A., Young, A., Sprosen, T., Peakman, T., & Collins, R. (2015). UK biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. *PLoS Med*, *12*(3), e1001779. https://doi.org/10.1371/journal.pmed.1001779

Suehs, B. T., Caplan, E. O., Hayden, J., Ng, D. B., & Gaddy, R. R. (2019). The Relationship Between Anticholinergic Exposure and Falls, Fractures, and Mortality in Patients with Overactive Bladder. *Drugs Aging*, *36*(10), 957-967. https://doi.org/10.1007/s40266-019-00694-5

Summers, W. K. (1978). A clinical method of estimating risk of drug induced delirium. *Life Sci*, *22*(17), 1511-1516. https://doi.org/10.1016/0024-3205(78)90006-1

Sumukadas, D., McMurdo, M. E., Mangoni, A. A., & Guthrie, B. (2014). Temporal trends in anticholinergic medication prescription in older people: repeated cross-sectional analysis of population prescribing data. *Age Ageing*, *43*(4), 515-521. https://doi.org/10.1093/ageing/aft199

Sura, S. D., Carnahan, R. M., Chen, H., & Aparasu, R. R. (2013). Prevalence and determinants of anticholinergic medication use in elderly dementia patients. *Drugs Aging*, *30*(10), 837-844. https://doi.org/10.1007/s40266-013-0104-x

Sura, S. D., Carnahan, R. M., Chen, H., & Aparasu, R. R. (2015). Anticholinergic drugs and health-related quality of life in older adults with dementia. *J Am Pharm Assoc (2003)*, *55*(3), 282-287. https://doi.org/10.1331/JAPhA.2015.14068

Swift, C. G. (1988). Prescribing in old age. *British Medical Journal*, *296*(6626). https://doi.org/10.1136/bmj.296.6626.913

Szabo, S. M., Gooch, K., Schermer, C., Walker, D., Lozano-Ortega, G., Rogula, B., Deighton, A., Vonesh, E., & Campbell, N. (2019). Association between cumulative anticholinergic burden and falls and fractures in patients with overactive bladder: US-based retrospective cohort study. *BMJ Open*, *9*(5), e026391. https://doi.org/10.1136/bmjopen-2018-026391

Tabatabaei-Jafari, H., Walsh, E., Shaw, M. E., & Cherbuin, N. (2017). The cerebellum shrinks faster than normal ageing in Alzheimer's disease but not in mild cognitive impairment. *Human Brain Mapping*, *38*(6), 3141-3150. https://doi.org/10.1002/hbm.23580

Taipale, H., Gomm, W., Broich, K., Maier, W., Tolppanen, A. M., Tanskanen, A., Tiihonen, J., Hartikainen, S., & Haenisch, B. (2018). Use of Antiepileptic Drugs and Dementia Risk-an Analysis of Finnish Health Register and German Health Insurance Data. *J Am Geriatr Soc*, *66*(6), 1123-1129. https://doi.org/10.1111/jgs.15358

Tan, M. P., Tan, G. J., Mat, S., Luben, R. N., Wareham, N. J., Khaw, K. T., & Myint, P. K. (2020). Use of Medications with Anticholinergic Properties and the Long-Term Risk of Hospitalization for Falls and Fractures in the EPIC-Norfolk Longitudinal Cohort Study. *Drugs Aging*, *37*(2), 105-114. https://doi.org/10.1007/s40266-019-00731-3

Tansey, E. M. (2006). Henry Dale and the discovery of acetylcholine. *C R Biol*, *329*(5-6), 419-425. https://doi.org/10.1016/j.crvi.2006.03.012

Taubman, S. L., Robins, J. M., Mittleman, M. A., & Hernan, M. A. (2009). Intervening on risk factors for coronary heart disease: an application of the parametric g-formula. *Int J Epidemiol*, *38*(6), 1599-1611. https://doi.org/10.1093/ije/dyp192

Taylor-Rowan, M., Edwards, S., Noel-Storr, A. H., McCleery, J., Myint, P. K., Soiza, R., Stewart, C., Loke, Y. K., & Quinn, T. J. (2021). Anticholinergic burden (prognostic factor) for prediction of dementia or cognitive decline in older adults with no known cognitive syndrome. *Cochrane Database Syst Rev*, *5*, CD013540. https://doi.org/10.1002/14651858.CD013540.pub2

Taylor-Rowan, M., Kraia, O., Kolliopoulou, C., Noel-Storr, A. H., Alharthi, A. A., Cross, A. J., Stewart, C., Myint, P. K., McCleery, J., & Quinn, T. J. (2022). Anticholinergic burden for prediction of

cognitive decline or neuropsychiatric symptoms in older adults with mild cognitive impairment or dementia. *Cochrane Database Syst Rev*, *8*, CD015196. https://doi.org/10.1002/14651858.CD015196.pub2

Thiele, A. (2013). Muscarinic signaling in the brain. *Annu Rev Neurosci*, *36*, 271-294. https://doi.org/10.1146/annurev-neuro-062012-170433

Thienhaus, O. J., Jerry, A. A., Bennett, J. A., Chopra, Y., M., & Zemlan, F. P. (1990). Anticholinergic serum levels and cognitive performance. *European Archives of Psychiatry and Clinical Neuroscience*, *240*(1), 28-33. https://doi.org/10.1007/BF02190089

Thom, A., & Crief, C. (1879). Article IX. - The Therapeutics of Belladonna in Intestinal Obstruction; Notes of a Case; Foecal Vomiting; Recovery. *Edinburgh Medical Journal*, *25*(6), 538-543.

Thomann, P. A., Dos Santos, V., Toro, P., Schönknecht, P., Essig, M., & Schröder, J. (2009). Reduced olfactory bulb and tract volume in early Alzheimer's disease—A MRI study. *Neurobiology of Aging*, *30*(5), 838-841. https://doi.org/10.1016/j.neurobiolaging.2007.08.001

Thomas, C., Hestermann, U., Kopitz, J., Plaschke, K., Oster, P., Driessen, M., Mundt, C., & Weisbrod, M. (2008). Serum anticholinergic activity and cerebral cholinergic dysfunction: an EEG study in frail elderly with and without delirium. *BMC Neuroscience*, *9*(86). https://doi.org/10.1186/1471-2202-9-86

Thompson, C. J. S. (1934). *The mystic mandrake*. Rider & co.

Tokuoka, K., Kasai, M., Kobayashi, K., & Isa, T. (2020). Anatomical and electrophysiological analysis of cholinergic inputs from the parabigeminal nucleus to the superficial superior colliculus. *J Neurophysiol*, *124*(6), 1968-1985. https://doi.org/10.1152/jn.00148.2020

Tombaugh, T. N., & Mclntyre, N. J. (1992). The Mini-Mental State Examination: a comprehensive review. *Progress in Geriatrics*, *40*(9), 922-935. https://doi.org/10.1111/j.1532-5415.1992.tb01992.x

Townsend, P. (1987). Deprivation. *Journal of Social Policy*, *16*(2), 125-146. https://doi.org/10.1017/s0047279400020341

Tracey, K. J. (2002). The inflammatory reflex. *Nature*, *420*(6917), 853-859. https://doi.org/10.1038/nature01321

Trenaman, S. C., Bowles, S. K., Andrew, M. K., & Goralski, K. (2021). The role of sex, age and genetic polymorphisms of CYP enzymes on the pharmacokinetics of anticholinergic drugs. *Pharmacol Res Perspect*, *9*(3), e00775. https://doi.org/10.1002/prp2.775

Trend, H. (1858). Application of belladonna as an antilactescent. *British Medical Journal*, *1*(76), 478.

Trendelenburg, A. U., Gomeza, J., Klebroff, W., Zhou, H., & Wess, J. (2003). Heterogeneity of presynaptic muscarinic receptors mediating inhibition of sympathetic transmitter release: a study with M2- and M4-receptor-deficient mice. *Br J Pharmacol*, *138*(3), 469-480. https://doi.org/10.1038/sj.bjp.0705053

Tristancho-Perez, A., Villalba-Moreno, A., Lopez-Malo de Molina, M. D., Santos-Ramos, B., & Sanchez-Fidalgo, S. (2022). The Predictive Value of Anticholinergic Burden Measures in Relation to Cognitive Impairment in Older Chronic Complex Patients. *J Clin Med*, *11*(12). https://doi.org/10.3390/jcm11123357

Tristancho-Pérez, Á., Villalba-Moreno, Á., Santos-Rubio, M. D., Belda-Rustarazo, S., Santos-Ramos, B., & Sánchez-Fidalgo, S. (2021). Concordance Among 10 Different Anticholinergic Burden Scales in At-Risk Older Populations. *Journal of Patient Safety*. https://doi.org/10.1097/PTS.0000000000000929

Tsukada, H., Nishiyama, S., & Takahashi, K. (2016). Imaging of Muscarinic Receptors in the Central Nervous System. In *Muscarinic Receptor: From Structure to Animal Models* (pp. 181-203). https://doi.org/10.1007/978-1-4939-2858-3_10

Tsunawaki, S., Sporn, M., Ding, A., & Nathan, C. (1988). Deactivation of macrophages by transforming growth factor-beta. *Nature*, *334*(6179), 260-262. https://doi.org/10.1038/334260a0

Tune, L., Carr, S., Cooper, T., Klug, B., & Golinger, R. C. (1993). Association of anticholinergic activity of prescribed medications with postoperative delirium. *The Journal of Neuropsychiatry and Clinical Neurosciences*, *5*(2), 208-210. https://doi.org/10.1176/jnp.5.2.208

Tune, L., Carr, S., Hoag, E., & Cooper, T. (1992). Anticholinergic Effects of Drugs Commonly the Elderly : Potential Means for Assessing Risk of Delirium. *American Journal of Psychiatry*, *149*(10), 1993-1394. https://doi.org/10.1176/ajp.149.10.1393

Tune, L., & Coyle, J. T. (1980). Serum Levels of Anticholinergic Drugs in Treatment of Acute Extrapyramidal Side Effects. *Archives of General Psychiatry*, *37*(3), 293-297. https://doi.org/10.1001/archpsyc.1980.01780160063007

Tune, L., & Coyle, J. T. (1981). Acute extrapyramidal side effects: Serum levels of neuroleptics and anticholinergics. *Psychopharmacology*, *75*(1), 9-15. https://doi.org/10.1007/BF00433493

Turchi, J., & Sarter, M. (1997). Cortical acetylcholine and processing capacity: effects of cortical cholinergic deafferentation on crossmodal divided attention in rats. *Brain Res Cogn Brain Res*, *6*(2), 147-158. https://doi.org/10.1016/s0926-6410(97)00027-x

Twig, G., Tirosh, A., Derazne, E., Haklai, Z., Goldberger, N., Afek, A., Gerstein, H. C., Kark, J. D., & Cukierman-Yaffe, T. (2018). Cognitive function in adolescence and the risk for premature diabetes and cardiovascular mortality in adulthood. *Cardiovasc Diabetol*, *17*(1), 154. https://doi.org/10.1186/s12933-018-0798-5

UK Biobank. (2006). UK Biobank: Protocol for a large-scale prospective epidemiological resource. In.

UK Biobank. (2019a). First Occurrence of Health Outcomes Defined by 3-character ICD10 code. In.

UK Biobank. (2019b). UK Biobank Primary Care Linked Data Version 1.0. In.

UK Biobank. (2020a). UK Biobank Hospital inpatient data Version 3.0. In.

UK Biobank. (2020b). UK Biobank Mortality data: linkage to death registries Version 2.0. In.

UK Biobank. (2022). Algorithmically-defined outcomes (ADOs). In.

Valenstein, E. S. (2002). The discovery of chemical neurotransmitters. *Brain Cogn*, *49*(1), 73-95. https://doi.org/10.1006/brcg.2001.1487

Valladales-Restrepo, L. F., Duran-Lengua, M., Castro-Osorio, E. E., & Machado-Alba, J. E. (2020). Consistency between anticholinergic burden scales in the elderly with fractures. *PLoS One*, *15*(2), e0228532. https://doi.org/10.1371/journal.pone.0228532

van de Waterbeemd, H., Camenisch, G., Folkers, G., Chretien, J. R., & Raevsky, O. A. (1998). Estimation of blood-brain barrier crossing of drugs using molecular size and shape, and H-bonding descriptors. *J Drug Target*, *6*(2), 151-165. https://doi.org/10.3109/10611869808997889

van der Meer, H. G., Wouters, H., Pont, L. G., & Taxis, K. (2018). Reducing the anticholinergic and sedative load in older patients on polypharmacy by pharmacist-led medication review: a randomised controlled trial. *BMJ Open*, *8*(7), e019042. https://doi.org/10.1136/bmjopen-2017-019042

van der Poll, T., Coyle, S. M., Barbosa, K., Braxton, C. C., & Lowry, S. F. (1996). Epinephrine inhibits tumor necrosis factor-alpha and potentiates interleukin 10 production during human endotoxemia. *J Clin Invest*, *97*(3), 713-719. https://doi.org/10.1172/JCI118469

van Diepen, M., Ramspek, C. L., Jager, K. J., Zoccali, C., & Dekker, F. W. (2017). Prediction versus aetiology: common pitfalls and how to avoid them. *Nephrol Dial Transplant*, *32*(suppl_2), ii1-ii5. https://doi.org/10.1093/ndt/gfw459

Vasavada, M. M., Wang, J., Eslinger, P. J., Gill, D. J., Sun, X., Karunanayaka, P., & Yang, Q. X. (2015). Olfactory Cortex Degeneration in Alzheimer's Disease and Mild Cognitive Impairment. *Journal of Alzheimer's Disease*, *45*(3), 947-958. https://doi.org/10.3233/jad-141947

Venkatesan, S., Jeoung, H. S., Chen, T., Power, S. K., Liu, Y., & Lambe, E. K. (2020). Endogenous Acetylcholine and Its Modulation of Cortical Microcircuits to Enhance Cognition. *Curr Top Behav Neurosci*, *45*, 47-69. https://doi.org/10.1007/7854_2020_138

Vickers, L. E., Martinez, A. I., Wallem, A. M., Johnson, C., & Moga, D. C. (2021). Potentially Inappropriate Medication Use in Older Adults with Alzheimer's Disease and Related Dementias Living in the Community: A Cross-Sectional Analysis. *Drugs Real World Outcomes*, *8*(4), 519-526. https://doi.org/10.1007/s40801-021-00265-4

Villalba-Moreno, A. M., Alfaro-Lara, E. R., Pérez-Guerrero, M. C., Nieto-Martín, M. D., & Santos-Ramos, B. (2016). Systematic review on the use of anticholinergic scales in poly pathological patients. *Arch Gerontol Geriatr*, *62*, 1-8. https://doi.org/10.1016/j.archger.2015.10.002

Vinogradov, S., FIsher, M., Warm, H., Holland, C., Kirshner, M. A., & Pollock, B. G. (2009). The cognitive cost of anticholinergic burden: Decreased response to cognitive training in schizophrenia. *American Journal of Psychiatry*, *166*(9), 1055-1062. https://doi.org/10.1176/appi.ajp.2009.09010017

Viswanath, H., Carter, A. Q., Baldwin, P. R., Molfese, D. L., & Salas, R. (2013). The medial habenula: still neglected. *Front Hum Neurosci*, *7*, 931. https://doi.org/10.3389/fnhum.2013.00931

Waade, R. B., Molden, E., Refsum, H., & Hermann, M. (2012). Serum concentrations of antidepressants in the elderly. *Therapeutic Drug Monitoring*, *34*(1). https://doi.org/10.1097/FTD.0b013e318241dce0

Wallace, T. L., Ballard, T. M., Pouzet, B., Riedel, W. J., & Wettstein, J. G. (2011). Drug targets for cognitive enhancement in neuropsychiatric disorders. *Pharmacol Biochem Behav*, *99*(2), 130-145. https://doi.org/10.1016/j.pbb.2011.03.022

Wallenstein, G. V., & Vago, D. R. (2001). Intrahippocampal scopolamine impairs both acquisition and consolidation of contextual fear conditioning. *Neurobiol Learn Mem*, *75*(3), 245-252. https://doi.org/10.1006/nlme.2001.4005

Wang, K., Alan, J., Page, A. T., Dimopoulos, E., & Etherton-Beer, C. (2021). Anticholinergics and clinical outcomes amongst people with pre-existing dementia: A systematic review. *Maturitas*, *151*, 1-14. https://doi.org/10.1016/j.maturitas.2021.06.004

Wang, L., Zhang, Z., McArdle, J. J., & Salthouse, T. A. (2009). Investigating Ceiling Effects in Longitudinal Data Analysis. *Multivariate Behav Res*, *43*(3), 476-496. https://doi.org/10.1080/00273170802285941

Wang, Y. C., Chen, Y. L., Huang, C. C., Ho, C. H., Huang, Y. T., Wu, M. P., Ou, M. J., Yang, C. H., & Chen, P. J. (2019). Cumulative use of therapeutic bladder anticholinergics and the risk of dementia in patients with lower urinary tract symptoms: a nationwide 12-year cohort study. *BMC Geriatr*, *19*(1), 380. https://doi.org/10.1186/s12877-019-1401-y

Ward, L. M., Stanley, B., Greenlaw, N., Cooper, S. A., Pacitti, C., Henderson, A., Gibson, J., & Kinnear, D. (2021). Risk of anticholinergic burden in adults with intellectual disabilities: a Scottish retrospective cohort study of n = 17 220. *J Intellect Disabil Res*, *65*(9), 813-830. https://doi.org/10.1111/jir.12861

Ward McKernan, L., Cooper, S. A., Henderson, A., Stanley, B., Greenlaw, N., Pacitti, C., & Cairns, D. (2022). A study on prescriptions contributing to the risk of high anticholinergic burden in adults with intellectual disabilities: retrospective record linkage study. *Ann Gen Psychiatry*, *21*(1), 41. https://doi.org/10.1186/s12991-022-00418-x

Wawruch, M., Macugova, A., Kostkova, L., Luha, J., Dukat, A., Murin, J., Drobna, V., Wilton, L., & Kuzelova, M. (2012). The use of medications with anticholinergic properties and risk factors

for their use in hospitalised elderly patients. *Pharmacoepidemiol Drug Saf*, *21*(2), 170-176. https://doi.org/10.1002/pds.2169

Weatherly, L. (1891). Use and Abuse of Hyoscine. *Journal of Mental Science*, *37*(158), 366-373. https://doi.org/10.1192/bjp.37.158.366

Weigand, A. J., Bondi, M. W., Thomas, K. R., Campbell, N. L., Galasko, D. R., Salmon, D. P., Sewell, D., Brewer, J. B., Feldman, H. H., Delano-Wood, L., & Alzheimer's Disease Neuroimaging, I. (2020). Association of anticholinergic medications and AD biomarkers with incidence of MCI among cognitively normal older adults. *Neurology*, *95*(16), e2295-e2304. https://doi.org/10.1212/WNL.0000000000010643

Welk, B. (2022). The Differential Risk of Mortality Among Users of Overactive Bladder Anticholinergic Medications and beta3 Agonists. *Eur Urol Focus*. https://doi.org/10.1016/j.euf.2022.08.002

Welk, B., Etaby, K., McArthur, E., & Chou, Q. (2022). The risk of delirium and falls or fractures with the use of overactive bladder anticholinergic medications. *Neurourol Urodyn*, *41*(1), 348-356. https://doi.org/10.1002/nau.24827

Welk, B., Richardson, K., & Panicker, J. N. (2021). The cognitive effect of anticholinergics for patients with overactive bladder. *Nat Rev Urol*, *18*(11), 686-700. https://doi.org/10.1038/s41585-021-00504-x

Welsh, T. J., van der Wardt, V., Ojo, G., Gordon, A. L., & Gladman, J. R. F. (2018). Anticholinergic Drug Burden Tools/Scales and Adverse Outcomes in Different Clinical Settings: A Systematic Review of Reviews. *Drugs Aging*, *35*(6), 523-538. https://doi.org/10.1007/s40266-018-0549-z

Wess, J., Duttaroy, A., Gomeza, J., Zhang, W., Yamada, M., Felder, C. C., Bernardini, N., & Reeh, P. W. (2003). Muscarinic receptor subtypes mediating central and peripheral antinociception studied with muscarinic receptor knockout mice. *Life Sciences*, *72*(18-19), 2047-2054. https://doi.org/10.1016/s0024-3205(03)00082-1

Wessler, I., & Kirkpatrick, C. J. (2008). Acetylcholine beyond neurons: the non-neuronal cholinergic system in humans. *Br J Pharmacol*, *154*(8), 1558-1571. https://doi.org/10.1038/bjp.2008.185

Westfall, T. C., Macarthur, H., & Westfall, D. P. (2018). Neurotransmission: The Autonomic and Somatic Motor Nervous Systems. In L. L. Brunton, R. Hilal-Dandan, & B. C. Knollmann (Eds.), *Goodman & Gilman's: The Pharmacological Basis of Therapeutics* (12 ed., pp. 115-147). McGraw-Hill.

Wezenberg, E., Verkes, R. J., Sabbe, B. G. C., Ruigt, G. S. F., & Hulstijn, W. (2005). Modulation of memory and visuospatial processes by biperiden and rivastigmine in elderly healthy subjects. *Psychopharmacology*, *181*(3), 582-594. https://doi.org/10.1007/s00213-005-0083-7

Whalley, L. J., Sharma, S., Fox, H. C., Murray, A. D., Staff, R. T., Duthie, A. C., Deary, I. J., & Starr, J. M. (2012). Anticholinergic drugs in late life: adverse effects on cognition but not on progress to dementia. *J Alzheimers Dis*, *30*(2), 253-261. https://doi.org/10.3233/JAD-2012-110935

Whitehouse, P. J., Price, D. L., Clark, A. W., Coyle, J. T., & DeLong, M. R. (1981). Alzheimer disease: evidence for selective loss of cholinergic neurons in the nucleus basalis. *Ann Neurol*, *10*(2), 122-126. https://doi.org/10.1002/ana.410100203

Whitehouse, P. J., Price, D. L., Struble, R. G., Clark, A. W., Coyle, J. T., & Delon, M. R. (1982). Alzheimer's disease and senile dementia: loss of neurons in the basal forebrain. *Science*, *215*(4537), 1237-1239. https://doi.org/10.1126/science.7058341

Wilczyński, K., Gorczyca, M., Gołębiowska, J., & Szewieczek, J. (2021). Anticholinergic Burden of Geriatric Ward Inpatients. *Medicina (Kaunas)*, *57*(10). https://doi.org/10.3390/medicina57101115

Wilkinson, T., Ly, A., Schnier, C., Rannikmae, K., Bush, K., Brayne, C., Quinn, T. J., Sudlow, C. L. M., Group, U. K. B. N. O., & Dementias Platform, U. K. (2018). Identifying dementia cases with routinely collected health data: A systematic review. *Alzheimers Dement*, *14*(8), 1038-1051. https://doi.org/10.1016/j.jalz.2018.02.016

Wilkinson, T., Schnier, C., Bush, K., Rannikmäe, K., Henshall, D. E., Lerpiniere, C., Allen, N. E., Flaig, R., Russ, T. C., Bathgate, D., Pal, S., O'Brien, J. T., Sudlow, C. L. M., Dementias Platform, U. K., & Biobank, U. K. (2019). Identifying dementia outcomes in UK Biobank: a validation study of primary care, hospital admissions and mortality data. *Eur J Epidemiol*, *34*(6), 557-565. https://doi.org/10.1007/s10654-019-00499-1

Wilkinson, T., Schnier, C., Bush, K., Rannikmäe, K., Lyons, R. A., McTaggart, S., Bennie, M., & Sudlow, C. L. (2022). Drug prescriptions and dementia incidence: a medication-wide association study of 17000 dementia cases among half a million participants. *J Epidemiol Community Health*, *76*(3), 223-229. https://doi.org/10.1136/jech-2021-217090

Williamson, J., & Chopin, J. M. (1980). Adverse reactions to prescribed drugs in the elderly: a multicentre investigation. *Age and Ageing*, *9*(2), 73-80. https://doi.org/10.1016/S0025-7125(16)31382-7

Wirsching, B. A., Beninger, R. J., Jhamandas, K., Boegman, R. J., & El-Defrawy, S. R. (1984). Differential effects of scopolamine on working and reference memory of rats in the radial maze. *Pharmacol Biochem Behav*, *20*(5), 659-662. https://doi.org/10.1016/0091-3057(84)90180-1

Wolf, A., Dedman, D., Campbell, J., Booth, H., Lunn, D., Chapman, J., & Myles, P. (2019). Data resource profile: Clinical Practice Research Datalink (CPRD) Aurum. *Int J Epidemiol*, *48*(6), 1740-1740g. https://doi.org/10.1093/ije/dyz034

Woodfield, R., Grant, I., Group, U. K. B. S. O., Follow-Up, U. K. B., Outcomes Working, G., & Sudlow, C. L. (2015). Accuracy of Electronic Health Record Data for Identifying Stroke Cases in Large-Scale Epidemiological Studies: A Systematic Review from the UK Biobank Stroke Outcomes Group. *PLoS One*, *10*(10), e0140533. https://doi.org/10.1371/journal.pone.0140533

Wright, F. L., Green, J., Canoy, D., Cairns, B. J., Balkwill, A., Beral, V., & Million Women Study, C. (2012). Vascular disease in women: comparison of diagnoses in hospital episode statistics and general practice records in England. *BMC Med Res Methodol*, *12*, 161. https://doi.org/10.1186/1471-2288-12-161

Xu, D., Anderson, H. D., Tao, A., Hannah, K. L., Linnebur, S. A., Valuck, R. J., & Culbertson, V. L. (2017). Assessing and predicting drug-induced anticholinergic risks: an integrated computational approach. *Ther Adv Drug Saf*, *8*(11), 361-370. https://doi.org/10.1177/2042098617725267

Xu, X. J., Myint, P. K., Kioh, S. H., Mat, S., Rajasuriar, R., Kamaruzzaman, S. B., & Tan, M. P. (2022). A five-year prospective evaluation of anticholinergic cognitive burden and falls in the Malaysian elders longitudinal research (MELoR) study. *Arch Gerontol Geriatr*, *98*, 104535. https://doi.org/10.1016/j.archger.2021.104535

Yang, Y., Schulhofer-Wohl, S., Fu, Wenjiang J., & Land, Kenneth C. (2008). The Intrinsic Estimator for Age-Period-Cohort Analysis: What It Is and How to Use It. *American Journal of Sociology*, *113*(6), 1697-1736. https://doi.org/10.1086/587154

Yoshida, A., Fujino, T., Maruyama, S., Ito, Y., Taki, Y., & Yamada, S. (2010). The forefront for novel therapeutic agents based on the pathophysiology of lower urinary tract dysfunction: bladder selectivity based on in vivo drug-receptor binding characteristics of antimuscarinic agents for treatment of overactive bladder. *J Pharmacol Sci*, *112*(2), 142-150. https://doi.org/10.1254/jphs.09r14fm

Yoshiyama, Y., Kojima, A., Itoh, K., Isose, S., Koide, M., Hori, K., & Arai, K. (2015). Does Anticholinergic Activity Affect Neuropathology? Implication of Neuroinflammation in Alzheimer's Disease. *Neurodegener Dis*, *15*(3), 140-148. https://doi.org/10.1159/000381484

Yoshiyama, Y., Kojima, A., Itoh, K., Uchiyama, T., & Arai, K. (2012). Anticholinergics boost the pathological process of neurodegeneration with increased inflammation in a tauopathy mouse model. *Neurobiol Dis*, *45*(1), 329-336. https://doi.org/10.1016/j.nbd.2011.08.017

Yrjana, K. R., Neal, S. R., Soiza, R. L., Keevil, V., Luben, R. N., Wareham, N. J., Khaw, K. T., & Myint, P. K. (2021). Baseline anticholinergic burden from medications predicts poorer baseline and long-term health-related quality of life in 16 675 men and women of EPIC-Norfolk prospective population-based cohort study. *Pharmacoepidemiol Drug Saf*, *30*(2), 135-143. https://doi.org/10.1002/pds.5085

Zarrindast, M. R., Ardjmand, A., Ahmadi, S., & Rezayof, A. (2012). Activation of dopamine D1 receptors in the medial septum improves scopolamine-induced amnesia in the dorsal hippocampus. *Behav Brain Res*, *229*(1), 68-73. https://doi.org/10.1016/j.bbr.2011.12.033

Zheng, Y. B., Shi, L., Zhu, X. M., Bao, Y. P., Bai, L. J., Li, J. Q., Liu, J. J., Han, Y., Shi, J., & Lu, L. (2021). Anticholinergic drugs and the risk of dementia: A systematic review and meta-analysis. *Neurosci Biobehav Rev*, *127*, 296-306. https://doi.org/10.1016/j.neubiorev.2021.04.031

Zinner, N. (2007). Darifenacin: a muscarinic M3-selective receptor antagonist for the treatment of overactive bladder. *Expert Opin Pharmacother*, *8*(4), 511-523. https://doi.org/10.1517/14656566.8.4.511