Medication use, falls and genetic variants in an older population

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Medication Use, Falls and
Genetic Variants in an Older Population

Medicatiegebruik, vallen en genetische variatie in een oudere populatie

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Content

1. GENERAL INTRODUCTION

Medication use in the older population

Our western population is ageing, as not only the number of older people is increasing, but also the life expectancy.[1] In 2012, the Netherlands counted 2.7 million people aged ≥65 years – corresponding to 16% of the total population –, and this is expected to increase to 4.7 million in 2041 – corresponding to ~26% of the total population –.[2] With respect to health, the ageing process is accompanied by morbidities, such as cardiovascular, musculoskeletal, and airway conditions, but also cognitive decline, cancer and diabetes.[1] In older individuals, multiple conditions are regularly present at the same time, of those aged ≥65 years ~65% has two or more conditions, which are often treated or managed with medication regimens.[3-6] Improvements in healthcare, disease management and treatment are additive factors that facilitate the ageing of the western population.[1] On the other hand, use of multiple medications for various conditions increases the risk of adverse drug reactions and drug-drug interactions.[3,4] Especially for older individuals this is of importance as – compared to younger individuals – they not only use more medicines, they are also more prone to adverse drug reactions due to physiological changes, including changes in body composition, blood flow, and liver and kidney function.[7,8] So, decisions on treatment – medication use – need to take into account this precarious balance in order to optimize the health effect. Therefore, insight into the potential risks and negative effects are needed. This thesis especially focuses on older individuals and fall incidents. Falls can have many causes but may also occur as an unintended consequence of medication use, and they are considered as a major health care problem in this age group[9]. Medication-related falls have gained more attention in the recent decades, though literature is inconclusive on the medication groups that are associated with an increased fall risk.[10-15] Moreover, the pathways underlying the association between medication use and fall incidents need to be further examined. Therefore, in this thesis we addressed medication-related falls and their potential underlying pathways. We examined the role of genetic variants in medication-related falls, and whether more knowledge on this subject could help to determine individuals at risk for medication-related falls. Furthermore, it could provide insight into the biological mechanism involved in medication-related falls. In addition to medication-related falls, we investigated homocysteine and bone mineral density (BMD). Homocysteine is a risk indicator for various age-related diseases and may be related to falls via physical function.[16,17] BMD is an indicator for bone health and fracture risk, which is of major importance for those at risk for falls.[18,19]

Medication use and fall incidents

As previously indicated, fall incidents are a major health problem in older individuals. Of all falls, 5 to 12%[20,21] result in serious injuries or fractures requiring medical attention, which lead to reduced quality of life and substantial health care costs.[22,23] Moreover, approximately one third of the older individuals (≥65 y) encounters at least one fall yearly.[9] There are multiple

risk factors for falling, including; a history of falling, muscle weakness, impaired balance, gait, vision and cognition, but also environmental hazards, and medication use.[20,24,25] Medication use is one of the potentially modifiable risk factors for falls.[26,27] Psychotropic[10-14] and cardiovascular[10,11,13-15] medication have been indicated as the main medication groups contributing to an increased fall risk. They may affect fall risk by inducing dizziness, sedation, instability, and hypotension.[10-15] However, evidence for the association between medication use and fall risk is mainly based on observational studies, which have applied varying methods for recording fall incidents and medication use. Only a limited number of the studies recorded falls prospectively and ascertained medication use at the time of the fall.[10] Therefore, new insight into potential associations between medication use and fall incidents, using prospectively gathered medication and fall data are of clinical value (*Chapter 2.1*).

Potential pathways of medication-related falls, including genetic variation

As described above, older individuals frequently use medication which could next to their intended effects, also have unintended effects – including falls –.[3,4] On the other hand, individuals might not respond to the given medication, which is also an undesired outcome. So, overall pharmacological effects of medications vary between individuals, which complicates attaining the desired outcome.[28-33] There are multiple factors that may play a role in medication response, including age, gender, health status, and lifestyle. Genetic variation is another factor that may explain interindividual variation and therefore may influence medication response. When we are able to identify genetic variants with substantial effects, individuals at increased – medication-related fall – risk could be identified based on their genetic makeup and treatment could be more targeted.[30-33]

Benzodiazepine-related falls

Use of benzodiazepines has most strongly and consistently been associated with an increased fall risk.[10,13,34,35] And, pharmacological effects of benzodiazepines have been observed to be influenced by genetic variation.[36,37] Nevertheless, limited information is available about the role of genetic variation in benzodiazepine-related falls.[38] So, more research is needed and various approaches can be used.[30,31] We chose to follow two approaches: a candidate gene study and a genome wide association study (GWAS). The first approach has previously been used in a pilot study.[38] The authors selected genetic variants in genes encoding medication metabolising enzymes, thereby plasma drug concentrations may be influenced and might subsequently affect fall risk. Two genetic polymorphisms were identified that modified benzodiazepine-related fall risk.[38] To confirm this finding, a replication study was performed (*Chapter 2.2*). Nevertheless, a candidate gene approach is restricted to prior knowledge on genetic variants that influence a pathway, which may affect the outcome – medication-related falls –.[30,31] The second approach is hypothesis free and can identify genetic variants

– out of millions of variants –[30,31,39] that significantly modified the association between medication use and fall risk (*Chapter 2.3*).

Beta-blocker-related falls

Use of beta-blockers has been associated with fall risk, but literature is contradictory.[10,40-42] Pharmacological effects and occurrence of adverse effects may vary between different beta-blocking agents. Differences between beta-blocking agents relate, for example, to their selectivity for adrenergic receptors, lipid solubility, intrinsic sympathetic activity (ISA), and their elimination route.[43,44] Some beta-blockers are eliminated through liver metabolism – e.g., metoprolol and propranolol –,[43,44] in which the Cytochrome P450 (CYP) 2D6 enzyme plays an important role.[45,46] Genetic variation in the CYP2D6 gene might thereby influence the elimination of beta-blockers, and potentially subsequent fall risk. In this thesis the association between use of beta-blockers and beta-blocker characteristics – selectivity, lipid solubility, intrinsic sympathetic activity (ISA), and CYP2D6 enzyme metabolism – and fall risk was analysed (*Chapter 2.4*).

Medication, homocysteine and falls

Homocysteine is an amino acid formed from methionine, an essential amino acid that is present in our diet.[47] Hyperhomocysteinemia, >15 μmol/L, is prevalent in 10-30% of Dutch older individuals[48] and is associated with cardiovascular disease,[49,50] cognitive decline,[51,52] and fractures.[53-55] Furthermore, homocysteine has been related to physical function,[16,17] and thereby it might be related to falls. We did not investigate the association between homocysteine and falls, as this was done by Swart et al in the B-PROOF study.[16,17] Instead, we were interested whether there was an association between medication use and homocysteine levels, as potential precursor to an association with falls. In addition to a reduced intake or absorption of vitamin B_{12} and/or folic acid,[47] medication use could, unintentionally, influence homocysteine levels.[56-58] However, evidence is limited per medication group and results are contradictory and inconclusive. More insight into possible medication-related changes in homocysteine levels could help to create clinical awareness, and might suggest monitoring these levels during the use of specific medications (*Chapter 3.1*).

Medication, bone mineral density and fractures

Fractures are another important problem in the elderly population, and are related to falls and osteoporosis.[18,19] Osteoporosis is characterized by loss of bone mass and structure, and is currently determined by assessing bone mineral density (BMD).[18,19] Use of selective serotonin reuptake inhibitors (SSRIs) has been associated with an increased fracture risk, potentially because of an underlying association with falls and/or BMD.[59-61] The association between SSRIs and falls has consistently been reported in literature,[11,62,63] while there are conflicting results regarding the association between SSRIs and BMD, and change in BMD.[64-66] In previous studies, use of SSRIs was assessed through interview data and limited information was available regarding the duration of use at the study visit or in between the

study visits.[64-66] Since bone remodeling is a slow process,[67] we expected that the duration of SSRI use is of importance when examining the association with BMD. Therefore, longitudinal studies investigating the association between use of SSRIs and duration of treatment, with BMD and change in BMD are of clinical relevance (*Chapter 3.2*).

Study populations

For this thesis, data from three Dutch studies were used, the Rotterdam Study, B-PROOF, and LASA.

The B-PROOF study population formed a basis for investigating the research questions within this thesis. B-PROOF is an acronym for 'B-vitamins for the PRevention Of Osteoporotic Fractures'. It is a multi-centre, randomized, placebo-controlled, double-blind trial investigating the efficacy of vitamin B_{12} and folic acid supplementation on the prevention of fractures in persons aged ≥65 years, with mildly elevated homocysteine levels (12-50 μmol/L).[68]

The Rotterdam Study is an ongoing prospective population-based cohort, executed within Rotterdam.[69] The study aims to investigate the incidence of, and risk factors for various age-related diseases. The study was initiated in 1989 and in total 7,983 participants, aged ≥55 years, were included (78% response rate, cohort I). In 2000, the study was extended with a second cohort of 3,011 participants, aged ≥55 years (67% response rate, cohort II). Additionally, in 2006 a second extension of the cohort was initiated, including 3,923 participants aged 45 years or older (65% response rate, cohort III). From baseline onwards, follow-up examinations were conducted every 4-5 years including interviews and an extensive set of examinations.

LASA, Longitudinal Aging Study Amsterdam, is also an ongoing prospective population-based cohort study and started in 1991.[70] The study was executed in the surrounding of three Dutch cities; Amsterdam, Zwolle and Oss, and aims to examine determinants and consequences of physical, cognitive and social aspects in an ageing population. From baseline onwards, data was collected approximately every 3 years on these aspects, using interviews, questionnaires and examinations.

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Objectives of this thesis

In this thesis, we addressed the association between medication use and fall incidents, and their potential underlying pathways. Thereby, we examined the role of genetic variants, homocysteine and bone mineral density. The following objectives were addressed:

Objective 1: Which medication is associated with fall risk in the B-PROOF study populations? (*Chapter 2.1*)
Objective 2: Are there genetic variants that modify the association between medication use and fall risk? (*Chapter 2.2, 2.3* and *2.4*)
Objective 3: Is medication use associated with homocysteine levels? (*Chapter 3.1*)
Objective 4: Is use of SSRIs associated with BMD and change in BMD over time? (*Chapter 3.2*)

Author contributions

In *chapter 2.1* the study concept and design was done by: BHS, AGU, and NvdV. Data analyses and interpretation was done by: ACH, BHS, AGU, and NvdV. Drafting the manuscript was done by: ACH and NvdV. Revising the manuscript was done by: all authors. The study was supervised by: BHS, AGU, and NvdV. In *chapter 2.2* the study concept and design was done by: BHS, GZ, AGU, and NvdV. Data analyses and interpretation was done by: ACH, GZ, BHS, AGU, and NvdV. Drafting the manuscript was done by: ACH and NvdV. Revising the manuscript was done by: all authors. The study was supervised by: BHS, AGU, and NvdV. In *chapter 2.3* the study concept and design was done by: BHS, AGU, and NvdV. Data analyses and interpretation was done by: ACH, BHS, AGU, and NvdV. Drafting the manuscript was done by: ACH, SCvD, and NvdV. Revising the manuscript was done by: all authors. The study was supervised by: BHS, AGU, SCvD, and NvdV. In *chapter 2.4* the study concept and design was done by: BHS, AGU, LB, NvdV. Data analyses and interpretation was done by: LB, ACH, BHS, AGU, and NvdV. Drafting the manuscript was done by: ACH, LB, BHS, AGU, and NvdV. Revising the manuscript was done by: all authors. The study was supervised by: LB, BHS, AGU, and NvdV. In *chapter 3.1* the study concept and design was done by: BHS, AGU, and NvdV. Data analyses and interpretation was done by: ACH, BHS, AGU, and NvdV. Drafting the manuscript was done by: ACH and NvdV. Revising the manuscript was done by: all authors. The study was supervised by: BHS, AGU, and NvdV. In *chapter 3.2* the study concept and design was done by: FR, GZ, BHS, AGU, LEV, NA, and ACH. Data analyses and interpretation was done by: NA, ACH, FR, BHS, MCZ, and LEV. Drafting manuscript was done by: NA, ACH, FR, and RN. Revising manuscript was done by: all authors. The study was supervised by: BHS, FR, MCZ, LEV, and AGU.

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2. MEDICATION-RELATED FALLS
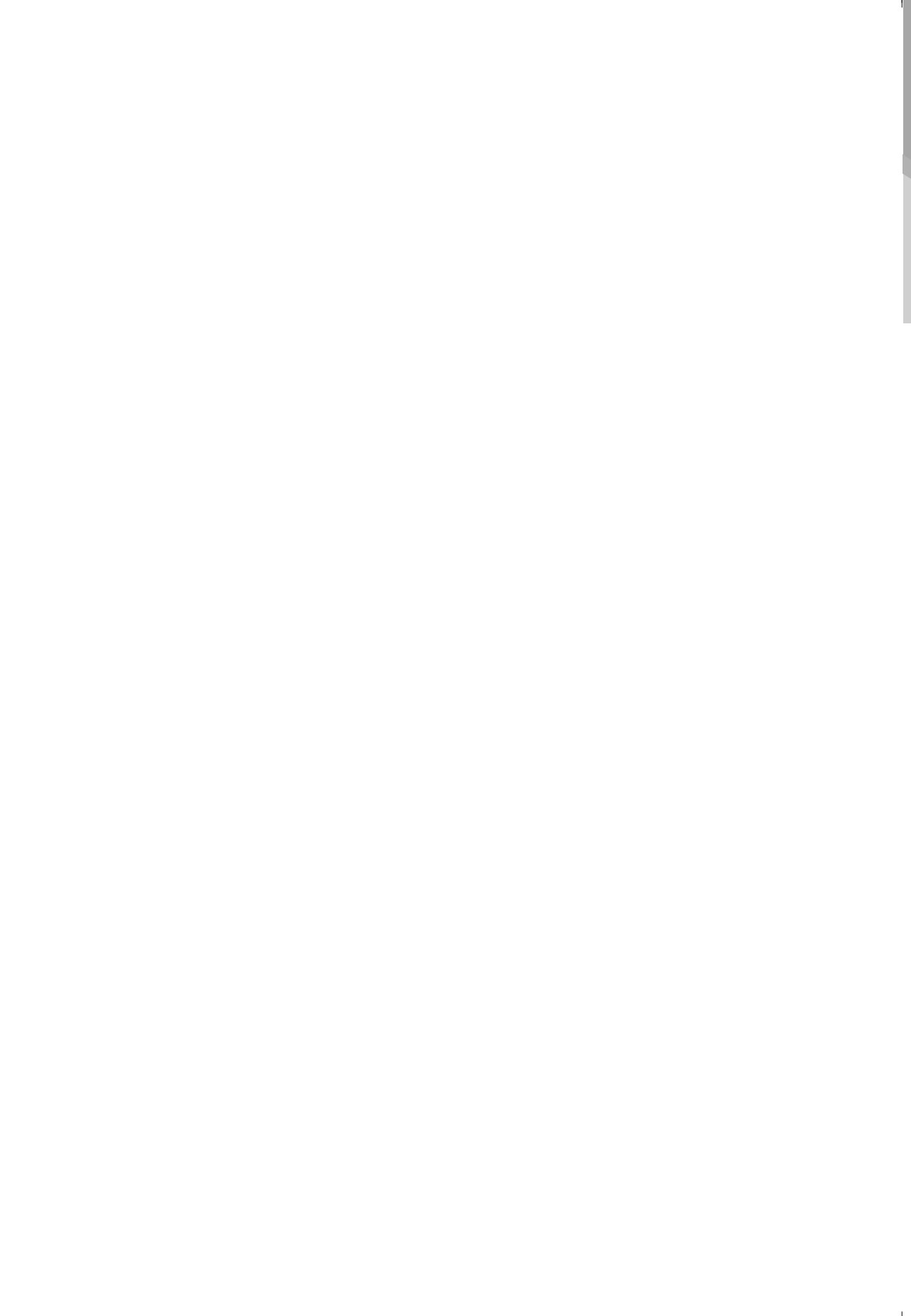
Medication-related fall incidents in an older ambulant population: the B-PROOF study

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Abstract

Background
Medication use is a potentially modifiable risk factor for falling; psychotropic and cardiovascular drugs have been indicated as main drug groups that increase fall risk. However, evidence is mainly based on studies that recorded falls retrospectively and/or did not determine medication use at the time of the fall. Therefore, we investigated the associations indicated in literature between medication use and falls, using prospectively recorded falls and medication use determined at the time of the fall.

Methods
Data from the B-PROOF (B-vitamins for the prevention of osteoporotic fractures) study were used, concerning community-dwelling elderly aged ≥65 years. We included 2,407 participants with pharmacy dispensing records. During the 2- to 3-year follow-up, participants recorded falls using a fall calendar. Cox proportional hazard models were applied, adjusting for potential confounders including age, sex, health status variables and concomitant medication use.

Results
During follow-up, 1,147 participants experienced at least one fall. Users of anti-arrhythmic medication had an increased fall risk (hazard ratio [HR]= 1.61; 95% confidence interval [CI] 1.12-2.32) compared with non-users. Similarly, non-selective beta-blocker use was associated with an increased fall risk (HR 1.41 [95% CI 1.12; 1.78]), while statin use was associated with a lower risk (HR=0.81 [95% CI 0.71; 0.94]). Benzodiazepine use (HR 1.32 [95% CI 1.02-1.71]), and antidepressant use (HR 1.40 [95% CI 1.07; 1.82]) were associated with an increased fall risk. Use of other cardiovascular and psychotropic medication was not associated with fall risk.

Conclusion
Our results strengthen the evidence for an increased fall risk in community-dwelling elderly during the use of anti-arrhythmics, non-selective beta-blockers, benzodiazepines, and antidepressant medication. Clinicians should prescribe these drugs cautiously and if possible choose safer alternatives for older patients.

Key points

- In a prospective setting including a community-dwelling population, aged ≥65 years, the use of antiarrhythmic medication, non-selective beta-blockers, benzodiazepines, and antidepressant medication was associated with an increased fall risk.
- Statin use was associated with a decreased fall risk.
- Clinicians should prescribe the fall-risk increasing drugs with caution and if possible choose safer alternatives for older patients.

Introduction

Fall incidents are a major problem in older individuals, as one in every three experiences at least one fall per year.[1] Of all falls, 5 to 12%[2,3] result in serious injuries or fractures requiring medical attention, which leads to reduced quality of life and substantial health care costs.[4,5] A potentially modifiable risk factor for falls is medication use.[6,7] Over the last decade, medication-related falls have received more and more attention. Psychotropic[8-10] and cardiovascular[8,9,11] medications have been indicated as the main drug groups contributing to an increased fall risk. However, evidence for these associations is mainly based on observational studies, which have applied varying methods for recording fall incidents and medication use. The most recent meta-analysis showed that only 6 out of 22 studies included recorded falls prospectively and ascertained medication use at the time of the fall.[8] In addition, current evidence is based on studies in community-dwelling older individuals as well as in those living in long-term care facilities, while these populations clearly differ in clinical characteristics. Therefore, the question arises whether these results can be validly combined. Although overall results point in a similar direction, only psychotropic drug use is consistently associated with an increased fall-risk.[8,9,12-14] Therefore, our objective is to investigate associations previously indicated in literature between medication use and fall incidents, using prospectively recorded fall incidents and pharmacy dispensing records to determine medication use at the time of the fall. The study setting concerns a large population of community-dwelling older individuals, with a follow-up period of 2-3 years.

Methods

Study population and setting
Data from the B-PROOF study were used. B-PROOF is an acronym for 'B-vitamins for the prevention of osteoporotic fractures', a study whose design has been described elsewhere in more detail.[15] Briefly, it is a multi-centre, randomized, placebo-controlled, double-blind trial investigating the efficacy of vitamin B_{12} and folic acid supplementation on the prevention of fracture incidence in individuals aged ≥65 years. In total, 2,919 participants were included from the area of three Dutch cities: Wageningen, Rotterdam and Amsterdam. All participants had mildly elevated homocysteine levels (12-50 µmol/L), sufficient renal function (creatinine ≤150 µmol/L), and did not report malignancies in the past 5 years. Participants were randomly selected to receive daily the intervention tablet containing 500 µg vitamin B_{12}, 400 µg folic acid and 600 IU vitamin D, or the placebo tablet containing only 600 IU of vitamin D. In total, the intervention period comprised 2-3 years. The Medical Ethics Committee of Wageningen University approved the study protocol, and the Medical Ethics Committees of Erasmus Medical Centre and VU University Medical Center gave approval for local feasibility. Before entering the study, all participants gave written informed consent.

Previous results indicated that the intervention had no effect on the time to the first or second fall or the number of falls experienced during the follow-up.[16] Therefore, in the current study, we treated the study population as a cohort and, to rule out potential residual confounding that might relate to the intervention, we adjusted for the intervention status. The intervention status indicated whether a participant received the intervention or the placebo tablet during follow-up.

Outcome

Fall incidents were prospectively recorded during the study period. Participants reported fall incidents each week on a fall and fracture calendar, which was returned to the research team every 3 months. When a calendar was incomplete or unclear, the participant was contacted by telephone. A fall incident was defined as an unintentional change in position resulting in coming to a rest at a lower level or on the ground.[17] Participants were followed until their first fall incident. The Thursday in that particular week was defined as the index date. Participants who encountered more than one fall during the follow-up period were censored after their first fall incident. Drop-outs (of the intervention study) without further calendar information after drop-out were censored at their drop-out date. Participants who kept filling out the calendar after their drop-out were followed until their last calendar. Therefore, a participant's follow-up time ended at the date of their first fall-incident, their drop-out date or the date of their last calendar, date of death, or the end of the study, whichever came first.

Medication use

Medication use was determined on the basis of pharmacy dispensing records. These records contain information regarding the product name, the anatomical therapeutic chemical code,[18] the administration route, the dispensing date, the total amount of drug units per prescription and the prescribed daily number of units. Electronic pharmacy dispensing records were obtained from the Dutch Foundation for Pharmaceutical Statistics (SFK). This foundation gathers data of all the pharmacies in their panel, which is approximately 95% of all Dutch community pharmacies.[19] Dispensing records were only obtained for participants who gave written informed consent for gathering these data. Additionally, the pharmacists approved the use of the data. The pharmacy dispensing data of a participant were defined as complete when all participant pharmacies were in the SFK panel and data could be obtained. Data were available for the participants throughout their follow-up period.

Medication usage periods were calculated from the dispensing date, the number of tablets prescribed, and the prescribed daily number of tablets. A participant was considered a current user of a medication group when the time of the fall (index date) fell within a prescription episode. The average prescribed daily dose was expressed in standardized defined daily doses (DDDs).[18]

The medication groups from Table 1, covering previously suggested fall-risk increasing drugs (FRID),[8-11,20,21] were used as potential exposure determinants. Medication groups with <1% users at baseline were not included in the analyses.

Covariates

Baseline demographic characteristics were ascertained using a questionnaire that gathered data on age, sex, use of a walking aid, history of falls and fractures, and health status variables (which included smoking habits, alcohol consumption, prevalent cardiovascular disease parameters, diabetes and hypercholesterolemia). A history of cardiovascular disease was defined as having a history of at least one of the following disorders: myocardial infarction, angina pectoris, heart failure, percutaneous coronary intervention, intermittent claudication, transient ischaemic attack, stroke, thrombosis or embolism. During the baseline study visit, various characteristics were measured, including weight, height, blood pressure, physical performance, handgrip strength, depressive symptoms and cognitive status. Weight was measured with a calibrated scale, and height was measured using a stadiometer. From this, the body mass index (BMI, kg/m^2) was calculated. Blood pressure was measured twice, using an Omron M1 plus device (Omron Healthcare Europe, Hoofddorp, The Netherlands), and the lowest diastolic and corresponding systolic blood pressure reading were included in the analyses. Hypertension was defined as systolic blood pressure >140mmHg and/or diastolic blood pressure >90 mmHg.[22] A physical performance score was calculated from the results of three physical function tests: walking test, chair stand test, and the tandem stand test.[23] For every test, a maximum score of 4 could be obtained, resulting in a physical performance score ranging from 0 to 12 (low physical performance – high physical performance).[24] Handgrip strength (kg) was assessed by performing two maximum trials per hand using a dynamometer (Takei TKK 5401, Takei Scientific Instrument CO. Ltd., Tokyo, Japan). The highest result of the four trials was used as the maximum handgrip strength. Depressive symptoms were measured using the 15-item version of the Geriatric Depression Scale (GDS),[25] and cognitive status was measured by using the Mini-Mental State Examination (MMSE).[26]

Blood was drawn when the participants had fasted or had consumed a light restricted breakfast. Plasma homocysteine levels were assessed from blood collected in an tube containing EDTA (ethylenediaminetetraacetic acid), which was stored on ice after blood collection and processed within 4 h. To determine homocysteine, Wageningen University used high-performance liquid chromatography (intra assay coefficient of variation [CV]= 3.1%, inter assay CV= 5.9%), Erasmus Medical Centre used liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) (intra assay CV= 5.5%, inter assay CV= 1.3%), and VU Medical Centre used the Architect i2000 RS analyser (intra assay CV= 2%, inter assay CV= 4%). Cross calibration of the assays indicated no significant difference between the outcomes.

Serum creatinine was measured using the enzymatic colorimetric Roche CREA plus assay (CV= 2%). It was used to calculate an age- and sex-adjusted estimate of the glomerular filtration rate (GFR) according to the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.[27] For men, it was calculated when their creatinine was ≤80 μmol/L using the formula 141 x [serum creatinine (μmol/L) / 80]$^{-0.411}$ x [0.993$^{\text{age (years)}}$], and if their creatinine was >80 μmol/L using the formula 141 x [serum creatinine (μmol/L) / 80]$^{-1.209}$ x [0.993$^{\text{age (years)}}$] in ml/min/1.73m^2. For women, it was calculated when their creatinine levels were ≤62 μmol/L

using the formula 144 x [serum creatinine (μmol/L) / 62]$^{-0.329}$ x [0.993$^{age (years)}$], and if their creatinine was >62 μmol/L using the formula 144 x [serum creatinine (μmol/L) / 62]$^{-1.209}$ x [0.993$^{age (years)}$] in ml/min/1.73m^2.

Serum 25-hydroxy vitamin D [25(OH)D], was used as marker for vitamin D status and determined by isotope dilution-online solid phase extraction liquid chromatography-tandem mass spectrometry (ID-XLS-MS/MS), which is described elsewhere in detail.[28]

Table 1. Drug categories of potentially fall-risk increasing drugs.[8-11,20,21]

Drug categories	Drug sub-categories
Cardiovascular	
Anti-arrhythmics	class 1A anti-arrhythmic, digitalis glycosides
Vasodilators	
Antihypertensives	α-blockers
β-blockers	selective, non-selective, α & β-blockers
Diuretics	thiazides, loop diuretics
Calcium antagonist	
Renin-angiotensin agents	ACE-inhibitors, angiotensin II antagonist
Lipid-lowering drugs	statins
Nervous system	
Analgesics	opioids, and others
Anticonvulsants	
Anti-Parkinson	
Antipsychotics	
Sedatives & hypnotics	benzodiazepines
Antidepressants	TCAs, SSRIs, others
Dementia drugs	cholinesterase inhibitors, others
Antivertigo drugs	
Respiratory system	sympathomimetics, antihistaminics
Miscellaneous	
Diabetic drugs	insulin, oral glucose-lowering
Antacids	H2-receptor antagonist, proton pump inhibitors
Urological	spasmolytic
Muscle-skeletal	muscle relaxants
Anti-inflammatory	NSAIDs

ACE-inhibitors= angiotensin-converting-enzyme inhibitors, TCAs= tricyclic antidepressants, SSRIs= selective serotonin reuptake inhibitors NSAIDs= non-steroidal anti-inflammatory drugs.

Statistical analyses

Baseline characteristics were determined for the overall group and for fallers and non-fallers separately. These characteristics were also assessed for the whole B-PROOF population, including those without electronic pharmacy dispensing records. Differences between groups were tested using a t test for continuous variables and a Chi-squared test for categorical variables. If a variable was non-normally distributed, a Mann-Whitney U test was used.

Cox proportional hazards models were used to calculate hazard ratios (HR). The model compares the prevalence of exposure to the medication group in the incident fall cases on the index date with the exposure prevalence in all other participants in the cohort on that date.[29] The models were adjusted for age, sex and intervention status. The variables that differed at baseline between fallers and non-fallers (p<0.2) were added to the model using the forward selection methods (model 2). Medication groups that resulted in significant HRs were added to the other significant medication groups, thereby adjusting for concomitant medication use (model 3). The effect of handgrip strength and physical performance was investigated separately by adding them separately as well as combined to the model. Physical function parameters are known fall-risk factors, but they might also be affected by medication use itself, and thereby might act as an intermediate. In addition, the effect of fall history was examined by adding it to the model. When the HR was changed >5%, the parameter was regarded to influence the association. Interaction with CKD-EPI was tested for medication groups with renal clearance, since associations may be different in those with reduced renal function. When the p-value of the interaction term was <0.1 the results were stratified.

To further investigate the robustness of our findings, we investigated the dose-response relationship for the medication groups that were significantly associated with fall risk. The categories for dose were created based on the median number of prescribed DDDs. Furthermore, additional analyses were conducted for non-selective β-blockers, diuretics, statins and antidepressants. Non-selective β-blocker use was subdivided in timolol use – administered as eye drops with potential systemic effects – and other non-selective β-blocker use, to investigate whether the association was driven by timolol use. For thiazide and loop diuretics, duration periods were investigated, since previous research indicated an increased fall risk on initiation of treatment.[30-32] The duration periods were defined as the first 21 days of use, 22-45 days and longer than 45 days of use, taking non-users as the reference.[30-32] In addition, the association between past use of statins and antidepressants with fall incidents was assessed. This reason for this was that the association between statins and antidepressants might have been confounded by the indication for their use. Past use was defined as use prior to the index date. All statistical analyses were carried out using the statistical software package SPSS version 21.0 (IBM, Armonk, NY, USA) and p values <0.05 were considered to be statistically significant.

Results

Study population characteristics
The study population with pharmacy dispensing records consisted of 2,407 participants. Their baseline characteristics, also subdivided into those who did and did not experience fall during follow-up, are presented in Table 2. Participants who experienced a fall during follow-up were slightly older, more likely to be women and to have a positive fall history. Furthermore, fallers were more likely to use a walking aid, have lower handgrip strength and lower physical performance score, while their MMSE and GDS score was slightly higher than those who did not fall during the follow-up.

Characteristics of the subgroup with pharmacy dispensing data were very similar to the overall B-PROOF population (n= 2,919, data not shown). The only significant difference was a slightly lower percentage of women (49.1 vs. 50.0, *p*= 0.026) and participants using a walking aid (13.6 vs. 14.6, *p*= 0.001) in the pharmacy dispensing data group. Furthermore, the representation of study centres was slightly altered and the physical performance score was slightly higher in those with pharmacy dispensing data than the whole B-PROOF study population, although the difference in physical performance score did not result in different median and interquartile range (IQR) values: 9 [6-11] vs. 9 [6-11], *p*= 0.017.

Fall risk-increasing drugs (FRID)
Of the cardiovascular drugs, the use of anti-arrhythmic medication was associated with an increased risk for falls compared with non-users (HR 1.61; 95% confidence interval [CI] 1.12; 2.32; *p*= 0.010) (Table 3, model 3). Similarly, use of non-selective beta-blockers was associated with an increased fall risk (HR 1.41 [95% CI 1.12; 1.78] *p*= 0.004), while statin use was associated with a lower fall risk (HR 0.81 [95% CI 0.71; 0.94] *p*= 0.004) (Table 3, model 3). Use of antihypertensive medication overall or any of the other cardiovascular medication groups was not significantly associated with fall incidents (Table 3, model 2). In addition, the use of 'other' analgesics was associated with an increased fall risk, HR= 1.45 (95% CI 1.00; 2.11) *p*= 0.049) (Table 3, model 3). This 'other' analgesics group included the non-opioid analgesics, covering the Anatomical Therapeutic Chemical (ATC) codes N02B and N02C. With regard to psychotropic drugs, the use of benzodiazepines was associated with an increased risk (HR 1.32 [95% CI 1.02; 1.71] *p*= 0.034) (Table 3, model 3). Likewise, antidepressant use was associated with an increased risk (HR 1.31 [95% CI 1.00; 1.70] *p*= 0.046) (Table 3, model 3). No other significant associations were observed between the use of psychotropic mediation or any of the other medication groups and fall incidents (Table 3, model 2 and 3).

Table 2. Baseline characteristics of the study population and subdivided in those who did and did not encountered a fall during follow-up.

Characteristic	A. Study cohort (N= 2,407)	B. Fall cases (N=1,147)	C. Non- fallers (N=1,260)	Comparison B vs. C p-value
Age (years)[a]	74.0 (6.4)	74.4 (6.7)	73.7 (6.1)	0.003*
Sex (women, %)	49.1	53.8	44.8	<0.001*
Study centre (%)				0.001*
Erasmus MC	47.1	43.8	50.2	
VUmc	27.0	30.3	24.1	
Wageningen UR	25.8	26.0	25.7	
History of falls (% yes)				<0.001*
No falls	67.9	55.8	78.6	
1 fall	20.4	25.6	15.8	
>1 fall	11.7	18.6	5.5	
Walking aid (% yes)	13.6	15.1	12.2	0.040*
BMI (kg/m^2)[a]	27.1 (4.0)	26.9 (4.0)	27.3 (4.0)	0.400
Smoking (%)				0.112
Never	33.9	35.3	32.7	
Past	56.8	56.6	57.0	
Current	9.3	8.1	10.3	
Alcohol use (%)				0.887
Light	67.3	67.8	66.9	
Moderate	29.0	28.5	29.5	
Excessive	3.3	3.4	3.3	
Very excessive	0.3	0.3	0.4	
MMSE score[b]	28 [27-29]	29 [27-29]	28 [27-29]	0.005*
GDS score[b]	1[0-2]	1[0-2]	1[0-2]	0.010*
Diabetes (% yes)	10.5	10.3	10.7	0.779
Hypertension (% yes)	63.8	64.0	63.6	0.865
Cardiovascular history (% yes)	38.3	38.9	37.9	0.643
Handgrip strength (kg)[a]	32.6 (10.7)	31.6 (10.7)	33.6 (10.6)	<0.001*
Physical performance (0-12)	9 [6-11]	9 [6-11]	9 [7-11]	0.012*
Homocysteine levels (µmol/L)[b]	14 [13-16]	14 [13-17]	14 [13-16]	0.620
Vitamin D, 25(OH)D	53 [37-71]	54 [36-72]	53 [37-70]	0.260
CKD-EPI GFR (ml/min/1.73m^2)[b]	71.4 [61.4-81.7]	70.8 [60.7-80.8]	72.2[61.7-82.4]	0.024*
Received intervention (%)	50.4	50.0	50.7	0.710

[a] Presented as mean (± standard deviation [SD]). [b] Presented as median and interquartile range [IQR]. *P-value <0.05.
Erasmus MC= Erasmus Medical Centre, VUmc= Vrije Universiteit Medical Centre, Wageningen UR= Wageningen University and Research centre, BMI= body mass index, MMSE= Mini-Mental State Examination, GDS= Geriatric Depression Scale , 25(OH)D= 25-hydroxyvitamin D, CKD-EPI= Chronic Kidney Disease Epidemiology Collaboration, GFR= glomerular filtration rate.

Table 3. Associations between the use of fall-risk increasing drugs and fall risk.

	Number of users (%) [a]	Model 1 [b] (HR [95% CI])	Model 2 [c] (HR [95% CI])	Model 3 [d] (HR [95% CI])	p-value
Cardiovascular					
Cardiac Glycosides	42 (1.7)	0.68 (0.42; 1.09)	0.62 (0.38; 1.00)		
Anti-arrhythmic	36 (1.5)	1.59 (1.11; 2.83)	1.59 (1.10; 2.29)	1.61 (1.12; 2.32)	0.010*
Vasodilators	66(2.7)	0.98 (0.70; 1.36)	0.90 (0.64; 1.25)		
Other cardiac drugs	1 (<0.1)	;	;		
Antihypertensive overall	1224 (50.9)	0.93 (0.83; 1.04)	0.92 (0.82; 1.04)		
α- blockers	149 (6.2)	0.77 (0.60; 0.98)	0.83 (0.64; 1.07)		
β- blockers	612 (25.5)	1.01 (0.88; 1.15)	1.00 (0.88; 1.13)		
Non-selective	130 (5.4)	1.37 (1.09; 1.72)	1.36 (1.08; 1.71)	1.41 (1.12; 1.78)	0.004*
Selective	486 (20.2)	0.93 (0.80; 1.07)	0.91 (0.79; 1.05)		
α- & β-blockers	13 (0.5)	;	;		
Diuretics	574 (23.9)	1.02 (0.90; 1.17)	0.99 (0.87; 1.13)		
Thiazides	430 (17.9)	0.99 (0.86; 1.15)	0.99 (0.86; 1.15)		
Loop diuretics	96 (4.0)	1.25 (0.96; 1.61)	1.13 (0.87; 1.47)		
Calcium antagonist	300 (12.5)	0.94 (0.79; 1.10)	0.93 (0.78; 1.09)		
Renin-angiotensin agents	731 (30.4)	0.91 (0.81; 1.03)	0.93 (0.83; 1.06)		
ACE-inhibitors	349 (14.5)	0.86 (0.73; 1.00)	0.90 (0.76; 1.06)		
Angiotensin II antagonist	393 (16.4)	1.01 (0.87; 1.18)	1.00 (0.86; 1.16)		
Lipid-lowering					
Statins	527 (21.9)	0.80 (0.70; 0.92)	0.83 (0.72; 0.95)	0.81 (0.71; 0.94)	0.004*
Nervous system					
Analgesics					
Opioids	45(1.9)	1.35 (0.97; 1.90)	1.26 (0.90; 1.77)		
Others	38 (1.6)	1.65 (1.14; 2.38)	1.47 (1.01; 2.13)	1.45 (1.00; 2.11)	0.049*
Anticonvulsants	42 (1.7)	1.44 (0.95; 2.18)	1.31 (0.87; 1.98)		
Anti-Parkinson	31 (1.3)	1.26 (0.81; 1.96)	1.25 (0.80; 1.95)		
Antipsychotics	13 (0.5)	;	;		
Sedatives & hypnotics	76(3.2)	1.47 (1.11; 1.94)	1.31 (0.99; 1.74)		
Benzodiazepines	100 (4.2)	1.46 (1.14; 1.89)	1.30 (1.01; 1.69)	1.32 (1.02; 1.71)	0.034*
Antidepressants	95 (4.0)	1.39 (1.07; 1.80)	1.30 (1.00; 1.69)	1.31 (1.01; 1.70)	0.046*
TCAs	26 (1.1)	1.56 (0.98; 2.49)	1.50 (0.94; 2.40)		
SSRIs	45 (1.9)	1.39 (0.96; 1.99)	1.28 (0.89; 1.85)		
Others	25 (1.0)	1.09 (0.63; 1.90)	1.00 (0.58; 1.74)		
Cholinesterase inhibitors	4 (0.2)	;	;		
Other dementia	6 (0.2)	;	;		
Antivertigo	22 (0.9)	;	;		

Table 3. *(Continued)*

	Number of users (%) [a]	Model 1 [b] (HR [95% CI])	Model 2 [c] (HR [95% CI])	Model 3 [d] (HR [95% CI])	*p*-value
Respiratory system					
Sympathomimetics	154 (6.4)	1.20 (0.97; 1.49)	1.16 (0.93; 1.44)		
Antihistaminics	52(2.2)	1.44 (1.01; 2.05)	1.39 (0.98; 1.98)		
Miscellaneous					
Diabetic					
Insulin	52 (2.2)	1.19 (0.83; 1.71)	1.18 (0.82; 1.70)		
Oral glucose-lowering	180 (7.5)	0.94 (0.76; 1.16)	0.95 (0.77; 1.17)		
Antacids	488 (20.3)	1.11 (0.97; 1.28)	1.07 (0.93; 1.22)		
H2-receptor antagonists	28 (1.2)	0.60 (0.31; 1.16)	0.62 (0.32; 1.19)		
Proton pump inhibitors	458 (19.1)	1.15 (1.00; 1.32)	1.10 (0.96; 1.26)		
Urologicals	185 (7.7)	0.87 (0.70; 1.08)	0.94 (0.75; 1.17)		
Spasmolytics	38 (1.6)	1.52 (1.00; 2.29)	1.43 (0.95; 2.17)		
Muscle-skeletal					
Muscle relaxants	3 (0.1)	;	;		
Anti-inflammatory					
NSAIDs	70 (2.9)	1.31 (0.97; 1.78)	1.26 (0.93; 1.71)		

[a] The number of users (%) at baseline. [b] Model 1: crude model. [c] Model 2: adjusted for age, sex, intervention status, MMSE, GDS, and study centre. [d] Model 3: confounders model 2, plus the other significantly associated drugs from model 2. *P-value <0.05.
HR= hazard ratio, CI= confidence interval, ACE-inhibitors= angiotensin-converting-enzyme inhibitors, TCAs= tricyclic antidepressants, SSRIs= selective serotonin reuptake inhibitors, NSAIDs= non-steroidal anti-inflammatory drugs, MMSE= Mini-Mental State Examination, GDS= Geriatric Depression Scale

The role of physical performance, fall history and renal function parameters

Adding handgrip strength, physical performance, or both parameters to the model did not change most of the HRs substantially; only the association with non-opioid analgesics and antidepressants was affected. After adding the physical performance score to the model, the association with non-opioid analgesics lost significance (HR 1.39 [95% CI 0.95; 2.05] *p*= 0.093). Conversely, when physical performance was added to the antidepressant model, the association became stronger (HR 1.40 [95% CI 1.07; 1.82] *p*= 0.013), indicating a potentially protective effect of physical performance.

Adding fall history to the model did not substantially change the HR of any of the medication groups that were significantly associated with fall risk (supplementary Table 1). The interaction term for renal function with a medication group did not suggest effect modification for any of the medication groups (data not shown).

Additional analyses

No clear dose-response association was observed for any of the medication groups that were significantly associated with fall risk (supplementary Table 2).

The use of non-selective β-blockers – excluding timolol use – was associated with an increased fall risk (HR 1.41 [95% CI 1.03; 1.95] *p*= 0.034), while timolol use was borderline significantly associated (HR 1.37 [95% CI 0.99; 1.90] *p*= 0.060). Both associations were adjusted for age, sex, intervention status, study centre, MMSE and GDS score, and the other medication groups that were significantly associated with fall risk (data not shown).

The first 21 days of thiazide use (HR 0.89 [95% CI 0.59; 1.35] *p*= 0.588), and loop diuretic use, (HR 1.36 [95% CI 0.74; 2.48] *p*= 0.321) were not associated with an increased fall risk; both associations were adjusted for age, sex, intervention, study centre, and MMSE and GDS score.

Past use of statins was not associated with fall risk (HR 1.07 [95% CI 0.78; 1.46] *p*= 0.690). Likewise, past use of antidepressants was not associated with fall risk (HR 1.37 [95% CI 0.88; 2.14] *p*= 0.161). Both associations were adjusted for age, sex, intervention status, study centre, MMSE and GDS score, and the other medication groups that were significantly associated with fall risk (data not shown).

Discussion

Our results indicate an increased fall risk in community-dwelling older adults during the use of anti-arrhythmic medication, non-selective beta-blockers, benzodiazepines and antidepressant medication. Additionally, a decreased fall risk was observed for statin use, whereas no significant association was observed for other antihypertensive medication, including diuretics, and for non-steroidal anti-inflammatory drugs (NSAIDs).

In concordance with our results, the meta-analysis by Leipzig et al.[11] indicated an increased fall risk from anti-arrhythmic class IA drug use. However, our exposure category, was slightly different because we combined all classes of anti-arrhythmic medication. Potential adverse effects of anti-arrhythmics that may contribute to fall risk are bradycardia, hypotension or torsade de pointes.[33]

The meta-analysis by Woolcott et al.[8] did not indicate an increased fall risk for beta-blocker use, whereas two recent self-controlled case series studies did observe an increased risk.[30,31] Our results indicated an increased risk for use of non-selective beta-blocker. Previous studies did not distinguish between different types of beta-blockers, and it is unclear whether eye drops such as timolol were included. Timolol is a non-selective beta-blocker known to be able to cause systemic – adverse – effects.[34,35] Its use has been associated with syncope.[36] Timolol is regularly used in the elderly population, including our population, mainly for glaucoma or ocular hypertension.[34,35] Although timolol by itself was borderline significantly associated with an increased fall risk, the HR was similar to that for non-selective beta-blocker use, excluding timolol. Furthermore, the combination of non-selective beta-blocker use and timolol has a higher level of significance than when separated. An increased fall risk by beta-blockers may be a result of bradycardia and hypotension. However, non-selective beta-blocker use might have additional adverse effects by which they may increase the fall risk. For example, sotalol also exhibits class III anti-arrhythmic properties, which is associated

with torsade de pointes,[33] and propranolol use may result in central nervous system adverse effects, such as dizziness and insomnia, due to its lipophilic properties.[37]

The use of antihypertensive medication overall has been indicated to increase fall risk.[8,30] Similarly, diuretic use overall has been associated with increased fall risk,[11] but not consistently.[8] Recent results indicated that initiation of antihypertensives[30] and especially thiazide diuretics[30-32] increased fall risk, which is potentially attributed to an initially induced hypotensive effect that stabilizes over time.[30,32] Nevertheless, according to our additional analyses, initiation of thiazides or loop diuretics was not associated with fall risk. This discrepancy in findings might be due to our low number of users who initiated thiazides or loop diuretics.

Remarkably, our results indicated a decreased fall risk during statin use, while previously an increased risk has been proposed due to potential negative effects on muscle strength and balance. Nevertheless, no significant associations were observed.[38] A beneficial effect on fall risk might be due to the cardioprotective effects of statin use.[38-40] An opposite explanation might be confounding by indication. It is possible that more frail older individuals may not have received a statin prescription, and therefore the non-statin users had an increased fall risk. However, this is speculative, and our additional analyses investigating the association with past use of statins did not support this speculation. Because our finding is new and potential mechanisms are lacking, more research is required before firm conclusions can be drawn.

Analyses with use of 'other' analgesics, covering the non-opioids, indicated a significant association with fall risk in the first analysis, but lost significance after including physical performance. For this particular drug group, a confounding effect is more likely than a mediating effect. Use of non-opioid analgesics may reflect an impaired overall health status, including physical performance state, and may thereby be related to fall risk. Previously, Leipzig et al.[11] also did not observe an association between use of non-opioid analgesics and fall risk.

The use of sedatives and hypnotics, and especially benzodiazepines, has been consistently shown to increase fall risk.[8,9,12-14] Benzodiazepine use could influence fall risk in several ways, by negatively affecting balance, gait and cognition.[9] In addition, it may induce hyponatremia,[41] which is also associated with falls.[42,43]

In line with previous findings,[8,9,32,44] we observed an increased fall risk for antidepressant medication use overall, while no significant association with its subgroups were seen. This may be due to low numbers of users in the subgroups. Antidepressant use has been proposed to affect fall risk in several ways including by inducing sedation, impaired sleep and balance, slower gait speed and reaction time, and orthostatic hypotension.[44-46] Nevertheless, it is difficult to separate the effect from depression itself, as that could result in falls via similar mechanisms.[44-46] However, our additional analyses did not indicate an increased fall risk for past users of antidepressants. In addition, two self-controlled case-series studies also reported an increased risk, and this method is less subject to confounding by indication.[46,47] Furthermore, a cohort study investigating the association with both depression and

antidepressants concluded that both aspects contributed to fall risk.[44] Thus, our result strengthens the evidence for an increased fall risk during antidepressant use.

This study has several limitations. First, the studied population included participants with slightly elevated homocysteine levels at baseline. Therefore, our results cannot be extrapolated to the general ambulant older population. Nevertheless, it is questionable whether the observed association would be different in populations that also included participants with lower homocysteine levels, as there is, to our knowledge, no association between homocysteine levels and medication-related falls. Second, half of the participants received folic acid and vitamin B_{12}, and all participants received vitamin D supplementation during their follow-up. However, no differences were observed between the intervention and placebo groups regarding the time to the first fall or second fall and the number of falls experienced during follow-up.[16] With respect to the vitamin D supplementation, this has been suggested to reduce fall risk, though evidence is inconsistent.[48] Because all participants received supplementation, and we do not expect interference with medication-related falls, we do not think this affected our results. Third, falls were self-reported in a weekly fashion, thereby the fall week was known instead of the exact fall date. However, we do not think that this minimal random misclassification in timeframe has altered the results. Fourth, confounding by indication or contra-indication could not be investigated thoroughly in our study and could have affected our results. Finally, based on the number of medication groups investigated, a chance finding could have occurred. Although there are limitations, our study has major strengths. It investigated a large community-dwelling population in which a wide range of health status determinants were assessed. Furthermore, falls were recorded prospectively, and medication use was determined using pharmacy dispensing records, making it possible to determine medication use at time of the fall. Thereby, we could more closely approach the true association between medication use and fall incidents, compared with cross-sectional studies.

Conclusion and perspective

Overall, our results strengthen the evidence for an increased fall risk in community-dwelling older individuals during the use of anti-arrhythmic medication, non-selective beta-blockers, benzodiazepines and antidepressant medication. Although medication use is a potentially modifiable fall-risk factor, single interventions targeting reductions in the number or doses of medications are limited, though more studies focused on fall prevention using medication reviews to modify prescription. Fall rate could be reduced by such interventions, but results regarding fall risk reduction are modest,[48,49] which is partly attributed to the complexity of dose reduction or stopping medication and the possible reintroduction of medication use after stopping.[49] Nevertheless, clinicians should be aware of drugs associated with fall risk during prescription, and consider the risk-benefit balance. If available, safer alternatives should be recommended.

Acknowledgments

2

Supplementary material

Table 1. Significant associations between the use of fall-risk increasing drugs and fall risk, additionally adjusted for 'history of falls.'

	Number of users (%) [a]	Model 1 [b] (HR [95% CI])	Model 2 [c] (HR [95% CI])	Model 3 [d] (HR [95% CI])	Model 4 [e]
Antiarrhythmic	36 (1.5)	1.59 (1.11; 2.83)	1.59 (1.10; 2.29)	1.61 (1.12; 2.32)	p= 0.010*	1.63 (1.13; 2.34)*
Non-selective β-blockers	130 (5.4)	1.37 (1.09; 1.72)	1.36 (1.08; 1.71)	1.41 (1.12; 1.78)	p= 0.003*	1.41 (1.12; 178)*
Statins	527 (21.9)	0.80 (0.70; 0.92)	0.83 (0.72; 0.95)	0.81 (0.71; 0.94)	p= 0.004*	0.82 (0.71; 0.94)*
Benzodiazepines	100 (4.2)	1.46 (1.14; 1.89)	1.30 (1.01; 1.69)	1.32 (1.02; 1.71)	p= 0.033*	1.31 (1.02; 1.70)*
Antidepressants	95 (4.0)	1.39 (1.07; 1.80)	1.30 (1.00; 1.69)	1.31 (1.00; 1.70)	p= 0.046*	1.40 (1.07; 1.80) [f]*

[a] The number of users (%) at baseline. [b] Model 1: crude model. [c] Model 2: adjusted for age, sex, intervention status, MMSE, GDS, and study centre. [d] Model 3: confounders model 2, plus the other significantly associated drugs from model 2. [e] Model 4: confounders model 3, plus 'history of falls.' [f] Model 4: confounders model 3, plus 'history of falls' and physical performance. *P-value <0.05.
HR= hazard ratio, CI= confidence interval, MMSE= Mini-Mental State Examination, GDS= Geriatric Depression Scale.

Table 2. Dose-response relationships for medication groups that were significantly associated with fall risk.

	Number of users [a]	Model 1 [b] (HR [95% CI])	Model 2 [c] (HR [95% CI])	Model 3 [d] (HR [95% CI])	*p*-value
Antiarrhythmic					
≤ median (0.75 DDD)	15	1.60 (0.96; 2.66)	1.49 (0.89; 2.48)	1.47 (0.88; 2.46)	0.137
> median (0.75 DDD)	15	1.58 (0.95; 2.63)	1.70 (1.02; 2.84)	1.78 (1.07; 2.97)	0.027*
Non-selective β-blocker					
≤ median (0.50 DDD)	20	1.34 (0.86; 2.08)	1.38 (0.89; 2.15)	1.45 (0.93; 2.26)	0.103
> median (0.50 DDD)	20	1.35 (0.86; 2.13)	1.35 (0.85; 2.12)	1.40 (0.89; 2.22)	0.144
Statins					
< median (1.00 DDD)	110	0.80 (0.66; 0.97)	0.82 (0.67; 0.99)	0.80 (0.66; 0.98)	0.029
≥ median (1.00 DDD)	143	0.80 (0.67; 0.96)	0.83 (0.70; 0.99)	0.82 (0.69; 0.99)	0.033
Benzodiazepines					
≤ median (0.50 DDD)	38	1.48 (0.11; 2.04)	1.30 (0.94; 1.80)	1.30 (0.94; 1.81)	0.116
> median (0.50 DDD)	24	1.40 (0.93; 2.09)	1.27 (0.85; 1.91)	1.32 (0.88; 1.98)	0.186
Antidepressants					
< median (1.00 DDD)	33	1.61 (1.13; 2.29)	1.52 (1.06; 2.16)	1.59 (1.11; 2.26)	0.011 [e]*
≥ median (1.00 DDD)	29	1.21 (0.84; 1.75)	1.12 (0.77; 1.63)	1.23 (0.85; 1.79)	0.272 [e]

[a] The number of cases using the medication group. [b] Model 1: crude model. [c] Model 2: adjusted for age, sex, intervention status, MMSE, GDS, and study centre. [d] Model 3: confounders model 2, plus the other significantly associated drugs from model 2. [e] Model 3: confounders model 2, plus physical performance. *P-value <0.05.
HR= hazard ratio, CI= confidence interval, DDD= defined daily dose, MMSE= Mini-Mental State Examination, GDS= Geriatric Depression Scale.

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2.2

CYP2C9 genotypes modify benzodiazepine-related fall risk; original results from three studies with meta-analyses

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Abstract

Objective
To investigate whether the CYP2C9*2 and *3 variants modify benzodiazepine-related fall risk.

Design
Three prospective studies; the Rotterdam Study, B-PROOF and LASA.

Setting
Community-dwelling individuals living in or near five Dutch cities.

Participants
There were 11,485 participants aged ≥55 years.

Measurements
Fall incidents were recorded prospectively. Benzodiazepine use was determined using pharmacy dispensing records or interviews. Cox proportional hazard models adjusted for age and sex were applied to determine the association between benzodiazepine use and fall risk stratified for CYP2C9 genotype and comparing benzodiazepine users to non-users. The results of the three studies were combined applying meta-analysis. Within benzodiazepine users the association between genotypes and fall risk was also assessed.

Results
Three thousand seven hundred five participants (32%) encountered a fall during 91,996 follow-up years, and 4-15% – depending on the study population – used benzodiazepines. CYP2C9 variants had frequencies of 13% for the *2 allele and 6% for the *3 allele. Compared to non-users, current benzodiazepine use was associated with an 18% to 36% increased fall risk across studies with a combined hazard ratio (HR)= 1.26 (95% confidence interval [CI], 1.13; 1.40). CYP2C9*2 or *3 allele variants modified benzodiazepine-related fall risk. Compared to non-users, those carrying a CYP2C9*2 or *3 allele and using benzodiazepines had a 45% increased fall risk (HR, 1.45 (95% CI 1.21; 1.73)), whereas CYP2C9*1 homozygotes using benzodiazepines had no increased fall risk (HR, 1.14 (95% CI 0.90; 1.45)). Within benzodiazepine users, having a CYP2C9*2 or *3 allele was associated with an increased fall risk, HR, 1.35 (95% CI 1.06; 1.72). Additionally, we observed an allele dose effect, heterozygous allele carriers had a fall risk of (HR= 1.30 95% CI 1.05; 1.61), and homozygous allele carriers of (HR= 1.91 95% CI 1.23; 2.96).

Conclusions

CYP2C9*2 and *3 allele variants modify benzodiazepine-related fall risk. Those using benzodiazepines and having reduced CYP2C9 enzyme activity – based on their genotype – are at increased fall risk. In clinical practice, genotyping might be considered for elderly patients with an indication for benzodiazepine use. However, since the exact role of CYP2C9 in benzodiazepine metabolism is still unclear, additional research is warranted.

2

Introduction

In older adults, fall incidents form a significant health problem, as approximately one third yearly encounters at least one fall.[1] Fall incidents frequently result in morbidity and mortality, and they are associated with reduced quality of life and increased health care costs.[2-5] There are multiple risk factors for falling, including benzodiazepine use.[6-8] Benzodiazepine use may affect fall risk by inducing sedation, dizziness and balance problems.[9,10] However, pharmacological effects of benzodiazepines vary between individuals. This may be due to varying plasma drug concentrations, which are potentially influenced by genetic variation.[11] Moreover, previous studies observed an increased fall risk with increasing benzodiazepine dosages.[12-14]

Drug metabolism is of importance for plasma drug concentrations. Metabolism of most benzodiazepines consists of two phases; first, oxidation and second, conjugation to glucuronide, which is excreted via the urine. The benzodiazepines oxazepam, temazepam and lorazepam can, however, be directly conjugated.[15] The oxidation phase is primarily catalyzed by liver enzymes from the Cytochrome P450 (CYP) 3A family, isoenzyme 2C19,[11,15,16] and CYP2C9 may also be involved.[11,17] Genetic polymorphisms encoding altered enzyme activity may thereby influence drug concentrations, and potentially, subsequent fall risk. Previously, in a pilot study we investigated the association between benzodiazepine-related fall risk and genetic polymorphisms CYP3A4*1b, CYP3A5*3, CYP3A7*1c, CYP2C9*2, CYP2C9*3 and CYP2C19*2. Interestingly, only CYP2C9*2 and *3 allele carriership was associated with an increased benzodiazepine-related fall risk.[18] These polymorphisms both result in a decreased enzyme activity.[19,20] Therefore, our current objective was to confirm this finding by replication in two independent studies; B-PROOF (B-vitamins for the PRevention Of Osteoporotic Fractures) and LASA (Longitudinal Aging Study Amsterdam). Furthermore, we investigated replication within the Rotterdam Study (original discovery) cohort, but after a longer follow-up time. All three studies included community-dwelling older individuals.

Methods

Study population and setting

Data from three independent Dutch studies were used, the Rotterdam Study, B-PROOF, and LASA.

The Rotterdam Study is an ongoing prospective population-based cohort, executed within Rotterdam. Previously, its design, objectives and methods have been described in detail.[21,22] For the current study, participants with pharmacy dispensing data and validated fall data were included, covering a study period from May 1991 until December 2010, including up to five measurement rounds.

B-PROOF is a multi-centre, randomized, placebo-controlled, double-blind trial investigating the efficacy of vitamin B_{12} and folic acid supplementation on the prevention

of fracture incidence in persons aged ≥65 years and having homocysteine levels of 12 to 50 μmol/L. The study was executed from 2008 until 2013, and participants participated for 2 to 3 years.[23] Previous results indicated no effect of the intervention on the time to the first or second fall, and on the number of falls encountered during the study.[24] Therefore, a cohort study design was used in the current study.

LASA is an ongoing prospective population-based cohort study started in 1991 and executed in the surrounding of Amsterdam, Zwolle and Oss. Its design, objectives and follow-up measurement procedures have been described previously.[25,26] For the current study, data from the fall follow-up study were used. This study was embedded within LASA and included those who participated from wave C (1995/1996) until wave D (1998/1999), and who were ≥65 years on the first of January 1996, community-dwelling, and participated in the data examination visit.[27]

All three studies were approved by a Medical Ethics Committee and all participants gave written informed consent.[21-23,25-27]

Fall incidents

In the Rotterdam Study, 'serious falls' were defined as 'a fall leading to a hospital admission or leading to a fracture'. Serious falls data were obtained from a computerized reporting system of the general practitioners within the Rotterdam Study. Additionally, participant data were linked to the Dutch National Morbidity Registration (LMR), containing information of all hospital admissions. Two members of the research team coded the serious falls and data were completed until 2010. The first serious fall date was defined as the index date and participants were followed until their first serious fall, death or the end of the study period, whichever came first.

In B-PROOF and LASA, a fall incident was defined as 'an unintentional change in position resulting in coming to a rest at a lower level or on the ground'.[28] Participants reported falls weekly on a calendar.[29,30] In B-PROOF, the Thursday in the first fall week was defined as the index date. Participants were followed until their first fall-incident date, drop-out date or last calendar date, date of death, or the end of the study, whichever came first.[30] In LASA, the first fall week was defined as the index date. Participants were followed until their first fall-incident week, first missing calendar, death, or the end of the study period (wave D, 1998/1999), whichever came first.[29]

Benzodiazepine use

Benzodiazepine use was defined according to the Anatomical Therapeutic Chemical codes,[31] 'N05BA' for anxiolytics, or 'N05CD' for hypnotics.

In the Rotterdam Study, benzodiazepine use was based on pharmacy dispensing records. These records from the regional pharmacies were available from January 1st 1991 onwards. Over 95% of the participants fill their drug prescriptions at one of these pharmacies. The records include date of dispensing, total number of drug units per dispensing, prescribed daily number of units, product name of the drugs and corresponding Anatomical Therapeutic

Chemical code. Current medication use was defined as use at the time of the fall (on the index date). Past use was defined as use ending prior to the index date, taking into account potential carry-over effects of one week. To investigate a dose-response relation, the average prescribed daily dose was expressed in standardized defined daily doses (DDDs).[31] To avoid potential misclassification of exposure, we ensured that all participants had pharmacy dispensing records for at least four months prior to their study start.

In B-PROOF, benzodiazepine use was also based on pharmacy dispensing records. These records were obtained from the Dutch Foundation for Pharmaceutical Statistics (SFK), as previously described.[30] Data were available for participants throughout their study period. The same definition as in the Rotterdam Study for current and past use, and dosages was used.

In LASA, benzodiazepine use was determined at the medical interview. Participants were asked to bring their medication containers to the interview.[27] Current use was defined as use at the start of their fall follow-up. Past use was defined as use at the previous study visit and no current use. No specific dose information was available.

Covariables

Characteristics including age, sex, walking aid use, fall history, smoking habits, alcohol consumption, and diabetes were assessed using a questionnaire.[21,23,27] During study visits, various characteristics were measured including weight, height, blood pressure, depressive symptoms and cognitive status. Additionally, serum creatinine levels were determined.[21,23,32] As physical function measures, lower-limb disability scores were determined in the Rotterdam Study,[33,34] whereas in B-PROOF and LASA physical performance scores and handgrip strength were assessed.[27,35,36] Ethnicity was self-reported using a questionnaire in all three studies,[22,23,25] though in B-PROOF ethnicity was assessed using the genetic data when available. A detailed description of the covariable assessment is presented in the supplement.

Genotyping

In the Rotterdam Study, genotyping of CYP2C9*2 (rs1799853) and CYP2C9*3 (rs1057910) allele variants was done using a validated polymerase chain reaction and restriction enzyme digestion analysis.[37,38] The reference group was defined as those being homozygous for the major allele (*1/*1), i.e., the absence of a CYP2C9*2 or CYP2C9*3 allele.

In B-PROOF, both variants were determined based on the Illumina-Omni express array and imputations to 1000 Genomes Project (PhaseIv3, March 2012) reference set.[39] The imputation quality of both variants was >0.99. The imputations were only done for Caucasians. In LASA, the variants were directly genotyped using the Illumina HumanOmni2.5Exome- 8 BeadChip array.

The three methods were validated in the Rotterdam Study, the concordances for CYP2C9*3 was >99%. For CYP2C9*2 exome chip data were lacking, but concordance between genotyping and imputation to 1000 Genomes Project data was >99%.

Statistical analyses

Baseline characteristics were determined for fallers and non-fallers. Differences between groups were tested using a t-test for continuous variables and a Chi-square test for categorical variables. Non-normally distributed variables were tested using a Mann-Whitney U test. Deviation from Hardy-Weinberg equilibrium was tested using a Chi-square test for the allele frequencies.

Cox proportional hazards models were used to calculate the fall hazard ratios (HRs) for benzodiazepine use compared to non-benzodiazepine use. In the Rotterdam Study and B-PROOF the model compares the prevalence of exposure to benzodiazepines in the incident fall cases on the index date with the exposure prevalence in all other participants in the cohort on that date.[40] The models were adjusted for age and sex (model 1). Covariables that significantly differed between fallers and non-fallers at baseline were included in the model when they changed the HR >10% (model 2). Additionally, subgroup analyses were done for anxiolytic and hypnotic drug use. In the Rotterdam Study and B-PROOF, a dose-response relation was also investigated. Dose categories were made according to median number of prescribed DDDs. We assessed the role of CYP2C9*1, *2 and *3 genotypes within the association between benzodiazepine use and fall risk, by stratifying on genotypes per study population. Furthermore, 'the Synergy Index' (SI) was calculated, which is a ratio measure for assessing relative excess risk due to interaction between two factors.[41] Those without benzodiazepine use and without a variant allele functioned as reference group, and the risk for the presence of one of the factors (benzodiazepine use or carriership of an allele variant) or both factors (benzodiazepine use and carriership of an allele variant) was calculated, also using Cox proportional hazards models. In addition, the association between genotypes and fall risk was investigated within benzodiazepine users, to account for potential confounding by indication. To combine the results of the three studies, a meta-analysis was done. The effect estimates – beta's – and their standard errors were used to calculate the overall effect, and to investigate the heterogeneity between the studies. Meta-analyses were done using the R package 'rmeta' applying a random effect model, R version 3.0.3. All other statistical analyses were done using the statistical software package SPSS version 21.0 (IBM, Armonk, NY, USA) and p-values <0.05 were considered to be statistically significant.

Additional analyses

We reassessed the negative findings of the other genetic variants – CYP3A4*1b, CYP3A5*3, CYP3A7*1c, CYP2C19*2 – applying the same method as for the other variants, but only within the Rotterdam Study and B-PROOF, since these variants were not available in LASA. The genotyping of these variants is described in the supplement.

Results

Study population
In the Rotterdam Study, data on fall incidents and medication use was available for 7,662 participants and of these, 6,368 participants had CYP2C9*2 and CYP2C9*3 data. In B-PROOF, fall and medication data were available for N= 2,407, and genetic data for N= 2,135. In LASA the numbers were N= 1,416 and N= 938, respectively (supplementary Figure 1). Some of the baseline characteristics between participants with and without genetic data differed slightly, which are depicted in supplementary Table 1 for all three study populations. In Table 1, the population characteristics of the three study populations (including those with medication and fall data) are depicted for fallers and non-fallers during follow-up. This indicated that fallers were more likely to be older, of female gender, have a history of falls, use a walking aid, have depressive symptoms, have poorer physical function parameters, and to have reduced kidney function. The total median follow-up time was 11.4 years with an inter quartile range (IQR) of 5.1 to 17.9 years (Rotterdam Study), 1.7 years (0.6-3.0 [LASA]) and 1.8 years (0.5-2.0 [B-PROOF]).

Benzodiazepine use and fall risk
At baseline, 11.5% (Rotterdam Study), 4.2% (B-PROOF), and 14.9% (LASA) of the participants used a benzodiazepine. In all three studies, current benzodiazepine use – compared to non-use – was associated with an increased fall risk, the combined risk of the studies was HR= 1.26 (95% CI, 1.13; 1.40; p= $1.91*10^{-5}$) (Table 2). Past use of benzodiazepines was also associated with an increased fall risk, combined HR= 1.10 (95% CI, 1.00; 1.20; p= 0.04) (Table 2). In all three studies, the hazard ratios were adjusted for age and sex, as the other considered covariates (including the fall-risk factors described previously) did not change the HR by more than 10%, and thereby did not act as a confounding factor.

Dose-response relation
The meta-analyses indicated no dose-response associations for anxiolytic use (supplementary Table 2). For hypnotics, those using a dose below the median of 1.0DDD compared to non-use had a fall risk of HR= 1.14 (95% CI, 0.87; 1.48; p= 0.34), whereas those using a dose ≥1.0DDD had a risk of HR= 1.33 (95% CI, 1.10; 1.59; p= 0.003) (supplementary Table 2).

Genotypes and benzodiazepine-related fall risk
Genotypes and allele frequencies of CYP2C9 *1, *2, and *3 variants are provided in supplementary Table 3. Genotypes for both variants were in Hardy-Weinberg equilibrium in the studies. Participants having one or two variant alleles (*2 or *3) were clustered, as the allele frequencies were relatively low, resulting in stratified analyses for those having no variant alleles (subjects homozygous for *1/*1) versus those having at least one variant allele. The results stratified for CYP2C9*2 and CYP2C9*3 genotypes are provided in supplementary Table 4. The meta-analysis indicated that the fall risk of those carrying a CYP2C9*2 allele

Table 1. Baseline characteristics of fall cases and non-fallers during follow-up for all three study populations.

	the Rotterdam Study		B-PROOF		LASA	
	Fall cases N= 1,770	Non-fallers N= 5,892	Fall cases N= 1,147	Non-fallers N= 1,260	Fall cases N= 788	Non-fallers N= 628
Age*	71.6 (9.3)	69.7 (9.5)§	74.4 (6.7)	73.7 (6.1)§	76.0 (6.7)	74.9 (6.3)§
Female gender†	1,360 (76.8)	3,270 (55.5)§	617 (53.8)	564 (44.8)§	449 (57.0)	282 (44.9)§
Caucasian ethnicity†	1,640 (92.7)	5,477 (93.0)	1,080 (94.2)	1,186 (94.1)	785 (99.6)	620 (98.7)
BMI*	26.3 (3.7)	26.3 (3.7)	26.9 (4.0)	27.3 (4.0)	27.0 (4.3)	26.9 (4.2)
History of falls (yes)†‖	392 (22.6)	907 (15.7)§	399 (44.2)	217 (21.4)§	322 (41.0)	131 (20.9)§
Walking aid use (yes)†	215 (13.2)	591 (10.7)§	172 (15.1)	153 (12.2)§	135 (18.6)	71 (12.0)§
MMSE score‡	28 [26-29]	28 [26-29]	29 [27-29]	28 [27-29]§	28 [26-29]	28 [26-29]
Depressive symptoms (yes)†¶	153 (13.6)	339 (9.0)§	90 (7.9)	65 (5.2)§	128 (16.8)	80 (13.0)
Hypertension (yes)†**	955 (58.2)	3077 (56.9)	599 (64.0)	670 (63.6)	487 (63.7)	446 (71.8)§
Diabetes (yes)†	115 (6.7)	378 (6.6)	93 (10.3)	109 (10.7)	66 (8.4)	55 (8.8)
Alcohol intake						
g/day‡	2.4 [0.1-13.2]	3.9 [0.2-15.2]§	-	-	-	-
light†	-	-	778 (67.8)	842 (66.9)	590 (75.2)	471 (75.0)
moderate†	-	-	327 (28.5)	371 (29.5)	158 (20.1)	113 (18.0)
excessive†	-	-	42 (3.7)	46 (3.7)	37 (4.7)	44 (7.0)
Current smoking†	351 (20.4)	1345 (23.5)§	93 (8.1)	130 (10.3)	129 (16.4)	127 (20.2)
Lower-limb disability (yes)†	601 (36.4)	1624 (29.4)§	-	-	-	-
Physical performance score‡	-	-	9 [6-11]	9 [7-11]§	8 [7-10]	8 [6-9]§
Handgrip strength (kg)‡	-	-	29 [24-40]	33 [25-42]§	26 [21-35]	30 [22-40]§
eGFR (ml/min/1.73m²)‡	71.7 [61.7-81.5]	72.7 [62.2-82.7]§	70.8 [60.7-80.8]	72.2 [61.7-82.4]§	62.2 [52.2-70.7]	63.3 [55.9-71.8]§

The numbers presented are based on the valid number of included fall cases and non-fallers. *Presented as mean (±SD). †Presented as N (%). ‡Presented as median [IQR]. §Differences between fall cases and non-fallers within a study population with a p-value <0.05. ‖Fall history concerns falls in the last month for the Rotterdam Study and falls in the preceding year in B-PROOF and LASA. ¶Clinically relevant depressive symptoms were bases on CES-D scores ≥16[2,43], HADS-D scores ≥9,[44] or GDS scores ≥5.[45] **Hypertension was defined as systolic blood pressure >140mmHg and/or diastolic blood pressure >90 mmHg.[46] BMI= body mass index, MMSE= mini-mental state examination, the eGFR is based on the chronic kidney disease epidemiology collaboration formula.

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Table 2. Association between benzodiazepine use, and its subgroups anxiolytic and hypnotic use, and fall risk. Hazard ratios and 95% confidence intervals (CI) are presented.

	Meta-analysis[†]		the Rotterdam Study			B-PROOF			LASA		
	Nr of cases[*]	Model 1[§]	Nr of cases[‡]	Crude	Model 1[§]	Nr of cases[‡]	Crude	Model 1[§]	Nr of cases[‡]	Crude	Model 1[§]
Benzodiazepine											
Non use	2,444	Ref.	828	Ref.	Ref.	1,000	Ref.	Ref.	616	Ref.	Ref.
Past	814	1.10 (1.00; 1.20)	697	1.32 (1.20; 1.47)	1.10 (0.99; 1.22)	84	1.16 (0.92; 1.45)	1.07 (0.85; 1.35)	33	1.28 (0.90; 1.81)	1.15 (0.80; 1.63)
Current	447	1.26 (1.13; 1.40)	245	1.74 (1.51; 2.01)	1.18 (1.02; 1.36)	63	1.48 (1.14; 1.90)	1.34 (1.04; 1.73)	139	1.52 (1.26; 1.83)	1.36 (1.12; 1.64)
Anxiolytic											
Non use	2,706	Ref.	1,042	Ref.	Ref.	1,031	Ref.	Ref.	633	Ref.	Ref.
Past	628	1.07 (0.97; 1.18)	547	1.20 (1.08; 1.33)	1.06 (0.95; 1.18)	60	1.19 (0.92; 1.55)	1.10 (0.85; 1.45)	21	1.27 (0.82; 1.96)	1.13 (0.73; 1.74)
Current	196	1.19 (1.02; 1.37)	104	1.43 (1.17; 1.76)	1.12 (0.92; 1.37)	28	1.30 (0.90; 1.90)	1.20 (0.82; 1.74)	64	1.46 (1.13; 1.89)	1.30 (0.99; 1.69)
Hypnotic											
Non use	2,783	Ref.	1,107	Ref.	Ref.	1,046	Ref.	Ref.	630	Ref.	Ref.
Past	501	1.13 (1.02; 1.26)	443	1.41 (1.26; 1.58)	1.14 (1.02; 1.27)	38	1.22 (0.88; 1.69)	1.13 (0.81; 1.56)	20	1.48 (0.95; 2.32)	1.34 (0.86; 2.10)
Current	294	1.32 (1.17; 1.50)	167	1.91 (1.63; 2.25)	1.24 (1.05; 1.46)	37	1.61 (1.16; 2.24)	1.45 (1.04; 2.02)	90	1.60 (1.28; 2.00)	1.42 (1.13; 1.78)

[*]Total number of fall cases per group of the Rotterdam Study, B-PROOF, and LASA combined. [†]Meta-analyses results of the Rotterdam Study, B-PROOF, and LASA. [‡]Number of fall cases per group. [§]Model 1: age, sex.

was similar to the fall risk of those carrying a CYP2C9*3 allele. Therefore, the genotypes for both variant alleles were combined into a "variant genotype" group (as compared to the homozygous *1/*1 "wild type" genotype group). Additionally, a heterozygous (i.e., *1/*2 and *1/*3) and homozygous (i.e., *2/*2, *2/*3, and *3/*3) group was composed of the genotypes.

The stratified results for the combined genotypes of CYP2C9*2 and *3 are provided in Table 3. The meta-analysis indicated that in those carrying no variant CYP2C9 alleles (*1/*1), the fall risk of current benzodiazepine use (n= 204) compared to non-use (n= 1,752) was HR= 1.14 (95% CI, 0.90; 1.45; p= 0.29). In comparison, the fall risk in those carrying at least one variant CYP2C9*2 or *3 allele of current benzodiazepine use (n= 150) compared to non-use (n= 1,224) was HR= 1.45 (95% CI, 1.21; 1.73; p= 4.98*10^{-5}). When investigating allele dose effects we observed that the fall risk of benzodiazepine use compared to non-use in heterozygous CYP2C9 variant allele carriers was HR= 1.42 (95% CI, 1.17; 1.72; p= 3.80*10^{-4}) and HR= 1.70 (95% CI, 0.85; 3.40; p= 0.13) in homozygous CYP2C9 variant allele carriers.

The results within benzodiazepine users are presented in Table 4 for the combined genotypes of CYP2C9*2 and CYP2C9*3. Carrying a variant *2 or *3 allele was significantly associated with an increased fall risk, HR= 1.35 (95% CI, 1.06; 1.72; p= 0.02). When investigating an allele dose effect, we observed an increased fall risk for heterozygous allele carriers, HR= 1.30 (95% CI, 1.05; 1.61; p= 0.02), and for homozygous allele carriers HR= 1.91 (95% CI, 1.23; 2.96; p= 0.004). Moreover, there was a linear trend in fall risk between heterozygous and homozygous allele carriers, HR=1.33 (95% CI, 1.07; 1.64; p=0.009).

For calculation of the Synergy Index (SI), those without benzodiazepine use and without a variant CYP2C9*2 or *3 allele functioned as reference group, the combined fall risk for benzodiazepines users was HR= 1.14 (95% CI, 0.90; 1.44; p= 0.29), and the fall risk for carriers of a *2 or *3 allele was HR= 1.03 (95% CI, 0.95; 1.11; p= 0.54), whereas the fall risk for those using benzodiazepines and carrying a *2 or *3 allele was HR= 1.51 (95% CI 1.27; 1.79; p= 2.17*10^{-6}). These risks resulted in a SI of 3.0 indicating an excess risk from exposure to both factors due to the interaction, relative to the excess risk from exposure – to both factors – without interaction.

None of the meta-analyses indicated significant heterogeneity between the studies, I^2 varied between 0-19%.

Additional analyses

Regarding the other genetic variants, the genotype distribution for all alleles were in Hardy-Weinberg equilibrium. Of the alleles analyzed only the stratification on CYP2C19*2 indicated significant differences. The meta-analyses indicated that the fall risk of benzodiazepine use compared to non-use in those carrying no variant CYP2C19 alleles (reference *1/*1 genotype) was increased: HR= 1.33 (95% CI, 1.14; 1.55; p= 3.42*10^{-4}). In comparison, the fall risk in those carrying at least one variant CYP2C19 allele was HR= 0.85 (95% CI, 0.65; 1.12: p= 0.34). Within benzodiazepine users, carriership of CYP2C19*2 alleles was inversely associated with fall risk: combined HR= 0.65 (95% CI, 0.48; 0.88; p= 0.01).

Table 3. Association between benzodiazepine use and fall risk, stratified for CYP2C9*2 / *3 genotype. Hazard ratios and 95% confidence intervals (CI) are presented.

CYP2C9*2/*3	Meta-analysis†		the Rotterdam Study			B-PROOF			LASA		
	Nr of cases*	Model 1§	Nr of cases‡	Crude	Model 1§	Nr of cases‡	Crude	Model 1§	Nr of cases‡	Crude	Model 1§
*1/*1 carriers											
Non use	1,752	Ref.	856	Ref.		634	Ref.		262	Ref.	
Current use	204	1.14 (0.90; 1.45)	114	1.33 (1.10; 1.62)	0.97 (0.80; 1.19)	30	1.23 (0.85; 1.77)	1.12 (0.78; 1.62)	60	1.60 (1.21; 2.12)	1.43 (1.06; 1.92)
Variant allele carriers (*2 or *3)‖											
Non use	1,224	Ref.	732	Ref.		327	Ref.		165	Ref.	
Current use	150	1.45 (1.21; 1.73)	83	1.97 (1.56; 2.49)	1.43 (1.12; 1.81)	27	1.92 (1.29; 2.84)	1.73 (1.16; 2.57)	40	1.50 (1.06; 2.12)	1.30 (0.91; 1.86)
Heterozygous carriers¶											
Non use	819	Ref.	384	Ref.		294	Ref.		141	Ref.	
Current use	127	1.42 (1.17; 1.72)	72	1.94 (1.51; 2.49)	1.43 (1.11; 1.85)	21	1.70 (1.09; 2.65)	1.52 (0.97; 2.38)	34	1.52 (1.04; 2.21)	1.33 (0.91; 1.97)
Homozygous carriers**											
Non use	101	Ref.	48	Ref.		29	Ref.		24	Ref.	
Current use	23	1.70 (0.85; 3.40)	11	2.20 (1.14; 4.26)	1.48 (0.76; 2.90)	6	3.44 (1.41; 8.38)	3.60 (1.41; 9.18)	6	1.37 (0.56; 3.38)	0.95 (0.37; 2.48)

*Total number of fall cases per group of the Rotterdam Study, B-PROOF, and LASA combined. †Meta-analyses results of the Rotterdam Study, B-PROOF, and LASA. ‡Number of fall cases per group. §Model 1: age, sex. ‖Variant allele carries of CYP2C9 *2 and *3, i.e. genotypes: *1/*2, *1/*3, *2/*3, *2/*2, and *3/*3. ¶Heterozygous variant allele carries of CYP2C9 *2 and *3, i.e. genotypes: *1/*2 and *1/*3. **Homozygous variant allele carries of CYP2C9 *2 and *3, i.e. genotypes: *2/*3, *2/*2, and *3/*3

Table 4. The association between CYP2C9*2 and *3 genotype, and fall risk within benzodiazepine users. Hazard ratios and 95% confidence intervals (CI) are presented.

CYP2C9*2/*3	Meta-analyses†		the Rotterdam Study			B-PROOF			LASA		
	Nr of cases*	Model 1§	Nr of cases‡	Crude	Model 1§	Nr of cases‡	Crude	Model 1§	Nr of cases‡	Crude	Model 1§
*1/*1 carriers	204	Ref.	114	Ref.		30	Ref.		60	Ref.	
Variant allele carriers (*2 or *3)‖	150	1.35 (1.06; 1.72)	83	1.50 (1.13; 1.99)	1.51 (1.13; 2.00)	27	1.63 (0.96; 2.77)	1.54 (0.90; 2.64)	40	1.03 (0.69; 1.54)	1.04 (0.69; 1.55)
Heterozygous carriers¶	127	1.30 (1.05; 1.61)	72	1.45 (1.08; 1.95)	1.44 (1.07; 1.93)	21	1.49 (0.84; 2.64)	1.39 (0.78; 2.47)	34	1.00 (0.66; 1.53)	1.01 (0.66; 1.54)
Homozygous carriers**	23	1.91 (1.23;2.96)	11	1.93 (1.04; 3.58)	2.16 (1.16; 4.02)	6	2.45 (0.98; 6.13)	2.49 (0.99; 6.25)	6	1.22 (0.53; 2.82)	1.23 (0.53; 2.84)
Hetero-, homozygous trend††		1.33 (1.07; 1.64)		1.42 (1.13; 1.79)	1.45 (1.15; 1.83)		1.54 (1.03; 2.30)	1.50 (0.99; 2.27)		1.05 (0.76; 1.46)	1.06 (0.76; 1.47)

*Total number of fall cases per group of the Rotterdam Study, B-PROOF, and LASA combined. † Meta-analyses results of the Rotterdam Study, B-PROOF, and LASA. ‡ Number of fall cases per group. § Model 1: age, sex. ‖ Variant allele carries of CYP2C9 *2 and *3, i.e. genotypes: *1/*2, *1/*3, *2/*3, *2/*2, and *3/*3. ¶ Heterozygous variant allele carries of CYP2C9 *2 and *3, i.e. genotypes: *1/*2 and *1/*3. **Homozygous variant allele carries of CYP2C9 *2 and *3, i.e. genotypes: *2/*3, *2/*2, and *3/*3. ††Linear trend analysis for hetero and homozygous variant allele carries of CYP2C9 *2 and *3.

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Discussion

Our study addresses an important and novel topic, namely the effect of genetic variation on medication-related falls. Benzodiazepine-related fall risk was significantly modified by CYP2C9 genotype. Carriers of at least one variant CYP2C9 *2 or *3 allele using benzodiazepines – compared to non-users – had a significantly increased fall risk, while users carrying no variant *2 or *3 alleles had no significantly increased fall risk. In addition, we observed an allele dose effect, since having more variant alleles was associated with a higher increased fall risk.

Both fall risk and CYP2C9 variant allele frequencies are relatively low and thus multiple and large samples are required. By including three large and independent study populations we were able to show replication and consistency of the association and gain power by performing meta-analyses, in line with a previous pilot study in one of the study populations.[18] This was not only indicated in the analyses comparing benzodiazepine users with non-users, but also within users stratified on CYP2C9 genotypes, and thereby accounting for potential confounding by indication. Moreover, we observed evidence for an allele dose effect comparing heterozygous and homozygous variant allele carriers, as the fall risk increased with increasing number of CYP2C9 variant alleles. Furthermore, the Synergy Index indicated a threefold excess risk for exposure to the combination of benzodiazepine use and *2 or *3 allele carriership, due to the interaction between both factors. Additionally, carriership of a *2 or *3 allele on itself was not associated with fall.

The percentage of benzodiazepine users differed between the three study populations (4-15%), which is probably due to the different points in time of the studies. In the Netherlands, the reimbursement policy changed in 2009 resulting in a decrease in benzodiazepine use.[47]

The CYP2C9 gene is positioned on chromosome 10 with the *2 and *3 variants located in exon 3 and 7, encoding an arginine to cysteine (*2) and isoleucine to leucine (*3) amino acid substitution respectively. Both protein variants of the CYP2C9 enzyme result in a decreased enzyme activity, and the combination of having both variant alleles exhibits the most pronounced effect.[19,20] The *2/*2 or *3/*3 genotypes are considered to result in a relatively poor metabolizer (PM) phenotype.[19,20] In this phenotype, the metabolism rate of this oxidative pathway is approximately half compared to individuals having no variant alleles (*1/*1). Additionally, variant genotypes, both heterozygous and homozygous for CYP2C9*2 and *3 has been shown to affect drug clearance, with lower clearance observed up to 90% for, for example S-acenocoumarol, S-warfarin, phenytoin, tolbutamide, ibuprofen, or fluvastatin.[19,20] Yet, the effect of the variant alleles on metabolism rate and/or total drug clearance depends on the relative contribution of the CYP2C9 dependent pathway for that particular compound. Furthermore, effect on metabolism and/or clearance could differ between CYP2C9*2 and *3 alleles and per substrate[20]. For benzodiazepines, it is yet unclear to what extent *2 and *3 alleles affect their total metabolism and/or *in vivo* clearance. To our knowledge, as of yet there is only limited evidence indicating that CYP2C9 plays a role in the metabolism of benzodiazepines, as CYP3A enzymes and 2C19 enzymes are currently

thought to be the main enzymes involved in benzodiazepine biotransformation.[11,15,16] However, *in vitro* studies using human liver microsomes indicated that CYP2C9 can catalyze the N-demethylation of diazepam,[17,48,49] flunitrazepam,[50] N-desmethyladinazolam[51] and temazepam.[17] Additionally, another study using human liver microsomes showed that CYP2C9 was involved in the oxidation and hydroxylation of quazepam.[52] A complicating factor may be that some of these biotransformations can yield pharmacologically active metabolites. In addition, CYP2C9 may be inhibited by concomitantly used drugs including amiodarone, fluvastatin and fluconazole.[19,20] We could not account for all these factors and, although we do not think that they affected our study results substantially, they should be considered in further research. Additionally, due to our sample size we were not able to perform subanalyses of different benzodiazepine classes – anxiolytics and hypnotics – or investigate individual benzodiazepines in order to assess a potential class or individual drug effect. Overall, additional research is needed to elucidate the exact role of CYP2C9 enzyme and its polymorphisms in the metabolism of benzodiazepines.

Remarkably, our additional analyses indicated an increased fall risk for subjects with benzodiazepine use in those not carrying a CYP2C19*2 allele (homozygous reference *1/*1). Like CYP2C9*2, CYP2C19*2 alleles encode for a decreased enzyme activity.[53] We do not have a clear explanation for this finding, though we hypothesize that potentially different benzodiazepines are metabolized by CYP2C9 and CYP2C19, and for those metabolized by CYP2C19 active metabolites are of more importance in fall risk than the parent drug. However, further research is necessary to elucidate this finding.

Our study has limitations: first, no benzodiazepine plasma levels were available to confirm our hypothesized mechanism. Future studies could investigate plasma levels across CYP2C9*2 and *3 genotypes, and preferably also in relation to therapeutic and adverse effects. Second, the number of fall cases per CYP2C9*2 and CYP29*3 genotype groups was limited, and the population frequency of the variant alleles is relatively low with the *3 allele being lower than the *2 allele (6% vs. 13%). We therefore combined genotypes for each variant allele since both alleles result in a decrease in enzyme activity.[19,20] In addition, the observed effect size in subjects carrying the CYP2C9*3 allele was similar to that in subjects carrying the CYP2C9*2 allele. In future studies it would be interesting to investigate both variants: *2 and *3 separately. Third, as the majority of the study population was of Caucasian ethnicity, our results cannot be extrapolated to other ethnic groups. Fourth, in the Rotterdam Study, a fall definition was used that differed from the one in the other two studies (i.e., serious fall incidents). Thereby, the total number of incident falls is likely to higher, as the noninjurious falls were not included. This may have affected the association between benzodiazepine use and falls. However, the effect sizes were relatively similar across the three studies. Additionally, differences in fall definition could affect the association between benzodiazepine use and falls when the underlying mechanism for benzodiazepine-related falls would differ between serious and less serious falls. Nevertheless, we are not aware of potentially different underlying mechanisms. Fifth, in LASA, medication data were gathered during study visits and not ascertained at the moment of the fall; consequently,

misclassification of the exposure may have occurred. By using pharmacy dispensing records in the Rotterdam Study and B-PROOF, exposure at the moment of the fall could be assessed, although these records do not necessarily reflect actual use, thus misclassification may also have occurred. Last, the B-PROOF study population was selected on having a slightly elevated homocysteine level. However, we are not aware of a mechanism through which increased homocysteine levels could have interfered with benzodiazepine-related fall risk that is modified by CYP2C9 genetic variants.

Conclusion

Taken together, our results show that CYP2C9 genotype for *2 and *3 allele variants modify benzodiazepine-related fall risk. Those using benzodiazepines and having reduced CYP2C9 enzyme activity (based on their genotype) are at increased fall risk. When the role of CYP2C9 in benzodiazepine metabolism is further revealed and verified, this drug-gene interaction may also be relevant for other unintended benzodiazepine effects. Moreover, it will be interesting to investigate whether it also affects other age-groups. For now, in clinical practice, genotyping might be considered for elderly patients with an indication for benzodiazepine use. Further research on the additional value of genotyping as a prognostic factor in the clinical-decision making process is warranted.

Acknowledgments

The authors have no potential conflicts of interest that are directly relevant to the content of this study.

Supplementary material

Methods

Covariable assessment

In the Rotterdam Study, alcohol consumption was based on food frequency data and reported in grams per day.[54] In B-PROOF and LASA, alcohol consumptions was categorized into 'light', 'moderate' and 'excessive'.[55] Diabetes was based on self-report,[21,23] though in LASA these data was validated with general practitioner data.[32] From weight and height the body mass index (BMI, kg/m^2) was calculated. Hypertension was defined as systolic blood pressure >140mmHg and/or diastolic blood pressure >90 mmHg.[46] Depressive symptoms were measured using the Center for Epidemiological Studies Depression Scale (CES-D, score range 0-60)[56] in the Rotterdam Study and LASA, and in a subsample of the Rotterdam Study also the Hospital Anxiety and Depression Scale (HADS-D, score range 0-21)[57] was used. While in B-PROOF, the 15-item version of the geriatric depression scale (GDS) was used.[58] Clinically relevant depressive symptoms were bases on CES-D scores ≥16,[42,43] HADS-D scores ≥9,[44] or GDS scores ≥5.[45] Cognitive status was measured by using the mini-mental state examination (MMSE).[59] Serum creatinine levels were used to calculate an age-adjusted estimate of the glomerular filtration rate (GFR) according to the chronic kidney disease epidemiology collaboration (CKD-EPI) formula.[60] Lower-limb disability scores were assessed using a modified version of the Stanford Health Assessment Questionnaire.[33,34] The score was based on answers to questions regarding rising, walking, bending, and getting in and out of a car. Disability was defined as a score of 3 or higher.[34] A physical performance score was calculated from the results of three physical function tests: walking test, chair stand test, and the tandem stand test.[36] The physical performance score ranged from 0 to 12 (low physical performance – high physical performance).[27,35] The maximum handgrip strength (kg) was defined as the highest results of two maximum trials per hand using a dynamometer (B-PROOF: Takei TKK 5401, LASA: Takei TKK 5001, Takei Scientific Instrument CO. Ltd.,Tokyo, Japan). For the Rotterdam Study, baseline fall history and serum creatinine levels were used, and the use of a walking aid was not assessed at the second measurement round. In addition, depressive symptoms were available from the second visit onwards. Data of the other covariables were available for all follow-up visits, but to minimize the number of missing, the values from the preceding visit were used.

Genotyping

In the Rotterdam Study, genotyping of CYP3A4*1b (rs2740574), CYP3A5*3 (rs776746), CYP3A7*1c (rs11568825), all located on chromosome 7, and CYP2C19*2 (rs4244285) located on chromosome 10, was done using a TaqMan allelic discrimination assays on a ABI Prism 9700 HT sequence detection system, as previously described.[61,62]

In B-PROOF, these variants were determined based on the Illumina-Omni express array and imputations to 1000 Genomes Project (Phaselv3, March 2012) reference set.[39] The imputation quality of all variants was >0.99.
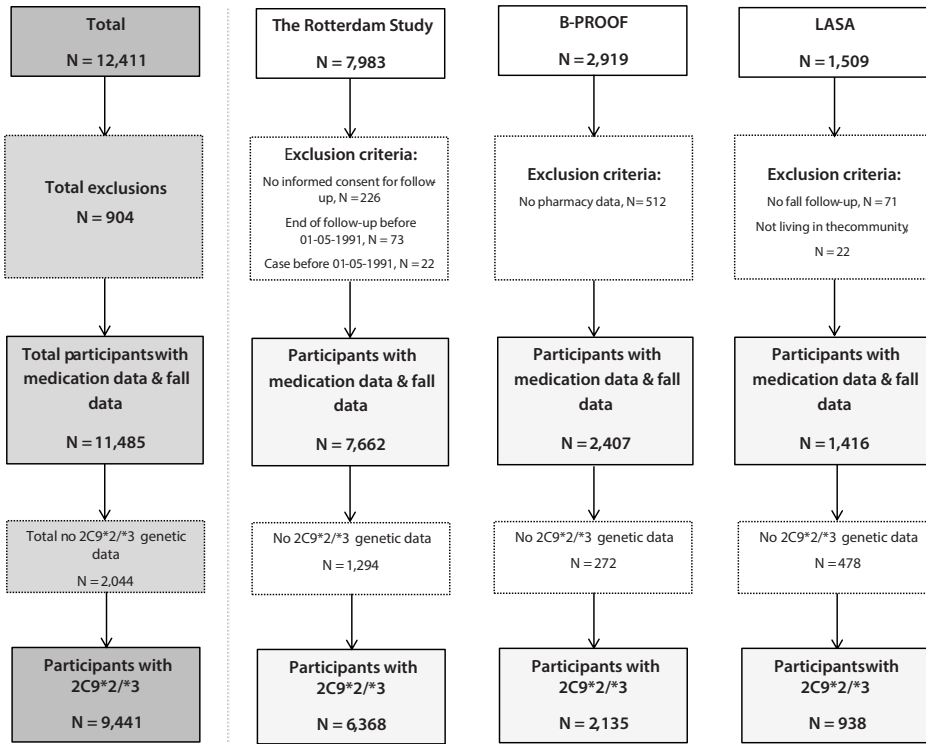

Figure 1. Flowchart of the included study populations.

Table 1. Baseline characteristics of those with and without genetic data for all three study populations.

	the Rotterdam Study		B-PROOF		LASA	
	With genetics N= 6,368	Without genetics N= 1,294	With genetics N= 2,135	Without genetics N= 272	With genetics N= 1,416	Without genetics N= 478
Age*	69.3 (9.0)	74.3 (10.5)§	74.5 (6.4)	73.9 (6.4)	75.1 (6.5)	76.4 (6.7)§
Female gender†	3751 (58.9)	879 (67.9)§	1045 (48.9)	136 (50)	487 (51.9)	244 (51.0)
Caucasian ethnicity†	5993 (94.1)	1124 (86.9)§	1516 (91.5)	272 (100)§	930 (99.1)	475 (99.4)
BMI*	26.3 (3.7)	26.3 (4.0)	27.1 (3.9)	27.1 (4.6)	26.9 (4.2)	27.1 (4.5)
History of falls (yes)†ll	1020 (16.3)	279 (22.6)§	540 (31.6)	76 (36.4)	318 (34.0)	135 (28.3)§
Walking aid use (yes)†	567 (9.4)	239 (21.5)§	292 (13.7)	33 (12.2)	117 (13.3)	89 (20.5)§
MMSE score‡	28 [27-29]	28 [26-29]§	28 [27-29]	28 [27-29]	28 [26-29]	27 [25-29]§
Depressive symptoms (yes)†¶	410 (9.5)	82 (13.7)§	139 (6.5)	16 (5.9)	134 (14.5)	74 (16.1)
Hypertension (yes)†**	3481 (56.0)	551 (65.8)§	1137 (64.0)	132 (62.0)	617 (66.8)	316 (68.4)
Diabetes (yes)†	237 (3.3)	99 (7.7)§	171 (10.0)	31 (14.9)§	71 (7.6)	50 (10.5)
Alcohol intake						
g/day‡	3.5 [0.2-14.9]	3.0 [0.0-14.8]				
light†			1426 (66.8)	194 (71.3)	683 (72.9)	378 (79.4)§
moderate†			628 (29.4)	70 (25.7)	192 (20.5)	79 (16.6)
excessive†			80 (3.7)	8 (2.9)	62 (6.6)	19 (4.0)
Current smoking†	1428 (23.0)	268 (21.9)§	198 (9.3)	25 (9.2)	182 (19.4)	74 (15.5)
Lower-limb disability (yes)†	1708 (28.2)	517 (45.9)§				
Physical performance score‡			9 [6-11]	9 [6-10]	8 [6-9]	8 [7-10]
Handgrip strength (kg)‡			31 [25-41]	30 [23-40]	28 [22-37]	26 [21-37]§
eGFR (ml/min/1.73m²)‡	72.8 [62.2-82.8]	70.4 [58.6-80.1]§	71.5 [61.5-81.8]	71.0 [60.1-80.0]	62.5 [53.6-70.4]	63.4 [54.0-73.0]

The numbers presented are based on the valid number of included fall cases and non-fallers. *Presented as mean (±SD). †Presented as N (%). ‡Presented as median [IQR]. §Differences between fall cases and non-fallers within a study population with a p-value <0.05. ll Fall history concerns falls in the last month for the Rotterdam Study and falls in the preceding year in B-PROOF and LASA. ¶Clinically relevant depressive symptoms were bases on CES-D scores ≥16,[42,43] HADS-D scores ≥9,[44] or GDS scores ≥5.[45] **Hypertension was defined as systolic blood pressure >140 mmHg and/or diastolic blood pressure >90 mmHg.[46] BMI= body mass index, MMSE= mini-mental state examination, SD= standard deviation, the eGFR is based on the chronic kidney disease epidemiology collaboration formula.

Table 2. Association between dose of anxiolytics and hypnotics use, and fall risk.

	Meta-analysis[†]		the Rotterdam Study			B-PROOF		
	Nr of cases[*]	Model 1[§]	Nr of cases[‡]	Crude	Model 1[§]	Nr of cases[‡]	Crude	Model 1[§]
Anxiolytics								
Non use	2,680	Ref.	1,589	Ref.		1,091	Ref.	
Dose ≤0.40 DDD	71	1.12 (0.89; 1.42)	56[**]	1.43 (1.09; 1.86)	1.08 (0.82; 1.41)	15	1.48 (0.89; 2.47)	1.32 (0.79; 2.19)
Dose >0.40 DDD	60	1.10 (0.85; 1.42)	47[**]	1.27 (0.95; 1.69)	1.11 (0.83; 1.48)	13	1.13 (0.65; 1.95)	1.08 (0.62; 1.86)
Dose trend[‖]		1.06 (0.95; 1.19)		1.18 (1.04; 1.34)	1.06 (0.93; 1.20)		1.13 (0.89; 1.43)	1.09 (0.86; 1.38)
Hypnotics								
Non use	2,634	Ref.	1,550	Ref.		1,084	Ref.	
Dose <1.00 DDD	80	1.14 (0.87; 1.48)	63	1.53 (1.19; 1.97)	1.05 (0.82; 1.35)	17[††]	1.61 (0.99; 2.59)	1.42 (0.88; 2.30)
Dose ≥1.00 DDD	123	1.33 (1.10; 1.59)	104	1.94 (1.59; 2.34)	1.31 (1.07; 1.60)	19[††]	1.53 (0.97; 2.41)	1.40 (0.89; 2.20)
Dose trend[¶]		1.15 (1.05; 1.25)		1.41 (1.28; 1.55)	1.13 (1.03; 1.25)		1.29 (1.05; 1.58)	1.22 (0.99; 1.50)

DDD, defined daily dose. The dose use is split on the median DDD. Hazard ratios and 95% confidence intervals (CI) are presented for the Rotterdam study and B-PROOF. [*]Total number of fall cases per group of the Rotterdam Study, and B-PROOF combined. [†]Meta-analyses results of the Rotterdam Study, and B-PROOF. [‡]Number of fall cases per group. [§]Model 1: age, sex. [‖]Linear trend analysis for the dose categories of anxiolytics. [¶]Linear trend analysis for the dose categories of hypnotics. [**]Dose of one anxiolytic users is missing. [††]Dose of one hypnotic users is missing.

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Table 3. Genotypes and allele frequencies of CYP2C9*2 and *3 in the total population, split per study population.

	the Rotterdam Study N= 7,662	B-PROOF N= 2,407	LASA N= 1,416
CYP2C9*2/*3	Tot available: 6,368[*]	Tot available: 2,135[*]	Tot available: 938[*]
*1/*1[†]	4,230 (66.4)	1,399 (65.5)	597 (63.6)
*1/*2[†]	1,320 (20.7)	435 (20.4)	195 (20.8)
*1/*3[†]	596 (9.4)	218 (10.2)	104 (11.1)
*2/*3[†]	92 (1.4)	34 (1.6)	14 (1.5)
*2/*2[†]	104 (1.6)	41 (1.9)	26 (2.8)
*3/*3[†]	26 (0.4)	8 (0.4)	2 (0.2)
Allele frequency *2 (%)	13	13	14
Allele frequency *3 (%)	6	6	7

[*]Total number of participants with genotype data available. [†]Presented as N (%).

Table 4. Association between benzodiazepine use and fall risk, stratified for CYP2C9*2 and CYP2C9*3 genotype.

CYP2C9*2/*3	Meta-analysis†		the Rotterdam Study			B-PROOF			LASA		
	Nr of cases*	Model 1§	Nr of cases‡	Crude	Model 1§	Nr of cases‡	Crude	Model 1§	Nr of cases‡	Crude	Model 1§
*1/*1 carriers											
Non use	1,752	Ref.	856	Ref.		634	Ref.		262	Ref.	
Current use	204	1.14 (0.90; 1.45)	114	1.33 (1.10; 1.62)	0.97 (0.80; 1.19)	30	1.23 (0.85; 1.78)	1.12 (0.78; 1.62)	60	1.60 (1.21; 2.12)	1.43 (1.06; 1.92)
*2 carriers											
Non use	616	Ref.	302	Ref.		212	Ref		102	Ref.	
Current use	90	1.45 (1.04; 2.01)	49	1.64 (1.21; 2.21)	1.17 (0.86; 1.59)	16	2.35 (1.41; 3.91)	2.12 (1.26; 3.56)	25	1.59 (1.03; 2.47)	1.42 (0.90; 2.23)
*3 carriers											
Non use	268	Ref.	109	Ref.		103	Ref.		56	Ref.	
Current use	49	1.41 (0.84; 2.01)	29	1.77 (1.84; 4.19)	2.09 (1.37; 3.20)	8	1.27 (0.62; 2.62)	1.07 (0.51; 2.22)	12	1.72 (0.63; 2.19)	1.03 (0.54; 1.97)
*2/*3 carriers											
Non use	40	Ref.	21	Ref.		12	Ref.		7	Ref.	
Current use	11	2.36 (0.94; 5.92)	5	2.57 (0.94; 7.00)	1.82 (0.65; 5.13)	3	2.72 (0.76; 9.73)	6.54 (1.36; 31.61)	3	3.15 (0.74; 13.30)	1.06 (0.14; 7.82)

Hazard ratios and 95% confidence intervals (CI) are presented. † Meta-analyses results of the Rotterdam Study, B-PROOF, and LASA. * Total number of fall cases per group of the Rotterdam Study, B-PROOF, and LASA combined. ‡ Number of fall cases per group. § Model 1: age, sex.

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Beta-blocker use and fall risk in older individuals; original results from two studies with meta-analysis

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Abstract

Background

To investigate the association between use of beta-blockers and beta-blocker characteristics – selectivity, lipid solubility, intrinsic sympathetic activity (ISA), and CYP2D6 enzyme metabolism – and fall risk.

Methods

Data from two prospective studies were used, including community-dwelling individuals, N= 7,662 (the Rotterdam Study) and 2,407 (B-PROOF), all aged ≥55 years. Fall incidents were recorded prospectively. Beta-blocker use was determined using pharmacy dispensing records. Cox proportional hazard models adjusted for age and sex were applied to determine the association between beta-blocker use, their characteristics – selectivity, lipid solubility, ISA, and CYP2D6 enzyme metabolism –, and fall risk. The results of the studies were combined using meta-analyses.

Results

In total 2,917 participants encountered a fall during a total follow-up time of 89,529 years. Meta-analysis indicated no association between use of any beta-blocker, compared to non-use, and fall risk, HR= 0.97 (95% CI 0.88; 1.06). Neither was use of a selective beta-blocker associated with fall risk, HR= 0.92 (95% CI 0.83; 1.01). Use of a non-selective beta-blocker was associated with an increased fall risk, HR= 1.22 (95% CI 1.01; 1.48). Other beta-blocker characteristics including lipid solubility and CYP2D6 enzyme metabolism were not associated with fall risk.

Conclusion

Our study suggests that use of a non-selective beta-blocker, contrary to selective beta-blockers, is associated with an increased fall risk in an older population. In clinical practice, beta-blockers have been shown effective for a variety of cardiovascular indications. Though, fall risk should be considered when prescribing a beta-blocker in this age group, and the pros and cons for beta-blockers classes should be taken into consideration.

What is already known about this subject

- Beta-blocker use has been associated with fall risk, although literature is contradictory.
- Pharmacological and adverse effects may vary between beta-blocker characteristics.
- Therefore, the association between beta-blocker characteristics – adrenergic receptor selectivity, lipid solubility, intrinsic sympathetic activity (ISA), and CYP2D6 enzyme metabolism – and fall risk should be evaluated.

What this study adds

- Use of a non-selective beta-blocker, in contrast to selective beta-blockers, is associated with an increased fall risk in an older population.
- Lipid solubility and CYP2D6 enzyme metabolism was not associated with fall risk.
- The number of participants using a beta-blocker with ISA was limited and therefore an association with fall risk could not be examined.

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Introduction

In the aging population, fall incidents form a growing healthcare problem.[1] Of those above 65 years of age, one in three encounters at least one fall annually.[2] Falls lead to significant morbidity and even mortality. Moreover, falls are associated with reduced quality of life and increased health care costs.[3-5] One of the risk factors for falls is the use of certain medication,[6,7] including beta-blockers, although literature is contradictory.[7-10] Beta-blocker use is thought to result in fall risk by inducing bradycardia, reducing the cardiac output, inducing hypotension and dizziness.[11] Pharmacological effects and occurrence of adverse effects may vary between different beta-blocking agents. Differences of beta-blocking agents relate for example to their selectivity for adrenergic receptors, lipid solubility, intrinsic sympathetic activity (ISA), and their elimination route.[11,12]

Previously, we observed an increased fall risk with the use of non-selective beta-blocking agents.[8] In addition, the more lipophilic beta-blockers may be associated with central nerve system side effects, such as dizziness and light-headedness.[11,13] Furthermore, beta-blocking agents with ISA might be less susceptible to cause bradycardia.[11] Regarding the elimination route, some beta-blockers are eliminated through liver metabolism – e.g., metoprolol and propranolol –, whereas others are predominantly eliminated by renal excretion – e.g., atenolol –.[11,12] For those subjected to liver metabolism, the Cytochrome P450 (CYP) 2D6 enzyme plays an important role.[14,15] The CYP2D6 gene displays multiple genetic variations, of which the *4 variant allele is suggested to be of main importance for Caucasians. The *4 variant results in a non-functional protein,[16] and in Caucasians it is responsible for the majority of poor metabolizer (PM) phenotypes.[14,15] Previous research indicated that metoprolol users with a poor metabolizers phenotype, according to their CYP2D6*4 genotypes, had a lower blood pressure and were at increased risk for bradycardia.[17,18] Overall, varying pharmacological effects of beta-blocking agents or individual differences on clearance may underlie the contradictory literature results.

Our objective was to investigate the association between use of beta-blocker and beta-blocker characteristics – selectivity, lipid solubility, ISA, and CYP2D6 enzyme metabolism – and fall risk. We hypothesized that use of non-selective agents, lipid soluble agents and those without ISA are associated with an increased fall risk. In addition, we hypothesized that users of beta-blockers metabolized by CYP2D6 who carry a CYP2D6*4 variant are also at increased risk for fall incidents. These research questions were investigated in two independent studies involving community-dwelling older individuals.

Methods

Study population and setting

Data were used from the Rotterdam Study and B-PROOF (B-vitamins in the PRevention Of Osteoporotic Fractures). The Rotterdam Study is an ongoing population-based cohort,

executed within a suburb of Rotterdam. Its design, objectives and methods have been described in detail.[19,20] Briefly, the study was initiated in 1989 and 7,983 participants aged ≥55 years were included. Subsequently, participants were interviewed and underwent an extensive set of examinations that were repeated during the follow-up visits every 4-5 years. For the current study, participants with pharmacy dispensing data and validated fall data were included, covering a study period from 1 May 1991 until 31 December 2010. The Rotterdam Study has been approved by the medical ethics committee according to the 'Wet Bevolkingsonderzoek ERGO' (Population Study Act: Rotterdam Study), executed by the Ministry of Health, Welfare and Sports of the Netherlands. All study participants gave written informed consent to participate in the study and obtain information from their treating physicians.[19,20]

B-PROOF has also been described in more detail.[21] In short, it is a multi-centre, randomized, placebo-controlled, double-blind trial investigating the efficacy of vitamin B_{12} and folic acid supplementation on the prevention of fractures in persons aged ≥65 years. In total 2,919 participants were included and followed for 2 to 3 years, covering a study period from 2008 until 2013. Inclusion criteria were homocysteine levels of 12-50 μmol/L, serum creatinine ≤150 μmol/L, and no reported malignancies in the past 5 years. For the current study, participants with pharmacy dispensing data were included. The Medical Ethics Committee of Wageningen University approved the study protocol, and the Medical Ethics committees of Erasmus Medical Centre and VU University Medical Center gave approval for local feasibility. Before entering the study, all participants gave written informed consent.[21]

Previous B-PROOF results indicated that the intervention had no effect on the time to first or second fall, or the number of falls encountered during the study.[22] For the current study we therefore used a cohort study design.

Fall incidents

In the Rotterdam Study, 'serious falls' were defined as 'a fall leading to a hospital admission or leading to a fracture'. Data were obtained from a computerized reporting system of the general practitioners within the Rotterdam Study. Participant data was also linked to the Dutch National Morbidity Registration (LMR), which contains information of all hospital admissions. Serious fall data were coded by two members of the research team and were completed until 2010. The first serious fall date was defined as the index date. A participant was followed from the baseline date (date of study enrolment) until the first serious fall (index date), death or the end of the study period, whichever came first.

In B-PROOF, a fall incident was defined as 'an unintentional change in position resulting in coming to a rest at a lower level or on the ground'.[23] Participants reported fall incidents prospectively on a fall calendar on a weekly basis. The calendar was returned to the research team every 3 months. Participants with incomplete or unclear calendars were contacted by telephone. Participants were followed until their first fall incident, the Thursday in that particular week was defined as the index date. Participants were followed from baseline until

the index date, their drop-out date or the date of their last calendar, date of death, or the end of the study, whichever came first.

Beta-blocker use

Beta-blocker use was defined according to the Anatomical Therapeutic Chemical (ATC) code,[24] 'C07'. Selective beta-blockers were defined with the ATC codes; 'C07AB', 'C07BB', 'C07CB', 'C07DB', 'C07EB', and 'C07FB'. Non-selective beta-blockers were defined as 'C07AA', 'C07BA', 'C07CA', 'C07DA', 'C07EA', 'C07FA', and 'C07AG'. Table 1 depicts the characteristics of individual beta-blocking agent, according to lipid solubility, ISA, and CYP2D6 enzyme metabolism.[11,12]

Five exposure definitions were used in the analyses: 1) beta-blocker use overall, 2) selective- and non-selective beta-blocker use, 3) lipophilic and non-lipophilic beta-blocker use, 4) beta-blockers with and without ISA, and 5) use of beta-blockers with and without CYP2D6 enzyme metabolism.

In both studies, beta-blocker use was based on pharmacy dispensing records. In the Rotterdam Study, these records from the regional pharmacies were available from January 1st 1991 onwards. More than 95% of the participants fill their drug prescriptions at one of these pharmacies.

In B-PROOF, pharmacy dispensing records were obtained from the Dutch Foundation for Pharmaceutical Statistics (SFK), as previously described.[8] Data were available throughout the study period of a participant.

The dispensing records contain information regarding date of dispensing, total amount of drug units per dispensing, prescribed daily number of units, product name of the drugs and corresponding ATC code. Current medication use was defined as use at the time of the fall (on the index date). Past use was defined as use prior to, but no longer on, the index date. To investigate a dose-response relation, the average prescribed daily dose was expressed in standardized defined daily doses (DDDs). In the Rotterdam Study, we ensured that all participants had pharmacy dispensing records available for at least four months prior to their study start, to avoid potential misclassification of exposure.

Covariables

Basis characteristics including age, sex, ethnicity, use of a walking aid, history of falls, smoking habits, alcohol consumption, and diabetes were ascertained using a questionnaire.[19,21] During study visits, various characteristics were measured including weight, height, blood pressure, depressive symptoms and cognitive performance. Additionally, serum creatinine,[19,21] and the use of concomitant medication was assessed. As a measure of physical function, lower limb disability scores were determined in the Rotterdam Study,[25,26] in B-PROOF physical performance scores and hand-grip strength were assessed.[27,28] In the Rotterdam Study, also orthostatic hypotension measures and dizziness were available.[19]

Table 1. Depicts the characteristics of individual beta-blocking agent, according to lipid solubility, ISA, and CYP2D6 enzyme metabolism.

Beta-blocker agent	Selectivity	Lipophilicity	ISA	CYP2D6 metabolism
Acebutolol	x	x	x	
Alprenolol		xx	x	x
Atenolol	x			
Betaxolol	x	x		
Bevantolol	x	x		
Bisoprolol	x	x		
Carteolol			x	
Carvedilol		xx		x
Celiprolol	x		x	
Labetalol		x		
Metoprolol	x	x		x
Nebivolol	x	x		x
Oxprenolol		x	x	
Penbutolol		xx	x	
Pindolol		x	x	
Propranolol		xx		x
Sotalol				
Timolol		x		x

A 'x' indicates the presence of a characteristic, and 'xx' indicates the highly lipophilic agents.

In the Rotterdam Study, alcohol consumption was based on food frequency data and reported in grams per day.[29] In B-PROOF, alcohol consumptions was categorized into 'light', 'moderate' and 'excessive'.[30] Diabetes was based on self-report.[19,21] Weight and height were measured and were used to calculate body mass index (BMI, kg/m^2). Hypertension was defined as systolic blood pressure >140mmHg and/or diastolic blood pressure >90 mmHg.[31] In the Rotterdam Study, depressive symptoms were assessed using the Center for Epidemiological Studies Depression Scale (CES-D, score range 0-60)[32] or in a subsample the Hospital Anxiety and Depression Scale (HADS-D, score range 0-21).[33] In B-PROOF, the 15-item version of the geriatric depression scale (GDS) was used.[34] Clinically relevant depressive symptoms were based on CES-D scores ≥16,[35,36] HADS-D scores ≥9,[37] or GDS scores ≥5.[38] Cognitive performance was assessed by the mini-mental state examination (MMSE).[39] Serum creatinine levels were used to calculate an age-adjusted estimate of the glomerular filtration rate (GFR) according to the chronic kidney disease epidemiology collaboration (CKD-EPI) formula.[40] Concomitant medication use was assessed with pharmacy dispensing records, and those considered as potential confounders were; antihypertensive medication 'C02', diuretics 'C03', calcium antagonist 'C08', renin-angiotensin agents 'C09' , benzodiazepines 'N05BA' or 'N05CD' , and antidepressants 'N06A'. Lower-limb disability scores were assessed using a modified version of the Stanford Health Assessment Questionnaire.[25,26] The score was based on answers to

questions regarding rising, walking, bending, and getting in and out of a car. Disability was defined as a score of 3 or higher.[26] A physical performance score was calculated from the results of three physical function tests: walking test, chair stand test, and the tandem stand test.[28] Physical performance score ranged from 0-12 (low physical performance – high physical performance).[27,41] Maximum handgrip strength (kg) was defined as the highest results of two maximum trials per hand using a dynamometer (Takei TKK 5401, Takei Scientific Instrument CO. Ltd.,Tokyo, Japan). Orthostatic hypotension was defined as: a decrease of ≥20 mmHg in systolic and/or a decrease of ≥10 mmHg in diastolic blood pressure.[42] Dizziness symptoms were assed using a questionnaire.[19]

For the Rotterdam Study, baseline fall history, dizziness and serum creatinine levels were used, and depressive symptoms were available from the second visit onwards. Data of the other covariables were available for all follow-up visits, but to minimize the number of missings, the values from the preceding visit were used.

Genotyping

CYP2D6*4 (rs3892097) allele variants were determined based on the illumina 550 (+duo) (the Rotterdam Study), and the Illumina-Omni express array (B-PROOF), and imputation to 1000 Genomes Project (Phaselv3, March 2012) reference set.[43] The imputation quality was 0.99. In B-PROOF imputations were only done for Caucasians. For both studies, the reference group was defined as those with homozygous major allele carrier ship, i.e. the absence of a CYP2D6*4 allele.

Statistical analyses

Baseline characteristics were determined for fallers and non-fallers. Differences between groups were tested using a t-test, a Chi-square test or a Mann-Whitney U test. Deviation from Hardy-Weinberg equilibrium was tested using a Chi-square test for allele frequencies.

Cox proportional hazards models were used to calculate fall hazard ratios (HR) for users compared to non-users.[44] The model compares the prevalence of exposure to beta-blockers in the incident fall cases on the index date with the exposure prevalence in all other participants in the cohort on the same date of follow-up. In this way, cases are censored but non-cases can serve as a reference on multiple occasions until the end of the study period. This method for cohort analysis with a Cox proportional hazards analysis with drug use as a time-varying determinant is valid and has been described earlier.[44] This analysis was done for the five exposure categories (i.e., beta-blocker use overall, selectivity, lipophilicity, ISA and CYP2D6 enzyme metabolism) separately. Per exposure category we stratified on beta-blocker characteristic (e.g., non-use vs. selective- and non-selective beta-blocker use). Non-use was defined as no current beta-blocker use. The models were adjusted for age and sex (model 1). Covariables were included in the models if they changed the hazard ratio of the association between beta-blocker use and falls by more than 10% (model 2). For the first exposure a dose-response relation was investigated. Dose categories were made according to median number of prescribed DDDs. In addition, the analysis of the fifth exposure was

stratified on CYP2D6 genotypes. Fall risk in current users was compared to non-users, within those carrying no variant CYP2D6*4 alleles. Likewise, within those carrying at least one variant CYP2D6*4 allele, fall risk in current users was compared to non-users.

The results of the two studies were combined using meta-analysis. The effect estimates – beta's (log HR) – and their standard errors were used to calculate the overall effect, and to investigate the heterogeneity between the studies. Meta-analyses were done using the R package 'rmeta' applying a random effect model, R version 3.0.3. All other statistical analyses were done using the statistical software package SPSS version 21.0 (IBM, Armonk, NY, USA) and p-values <0.05 were considered statistically significant.

Finally, sensitivity analyses were applied, in which we categorized the selectivity beta-blocker group into; no beta-blocker use (reference), selective beta-blocker use, non-selective, and non-selective and also high lipophilic beta-blocker use. The lipophilic beta-blocker group was categorized into; no beta-blocker use (reference), non-lipophilic, medium lipophilic, and highly lipophilic. In addition, a dose-response relation was investigated for non-selective beta-blockers. Furthermore, an association with falls for past en current use was investigated for selective and non-selective beta-blockers.

Results

Study population
The total Rotterdam Study population comprised 7,983 participants. Of those, 7,662 had both medication and fall data, with 6,170 having also genetic data. B-PROOF included 2,919 participants, of whom 2,407 had medication and fall data, and 2,135 also had genetic data (flow-chart supplementary figure 1). The Rotterdam population with medication, fall, and genetic data differed slightly from those without genetic data. Those without genetic data were slightly older, more likely to be of female gender, to have a positive fall history, use a walking aid, have a lower MMSE score, depressive symptoms, hypertension, self-reported diabetes, lower limb disabilities, and to be of non-Caucasian origin. In addition, there were fewer current smokers. In B-PROOF, those without genetic data were more like to have self-reported diabetes.

In the Rotterdam Study the median follow-up time was 11.4 year with an inter quartile range (IQR) of 5.1-17.9 years, and in B-PROOF it was 1.8 years [0.5-2.0]. Table 2 presents the baseline characteristics for both study populations, separated on occurrence of a fall during follow-up.

Beta-blocker use and fall risk
In both studies, current and past use of beta-blockers was not associated with fall risk, Table 3. For current use – compared to non-use – the combined HR was 0.97 (95% CI 0.88; 1.06). The use of selective beta-blockers was also not associated with fall risk, combined HR= 0.92 (95% CI 0.83; 1.01). Use of non-selective beta-blockers was associated with an increased

fall risk, combined HR= 1.22 (1.01; 1.48). Use of a lipophilic or non-lipophilic beta-blocker was not associated with fall risk, combined HR was 0.99 (95% CI 0.71; 1.37), and HR= 0.99 (95% CI 0.88; 1.09) respectively. In both studies the hazard ratios were adjusted for age and sex, as the other considered covariates did not change the HR by more than 10%. As, in total, there were only four fall cases who used a beta-blocker with ISA capacity, the association between beta-blockers with and without ISA could not be investigated.

Dose response relation

In both studies, beta-blockers were used in relatively low dosages, the median number of prescribed DDDs was 0.50. No dose-response relation was observed. The combined analyses indicated that those using the median dose or less – compared to non-use – had a fall risk of HR= 1.03 (95% CI 0.92; 1.16). Those using a dose above the median had a fall risk of HR= 0.88 (95% CI 0.72; 1.08). In addition, there was no significant linear trend for the dose categories, p= 0.159. Data of the individual studies is not shown.

Beta-blocker use, fall risk and CYP2D6*4 genotype

In the Rotterdam Study, CYP2D6*4 allele had a frequency of 20%, and in B-PROOF 22%. In both studies, the allele frequency was in Hardy and Weinberg-equilibrium.

The association between use of beta-blockers, subjected to CYP2D6 enzyme metabolism or not, and fall risk, was stratified on CYP2D6*4 genotype. No significant associations were observed, Table 4.

None, except one, of the meta-analyses indicated significant heterogeneity between the studies, I^2 varried between 0-4%. The meta-analyses of non-lipophilic beta-blockers indicated significant heterogeneity, p= 0.04 and I^2= 4%.

Sensitivity analyses

Additional categorization of the non-selective beta-blocker group into non-selective, and non-selective highly lipophilic beta-blockers, did not result in materially different results. Use of non-selective beta-blockers – compared to non-use – was non-significantly associated with an increased fall risk, combined HR= 1.22 (95% CI 0.97; 1.53). Use of non-selective highly lipophilic beta-blockers was non-significantly associated with fall risk, combined HR= 1.23 (95% CI 0.87; 1.74). Likewise, subdividing lipophilic beta-blockers into medium and highly lipophilic beta-blockers did not show substantially different results, combined HR= 0.99 (95% CI 0.85; 1.15), and HR= 1.22 (95% CI 0.86; 1.71), respectively. In addition, no dose-response relation was observed for non-selective beta-blocker use (data not shown). The combined analyses indicated that those using the median dose (0.50 DDD) or less – compared to non-use – had a fall risk of HR= 1.25 (95% CI 0.96; 1.62). Those using a dose above the median had a fall risk of HR= 1.22 (95% CI 0.87; 1.69). The results of the association between past and current use of selective and non-selective beta-blockers were not different when compared to the results for current users, supplemental Table 1. All these analyses were adjusted for age and sex.

Table 2. Baseline characteristics of the Rotterdam Study and B-PROOF grouped on the basis of fall incidents during follow-up.

	the Rotterdam Study		B-PROOF	
	Fallers **N= 1,770**	**Non-fallers** **N= 5,892**	**Fallers** **N= 1,147**	**Non-fallers** **N= 1,260**
Age, years[a]	71.6 (9.3)	69.7 (9.5)[d]	74.4 (6.7)	73.7 (6.1)[d]
Female gender[b]	1,360 (76.8)	3,270 (55.5)[d]	617 (53.8)	564 (44.8)[d]
Caucasian ethnicity[b]	1,640 (92.7)	5,477 (93.0)	1,080 (94.2)	1,186 (94.1)
BMI[a]	26.3 (3.7)	26.3 (3.7)	26.9 (4.0)	27.3 (4.0)
History of falls (yes)[b,e]	392 (22.6)	907 (15.7)[d]	399 (44.2)	217 (21.4)[d]
Walking aid use (yes)[b]	215 (13.2)	591 (10.7)[d]	172 (15.1)	153 (12.2)[d]
MMSE score[c]	28 [26-29]	28 [26-29]	29 [27-29]	28 [27-29][d]
Depressive symptoms (yes)[b,f]	153 (13.6)	339 (9.0)[d]	90 (7.9)	65 (5.2)[d]
Hypertension (yes)[b,g]	955 (58.2)	3077 (56.9)	599 (64.0)	670 (63.6)
Diabetes (yes)[b]	115 (6.7)	378 (6.6)	93 (10.3)	109 (10.7)
Alcohol intake				
g/day[c]	2.4 [0.1-13.2]	3.9 [0.2-15.2][d]	-	-
light[b]	-	-	778 (67.8)	842 (66.9)
moderate[b]	-	-	327 (28.5)	371 (29.5)
excessive[b]	-	-	42 (3.7)	46 (3.7)
Current smoking[b]	351 (20.4)	1345 (23.5)[d]	93 (8.1)	130 (10.3)
Lower limb disability (yes)[b]	601 (36.4)	1624 (29.4)[d]	-	-
Physical performance score[c]	-	-	9 [6-11]	9 [7-11][d]
Handgrip strength (kg)[c]	-	-	29 [24-40]	33 [25-42][d]
Dizziness (yes)[b]	616 (35.8)	1792 (31.1)[d]	-	-
Orthostatic hypotension (yes)[b]	203 (13.9)	656 (13.5)	-	-
eGFR (ml/min/1.73m²)[c]	71.7 [61.7-81.5]	72.7 [62.2-82.7][d]	70.8 [60.7-80.8]	72.2 [61.7-82.4][d]
Selective beta-blockers (yes)[b]	161 (9.1)	623 (10.6)	235 (20.5)	247 (19.6)
Non-selective beta-blockers (yes)[b]	40 (2.3)	145 (2.5)	46 (4.0)	35 (2.8)
Antihypertensive use (yes)[b]	20 (1.1)	84 (1.4)	9 (0.8)	14 (1.1)
Diuretic use (yes)[b]	224 (12.7)	806 (13.7)	181 (15.8)	181 (14.4)
Benzodiazepine use (yes)[b]	236 (13.3)	648 (11.0)[d]	54 (4.7)	46 (3.7)
Antidepressant use (yes)[b]	35 (2.0)	128 (2.2)	57 (5.0)	38 (3.0)[d]

The numbers presented are based on the valid number of included fall cases and non-fallers. [a] Presented as mean (±SD). [b] Presented as N (%). [c] Presented as median [IQR]. [d] Differences between fall cases and non-fallers within a study population with a *p*-value <0.05. [e] Fall history concerns falls in the last month for the Rotterdam Study and falls in the preceding year in B-PROOF. [f] Clinically relevant depressive symptoms were bases on CES-D scores ≥16,[35,36] HADS-D scores ≥9,[37] or GDS scores ≥5.[38] [g] Hypertension was defined as systolic blood pressure >140mmHg and/or diastolic blood pressure >90 mmHg.[31]
BMI= body mass index, MMSE= mini-mental state examination, the eGFR is based on the chronic kidney disease epidemiology collaboration formula.

Table 3. Association between beta-blocker use, beta-blocker characteristics – selectivity, lipid solubility, and CYP2D6 enzyme metabolism – and fall risk. Hazard ratios and 95% confidence intervals (CI) are presented.

	Nr of cases[a]	Meta-analysis[b] Model 1[d]	Nr of cases[c]	the Rotterdam Study Crude	the Rotterdam Study Model 1[d]	Nr of cases[c]	B-PROOF Crude	B-PROOF Model 1[d]
Beta-blockers								
Non use	1,966	ref	1,161	ref		805	ref	
Past	363	1.07 (0.95; 1.20)	319	1.12 (0.98; 1.27)	1.07 (0.94; 1.21)	44	1.10 (0.81; 1.49)	1.07 (0.79; 1.46)
Current	588	0.97 (0.88; 1.06)	290	0.95 (0.84; 1.09)	0.96 (0.85; 1.10)	298	0.99 (0.87; 1.13)	0.98 (0.86; 1.12)
Selectivity								
No beta-blockers use	2,329	ref	1,480	ref		849	ref	
Selective beta-blockers	480	0.92 (0.83; 1.01)	230	0.87 (0.76; 1.00)	0.90 (0.79; 1.04)	250	0.95 (0.82; 1.09)	0.94 (0.81; 1.08)
Non-selective beta-blockers	108	1.22 (1.01; 1.48)	60	1.24 (0.96; 1.61)	1.18 (0.91; 1.52)	48	1.30 (0.97; 1.74)	1.28 (0.96; 1.72)
Lipophilicity								
No beta-blockers use	2,329	ref	1,480	ref		849	ref	
Lipophilic beta-blockers	414	0.99 (0.88; 1.09)	173	1.01 (0.86; 1.18)	1.04 (0.88; 1.21)	241	0.94 (0.82; 1.09)	0.93 (0.81; 1.08)
Non lipophilic beta-blockers	174	0.99 (0.71; 1.37)	117	0.84 (0.69; 1.01)	0.85 (0.70; 1.02)	57	1.23 (0.94; 1.60)	1.19 (0.91; 1.55)
CYP2D6 metabolism								
No beta-blockers use	2,329	ref	1,480	ref		849	ref	
2D6 metabolism[e]	334	0.98 (0.88; 1.11)	118	0.99 (0.82; 1.19)	1.00 (0.83; 1.21)	216	0.98 (0.85; 1.14)	0.97 (0.84; 1.13)
No 2D6 metabolism	254	0.94 (0.82; 1.07)	172	0.89 (0.76; 1.05)	0.92 (0.78; 1.07)	82	0.99 (0.80; 1.25)	0.98 (0.78; 1.23)

[a] Total number of fall cases per group of the Rotterdam Study and B-PROOF combined. [b] Meta-analyses results of the Rotterdam Study, and B-PROOF. [c] Number of fall cases per group. [d] Model 1: age, sex. [e] Beta-blockers that are –partially– metabolized by the CYP2D6 enzyme, i.e., metoprolol, propranolol, nebivolol, carvedilol, timolol, and alprenolol (in the Rotterdam Study).

Table 4. Association between beta-blocker use, categorised by CYP2D6 enzyme metabolism and fall risk, stratified for CYP2D6*4 genotype. Hazard ratios and 95% confidence intervals (CI) are presented.

CYP2D6*4	Nr of cases[a]	Meta-analyses[b] Model 1[d]	Nr of cases[c]	the Rotterdam Study Crude	the Rotterdam Study Model 1[d]	Nr of cases[c]	B-PROOF Crude	B-PROOF Model 1[d]
Betablockers 2D6 Metabolism								
No *4 carriers								
Non use	1,223	ref	757	ref	ref	466	ref	ref
2D6 metabolism[e]	176	0.92 (0.78; 1.08)	64	1.03 (0.79; 1.33)	1.02 (0.79; 1.32)	112	0.87 (0.71; 1.07)	0.86 (0.70; 1.05)
No 2D6 metabolism	144	0.96 (0.81; 1.14)	95	0.87 (0.71; 1.08)	0.91 (0.73; 1.12)	49	1.07 (0.80; 1.43)	1.08 (0.81; 1.45)
*4 carriers								
Non use	745	ref	459	ref	ref	286	ref	ref
2D6 metabolism[e]	116	1.16 (0.95; 1.42)	38	1.00 (0.72; 1.40)	1.07 (0.77; 1.49)	78	1.20 (0.94; 1.55)	1.22 (0.95; 1.56)
No 2D6 metabolism	81	0.98 (0.78; 1.24)	54	1.02 (0.77; 1.36)	0.99 (0.75; 1.31)	27	1.03 (0.69; 1.52)	0.97 (0.65; 1.44)

[a] Total number of fall cases per group of the Rotterdam Study and B-PROOF combined. [b] Meta-analyses results of the Rotterdam Study, and B-PROOF combined. [c] Number of fall cases per group. [d] Model 1: age, sex. [e] Beta-blockers that are –partially– metabolized by the CYP2D6 enzyme, i.e., metoprolol, propranolol, nebivolol, carvedilol, timolol, and alprenolol (in the Rotterdam Study).

Discussion

In two large older populations, the use of non-selective beta-blockers was associated with an increased fall risk. Use of selective, lipophilic or beta-blockers overall, was not associated with fall risk. Furthermore, we did not observe an association between beta-blocker use and fall risk across genotypes of CYP2D6.

To our knowledge, our study group is the first to evaluate the association between beta-blocker characteristics and fall risk. We observed an increased fall risk for current use of non-selective beta-blockers, although there was no dose-response relation. Furthermore, no association was observed for selective beta-blocker use or beta-blocker use overall. These findings might be explained by the receptor binding profile and accompanying systemic effects of non-selective beta-blockers. Non-selective beta-blockers bind, in addition to binding to β1-receptors, also to β2-receptors and some also to α-receptors. β1-receptors are mainly located in the heart, while β2-receptors are also present in the lungs, smooth muscle cells of the peripheral circulation, liver and in skeletal muscle cells.[11,12,45] As a consequence non-selective beta-blockers not only reduce heart rate and contractility, they also induce peripheral vasoconstriction, including in blood vessels towards and in skeletal muscle.[11,12] Contrarily, β- and α-blockers also exhibit vasodilating properties.[11,12] In theory, β2-antagonist may as well have a direct negative effect on skeletal muscle and might thereby be related to fall risk, as β2-agonist are suggested to have a positive effect on muscle function.[46,47] Thus, non-selective beta-blockers may be related to fall risk by their broader range in effects and their potential negative effect on skeletal muscle.

Another aspect of selective and non-selective beta-blockers is that the indication for use can differ. Selective beta-blockers are mainly used for hypertension, although metoprolol, for example, is also used in patients with heart failure or those with angina pectoris or a previous myocardial infarction. Non-selective beta-blockers on the other hand are contraindicated for asthmatics and diabetics. Sotalol, a non-selective agent is used for arrhythmias and carvedilol is used for heart failure, but also for hypertension and angina pectoris.[11,12,48] These potential indication differences of beta-blockers may be related to fall risk. Nevertheless, our sensitivity analyses did not indicate an association with fall risk for past use of selective or non-selective beta-blockers. If the association was spuriously caused by confounding by indication, we would expect a similar risk estimate in past users as in current users.

Within the B-PROOF population we previously observed an association between non-selective beta-blocker use and fall risk, though then non-selective beta-blockers were slightly differently defined.[8] Currently we also included in alfa- and beta-blockers, and excluded ocular administered beta-blockers.

In previous studies, overall use of beta-blockers has been associated with fall risk,[9,10] but not consistently.[7] In the studies that reported an association, an increased fall risk was observed during initiation of use, which was thought to be due the increased risk of hypotension.[9,10] We investigated current use and not initiation of use, which may partly explain the discrepancy in results.

With respect to the lipophilicity of beta-blockers, some non-selective beta-blockers are highly lipophilic, such as carvedilol and propranolol.[11,12] Because lipophilic agents can cross the blood-brain barrier,[11,13] we hypothesized that their use was associated with more central adverse effects, including dizziness. Nevertheless, our sensitivity analyses investigating the use of beta-blockers, combining strong lipophilic and non-selective characteristics, in relation to fall risk did not result in a different association than for non-selective beta-blockers overall. Thus, our results do not confirm this hypothesis.

Regarding pharmacokinetic properties of beta-blockers, we hypothesized that users of beta-blockers that are subjected to 2D6 enzyme metabolism who carry a CYP2D6*4 variant are at increased risk for fall incidents, due to decreased metabolism and potentially increased drug concentrations. Previous studies indicated that the combination of metoprolol – a beta-blocker predominantly metabolized by CYP2D6 – use and a poor metabolizer phenotype – based on genotype – was associated with a lower clearance, a longer half-life,[49] and with lower blood pressure and, heart rate.[17,18] Although we were not able to investigate these specific endpoints, our results do not indicate that these clinical effects – whether they occurred or not – were translated into fall risk.

Our study has strengths and limitations. Its strength is the combination of two large, independent, community-dwelling study populations, and thereby the possibility to investigate consistency of a potential signal (finding) across the two studies. Our study also has limitations, as the B-PROOF study participants were included according to their homocysteine levels. However, we do not think that this inclusion criterion would have interfered with a mechanism underlying a potential association between beta-blocker use and falls. In the Rotterdam Study, fall incidents were differently assessed than in B-PROOF, as serious fall incidents were gathered, and falls not leading to serious consequences were not included. This may lead to a different association if the underlying mechanism for beta-blocker-related falls would differ between serious and less serious falls. However, we are not aware of different mechanisms, and the effect sizes were relatively similar across both studies. In addition, we investigated current use. Possibly, participants encountering side effects already stopped using, switched to another beta-blocker, or received lower doses. This may have resulted in underestimation of the association. Another limitation is the relatively low number of users carrying a CYP2D6*4 allele, consequently we clustered intermediate metabolizers (IMs) phenotype – carriers of one *4 allele – with PMs – carriers of two *4 alleles –. Although the clustering, the numbers were too low to draw conclusion. And lastly, we do not have information on actual plasma levels of the beta-blockers.

Conclusion

Our study indicates an increased fall risk in older people during the use of non-selective beta-blockers, contrary to selective beta-blockers. In clinical practice, beta-blockers have been shown effective for a variety of cardiovascular indications. Though, fall risk should be

considered when prescribing a beta-blocker in this age group, and the pros and cons for beta-blockers classes should be taken into consideration.

Acknowledgments

Supplementary material

Total	The Rotterdam Study	B-PROOF
N = 10,902	N = 7,983	N = 2,919

Total exclusions	Exclusion criteria:	Exclusion criteria:
N = 833	No informed consent for follow-up, N = 226 / End of follow-up before 01-05-1991, N = 73 / Case before 01-05-1991, N = 22	No pharmacy data, N = 512

Total participants with medication data & fall data	Participants with medication data & fall data	Participants with medication data & fall data
N = 10,069	N = 7,662	N = 2,407

Total no 2D6*4 genetic data	No 2D6*4 genetic data	No 2D6*4 genetic data
N = 1,764	N = 1,492	N = 272

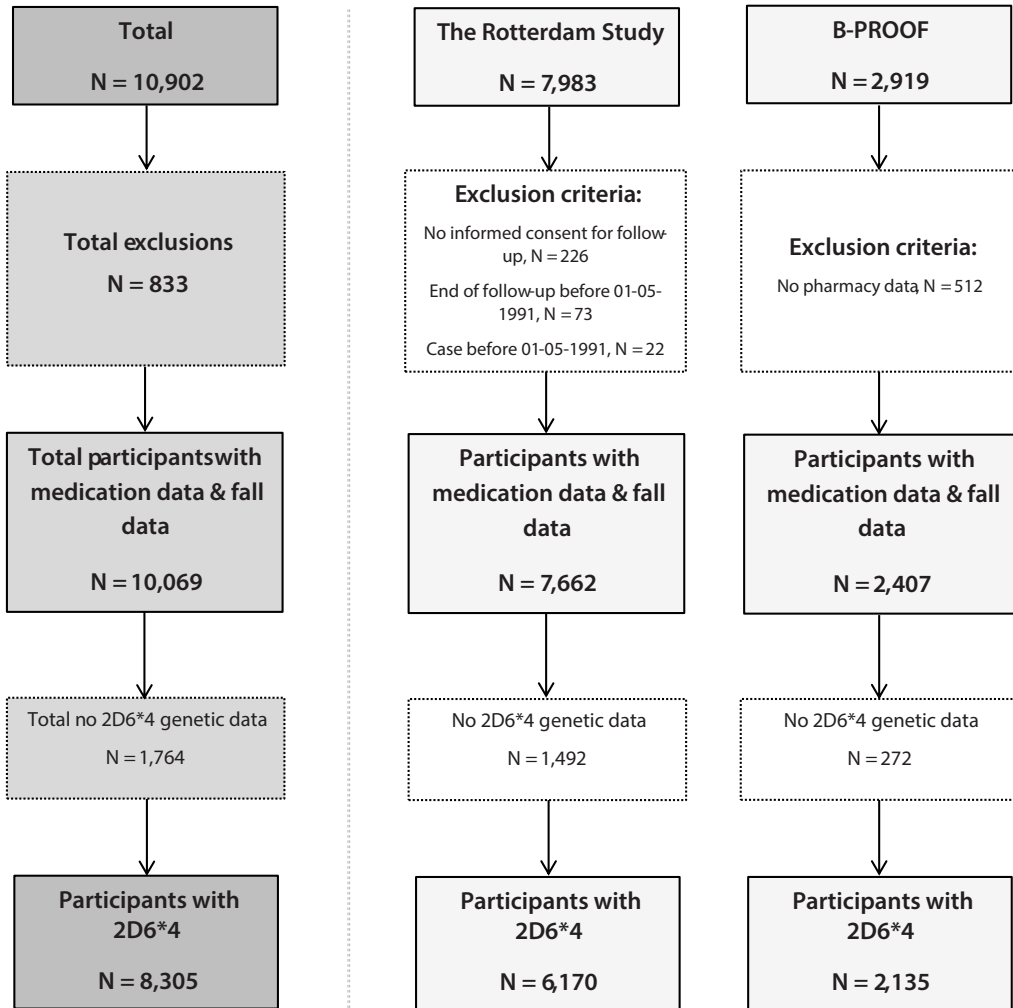| Participants with 2D6*4	Participants with 2D6*4	Participants with 2D6*4
N = 8,305	N = 6,170	N = 2,135

Figure 1. Flowchart of the included study populations.

Table 1. Association between selective and non-selective beta-blocker use for past and current use, and fall risk. Hazard ratios and 95% confidence intervals (CI) are presented.

|	Nr of cases[a]	Meta-analysis[b] Model 1[d]	Nr of cases[c]	the Rotterdam Study Crude	the Rotterdam Study Model 1[d]	Nr of cases[c]	B-PROOF Crude	B-PROOF Model 1[d]
Beta-blockers								
Non use	1,966	ref	1,161	ref	ref	805	ref	ref
Past selective beta-blockers	296	1.08 (0.95; 1.23)	257	1.11 (0.97; 1.27)	1.07 (0.93; 1.23)	39	1.19 (0.86; 1.65)	1.17 (0.85; 1.62)
Current selective beta-blockers	480	0.93 (0.84; 1.03)	230	0.89 (0.77; 1.03)	0.92 (0.80; 1.06)	250	0.95 (0.83; 1.10)	0.94 (0.81; 1.08)
Past non-selective beta-blockers	67	1.01 (0.76; 1.34)	62	1.15 (0.89; 1.48)	1.06 (0.82; 1.37)	5	0.66 (0.27; 1.59)	0.65 (0.27; 1.58)
Current non-selective beta-blockers	108	1.23 (1.01; 1.50)	60	1.27 (0.98; 1.65)	1.19 (0.92; 1.54)	48	1.31 (0.98; 1.75)	1.29 (0.96; 1.72)

[a] Total number of fall cases per group of the Rotterdam Study and B-PROOF combined. [b] Meta-analyses results of the Rotterdam Study, and B-PROOF. [c] Number of fall cases per group. [d] Model 1: age, sex.

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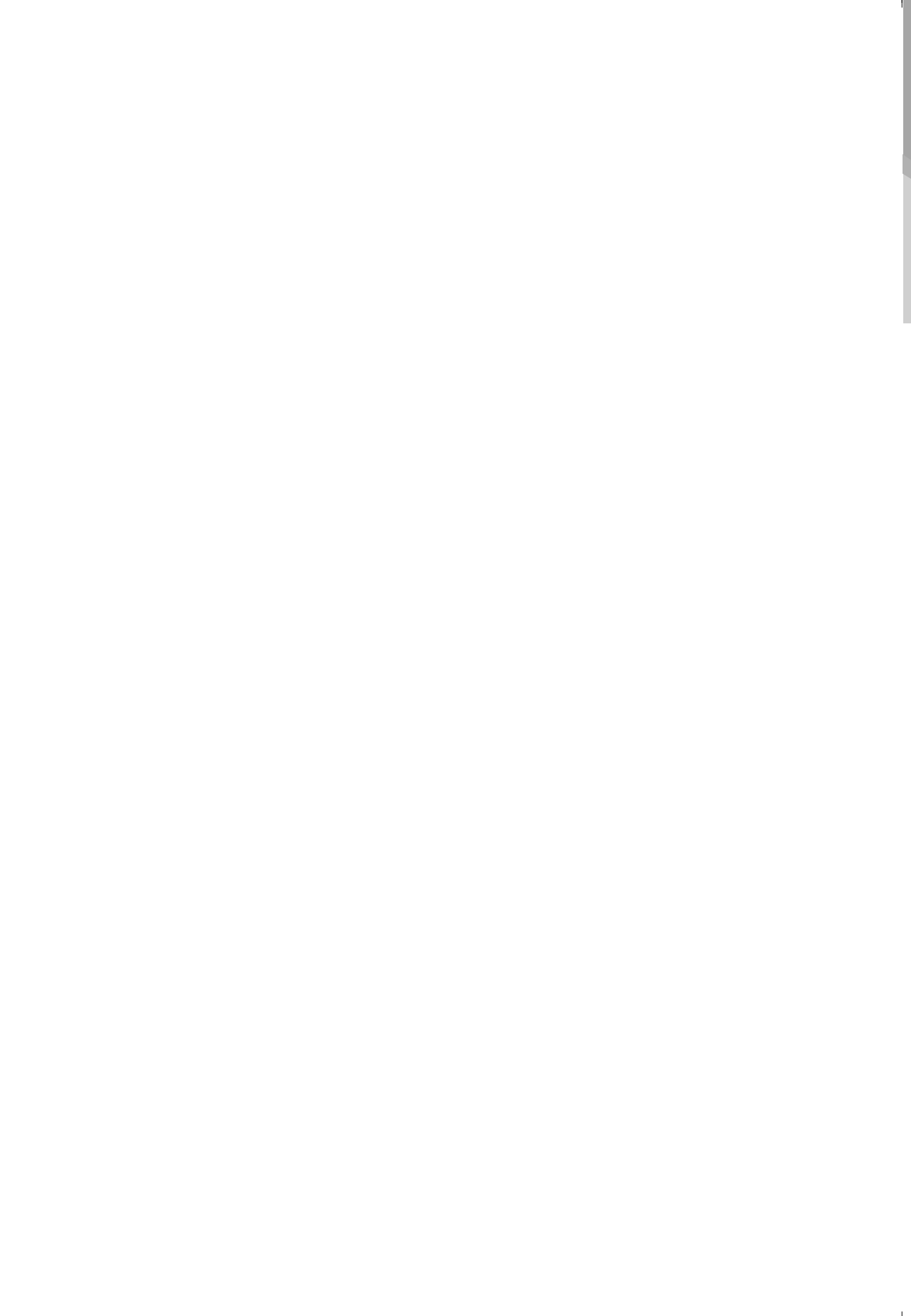
2.4

Identification of genetic risk factors for benzodiazepine-related falls

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B.H. Stricker, N. van der Velde

Abstract

Objective
To identify genetic variants that influence the association between benzodiazepine use and fall risk, applying a genome wide association study (GWAS) approach.

Design
GWAS on two nested case-control studies; the Rotterdam Study and B-PROOF.

Setting
Community-dwelling individuals living in or near three Dutch cities.

Participants
Benzodiazepine users, 201 fall cases and 3,187 controls (the Rotterdam Study), and 57 fall cases, 241 controls (B-PROOF).

Measurements
Fall incidents were prospectively recorded and benzodiazepine use was determined using pharmacy dispensing records. Only benzodiazepine users were included. Cases were defined as those who used a benzodiazepine at the date of the fall. All other participants were classified as control. GWAS was done for both studies separately, adjusting for age, sex, and the first four principal components. Results were combined with meta-analysis.

Results
In the Rotterdam Study, a variant was associated with benzodiazepine-related falls, OR 0.34 (95% CI 0.22; 0.52), $p= 6.56*10-7$. In B-PROOF this variant was also associated, OR 0.11 (95% CI 0.03; 0.46) $p= 2.51*10-3$. This variant was genome wide significantly associated in the combined results, OR= 0.31 (95% CI 0.21; 0.47) with MAF= (minor allele frequency) 0.03, $p= 2.15*10-8$, and $I^2= 54.3\%$, $p= 0.14$. This indicates a 69% lower benzodiazepine-related fall risk per minor allele, compared to non-carriers. The variant is located in an intron on chromosome 9 within the FAM73B gene.

Conclusions
Within our study population, a genome wide significantly associated with benzodiazepine-related falls was observed. Replication and functional studies are warranted to verify and support our observed finding.

Introduction

Fall incidents are a growing health problem in the light of the aging population and their substantial impact on morbidity and mortality. Moreover, falls are associated with reduced quality of life and increased health care costs.[1-4] Annually, one third of the older adults – aged ≥65 years – encounters at least one fall.[5] Fall risk is related to multiple factors, including, muscle weakness, problems with balance, vision and cognition, but also use of certain medication, in particular benzodiazepines.[6-8] Benzodiazepines are frequently used in older individuals, mainly for sleeping problems and as anxiolytic.[9,10] Their use is thought to affect fall risk by inducing sedation, dizziness and balance problems.[11,12]

It has been shown that response to benzodiazepines varies between individuals, which may be related to genetic variation.[13,14] Previous studies investigated and indicated several candidate genes that may affect benzodiazepine response. Most of these genes coded for drug metabolizing enzymes (e.g., Cytochrome P450 enzymes),[13,14] although genetic associations with drug receptors (e.g., GABA receptors) have also been investigated.[14-16]

However, candidate gene studies are generally biased towards existing knowledge, usually lack replication, and/or often only a few single nucleotide polymorphisms (SNPs) are investigated. In case of metabolizing enzymes different isoforms are usually investigated, but variants in enhancers or promotors of the gene are not always taken into account.[17,18] Such variants may influence the amount of protein present in the cell[19] and thus potentially also the rate of drug metabolism. Here, we aimed to gain more insight into pathways and mechanisms underlying benzodiazepine-related falls. In this study, we therefore opted for a hypothesis-free approach, namely genome-wide association studies (GWAS) in order to identify any genetic variants that influence the association between benzodiazepine use and fall risk.

Methods

Study population and setting

Data from two independent study populations were used: the Rotterdam Study and B-PROOF (B-vitamins in the PRevention Of Osteoporotic Fractures). The Rotterdam Study is an ongoing population-based cohort study, executed within a suburb of Rotterdam. Its design, objectives and methods have been described in detail.[20,21] In short, the study started in 1989 and 7,983 participants aged ≥55 years were included. Participants were interviewed and underwent examinations that were repeated every 4-5 years during the follow-up visits. Later, in 2002 and 2008 two other cohorts were enrolled. For the current study, participants with pharmacy dispensing data and validated fall data from the first cohort were included, covering a study period from 1 May 1991 until 31 December 2010. The Rotterdam Study has been approved by the medical ethics committee according to the 'Wet Bevolkingsonderzoek ERGO' (Population Study Act: Rotterdam Study), executed by the Ministry of Health, Welfare

and Sports of the Netherlands. All study participants gave written informed consent to participate in the study and obtain information from their treating physicians.[20,21]

B-PROOF has also been described in more detail.[22] Briefly, it is a multi-centre, randomized, placebo-controlled, double-blind trial investigating the efficacy of vitamin B_{12} and folic acid supplementation on the prevention of fractures. In total, 2919 participants aged ≥65 years were included and followed for 2 to 3 years, covering a study period from 2008 until 2013. Inclusion criteria were homocysteine levels of 12-50 μmol/L, serum creatinine ≤150 μmol/L, and no reported malignancies in the past 5 years. For the current study, participants with pharmacy dispensing data were included. The Medical Ethics Committee of Wageningen University approved the study protocol, and approval for local feasibility was obtained from the Medical Ethics committees of Erasmus Medical Centre and VU University Medical Centre. All participants gave written informed consent before entering the study.[22]

B-PROOF results indicated that the intervention had no effect on the time to first or second fall, or the number of falls encountered during the study.[23]

Benzodiazepine use

Benzodiazepine use was defined according to the Anatomical Therapeutic Chemical (ATC) codes,[24] 'N05BA' for anxiolytics, or 'N05CD' for hypnotics, and was based on pharmacy dispensing records. In the Rotterdam Study, records from the regional pharmacies were available from January 1st 1991 onwards. Over 95% of the participants fill their drug prescriptions at one of these pharmacies. To avoid potential misclassification of exposure, we ensured that all participants had pharmacy dispensing records for at least four months prior to their study start. In B-PROOF, pharmacy dispensing records were obtained from the Dutch Foundation for Pharmaceutical Statistics (SFK), as previously described.[25] Records were available for participants throughout their study period.

Fall incidents

In the Rotterdam Study, 'serious falls' were defined as 'a fall leading to a hospital admission or leading to a fracture'. Data were obtained from a computerized reporting system of the general practitioners within the Rotterdam Study. Participant data were also linked to the Dutch National Morbidity Registration (LMR), which contains information of all hospital admissions. Serious fall data were coded by two members of the research team and were completed until 2010. The first serious fall date was defined as the index date and a participant was followed until the first serious fall, death or the end of the study period, whichever came first.

In B-PROOF, a fall incident was defined as 'an unintentional change in position resulting in coming to a rest at a lower level or on the ground'.[26] Participants reported fall incidents prospectively on a fall calendar on a weekly basis, as previously described.[25] Participants were followed until the index date, their drop-out date or the date of their last calendar, date of death, or the end of the study, whichever came first.

Case and control definition

From both studies only benzodiazepine users were selected, which were defined as those having at least one benzodiazepine dispensing record during the follow-up of the study period. For each participant, the usage period was calculated by dividing the total number of pills in a prescription by the prescribed daily number of pills, starting at the prescription filling date at the pharmacy.

Cases were defined as those who used a benzodiazepine at the date of the first fall (index date), i.e. when the fall day fell in a usage period. All other participants were classified as controls.

Genotyping and imputation

Genotyping was done using the illumina 550 (+duo) array (the Rotterdam Study), and the Illumina-Omni express array (B-PROOF). Standard QC was performed.[27,28] Subsequently imputations were performed using the 1000 Genomes Project (PhaseIv3, March 2012) as a reference set.[29] In both studies only samples of European Ancestry were taken into account.

Statistical analyses

Baseline characteristics were determined for cases and controls per study population. Differences between groups were tested using a T-test, a Chi-square test or a Mann-Whitney U test.

GWAS was done for both studies separately applying logistic regression analysis, using an additive genetic model. Analyses were adjusted for age, sex and the first four principal components to account for any remaining population substructure. The analyses were done using GRIMP, a web-based tool for high-speed analysis of large-scale genome-wide association using imputed data.[30] Quality control was conducted for both studies according to the EasyQC protocol.[31] Finally, results were combined with meta-analysis using the software package METAL, applying a fixed-effect, inverse variance weighted approach and including genomic control correction.[32] Visualization of the results was done using R, the R package 'qqman',[33] and LocusZoom.[34]

Database lookups of the associated variants

To gain information about the location, linkage to other variants, and potential function of the associated variant – top hit – , the UCSC (University of Californa Santa Cruz) genome browser (Build hg19),[35] dbSNP[36] (human annotation release 107) ,OMIM (online mendelian inheritance in man),[37] MGI (mouse genome information),[38,39] and GeneCards[40] were reviewed. These databases were also used to gain information on the function of the genes near the top hit.

For further exploration of potential functional elements (e.g., gene regulatory elements, promotors, or transcripts) in various cell types and tissues of the top hit, and variants in high LD (R^2 >0.8), HaploReg was used,[41] which compiles data from ENCODE (Encyclopedia of DNA Elements), and the NIH Roadmap Epigenomics Mapping Consortium.[42] UCSC genome

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bowers was also used to access information from GTEX (genotype-tissue expression),[43] and ENCODE and Roadmap data directly. Additionally, to lookup whether the top hit was previously identified in other – published – GWAS studies, the GWAS catalog was consulted[44].

Results

Study population
In the Rotterdam Study 7,983 participants were included, of whom 6,170 had medication, fall and genetic data. Subsequently, 201 cases and 3,187 controls – participants with a benzodiazepine dispensing during follow-up – were available for the GWAS analysis. In B-PROOF, 2,919 participants were included of whom 2,135 had medication, fall and genetic data. Subsequently, 57 cases and 241 controls were available for the GWAS analysis, supplementary Figure 1. Table 1 depicts the baseline characteristics of both study populations separately for cases and controls.

Meta-analysis
Separate and combined analyses of the Rotterdam study and B-PROOF resulted in several suggestive hits, $p \leq 1.00*10\text{-}6$, supplementary Table 1a, 1b and 1c. For the combined results, the QQ (quantile-quantile) plot, Figure 1, did not show evidence for strong inflation and showed some evidence for associations. The Manhattan plot (Figure 2) showed one genome-wide significant signal on chromosome 9 as well as several suggestive loci. Figure 3 displays the regional plot. Table 3 depicts the combined results of the top hit, and those for both studies (the Rotterdam Study and BPROOF) separately. The top hit was negatively associated with benzodiazepine-related falls, OR 0.31 (95% CI 0.21; 0.47), with MAF= (minor allele frequency) 0.03 and $P= 2.15*10\text{-}8$. This indicates a 69% lower benzodiazepine-related fall risk per minor allele, compared to non-carriers (Table 2). The heterogeneity between the studies was, $I^2= 54.3\%$, $p= 0.14$. Figure 4 depicts a regional plot of for the genome-wide significant association.

Database lookups
The variant is located within an intron of FAM73B gene (homo sapiens family with sequence similarity 73 member B) with the alternative name of MIGA2 (mitoguardin 2). The gene encodes a protein involved in regulation of mitochondrial fusion. No pathway databases were available for the gene. With respect to the gene ontology, the protein of the gene is located in the cell membrane, and integral component of membrane, and it is involved in bone development. The OMIM database referred to research by Zang et al., indicating that malfunctioning of mitochondrial fusion is involved in neurodegenerative disorders and fat metabolism.[50] Knock out models are present in MGI database, and the following associated phenotypes are reported; craniofacial, homeostasis/metabolism, immune system and

skeleton.[51,52] With respect to skeleton phenotypes, a.o., decreased bone mineral content and density, and decreased bone strength have been reported.[51,52]

Table 1. Baseline characteristics of the Rotterdam Study and B-PROOF separately for cases and controls.

	the Rotterdam Study		B-PROOF	
	Cases N= 201	Controls N= 3,187	Cases N= 57	Controls N= 241
Age (years)[a]	73.8 (9.0)	69.3 (8.9)[d]	77.5 (7.3)	74.8 (6.5)[d]
Sex (women, %)[b]	172 (85.6)	2085 (65.4)[d]	44 (77.2)	153 (63.5)[d]
BMI (Kg/m²)a	26.3 (3.8)	26.4 (3.8)	26.3 (3.3)	27.5 (4.0)[d]
History of falls (%yes)[b,e]	63 (32.5)	546 (17.4)[d]	25 (52.1)	58 (30.7)[d]
Walking aid (%yes)[b]	29 (16.0)	310 (10.2)[d]	19 (33.3)	44 (18.3)[d]
MMSE score[c]	27 [26-29]	28 [27-29][d]	28 [27-29]	28 [27-29]
Depressive symptoms (%yes)[b,f]	27 (24.1)	250 (11.8)[d]	12 (21.1)	26 (10.8)[d]
Hypertension (yes)[b,g]	119 (60.4)	1752 (56.3)	32 (68.1)	117 (56.0)
Diabetes (yes)[b]	15 (7.8)	179 (5.7)	5 (10.4)	21 (11.0)
Alcohol intake				
g/day[c]	0.8 [0.1-11.3]	3.2 [0.1-14.5][d]		
light[b]			44 (77.2)	158 (65.6)
moderate[b]			11 (19.3)	69 (28.6)
excessive[b]			2 (3.5)	14 (5.8)
Current smoking[b]	46 (23.8)	728 (23.4)	11 (19.3)	24 (10)
Lower limb disability (%yes)[b]	92 (48.9)	917(30.2)[d]	-	-
Physical performance score[c]	-	-	6.0 [3.0-9.0]	8.0 [6.0-10.0][d]
Handgrip strength (kg)[c]	-	-	23.1 [18.5-27.6]	28.1 [23.0-36.0][d]
eGFR (ml/min/1.73m²)[c]	71.1 [58.9-82.2]	72.2 [62.0-82.6]	69.2 [56.9-82.2]	72.3 [61.6-82.1]

The numbers presented are based on the valid number of included cases and controls. [a] Presented as mean (±SD). [b] Presented as N (%). [c] Presented as median [IQR]. [d] Differences between cases and controls within a study population with a *p*-value <0.05. [e] Fall history concerns falls in the last month for the Rotterdam Study and falls in the preceding year in B-PROOF. [f] Clinically relevant depressive symptoms were bases on CES-D scores ≥16,[45,46] HADS-D scores ≥9,[47] or GDS scores ≥5.[48] [g] Hypertension was defined as systolic blood pressure >140mmHg and/or diastolic blood pressure >90 mmHg.[49]
BMI= body mass index, MMSE= mini-mental state examination, the eGFR is based on the chronic kidney disease epidemiology collaboration formula.

HaploReg reports no regulatory features or regions for the variant. Additionally, none of the SNPs in high LD (linkage disequilibrium; R^2 >0.8), had regulatory features or were in regulatory regions.

Using ENCODE, FAM73B appears to be expressed in various cell types; blood cell types, kidney cells, skeletal muscle cells, lung cells, and breast cells. Similarly, using the roadmap 'ChromHmm Primary Core Marks' track FAM73B appears to be expressed in various cells types, e.g., blood, adipose, skeletal muscle, brain, liver and pancreas cells. GTEX, also reports expression in a variety of tissues, though with a highest median expression in brain – cerebellum. Likewise, expression patterns in GeneCard report expression in a variety of

(normal human) tissues – GeneCard also includes data from GTEX –. In addition, it reports overexpression in bone in embryonic tissue and stem cells.
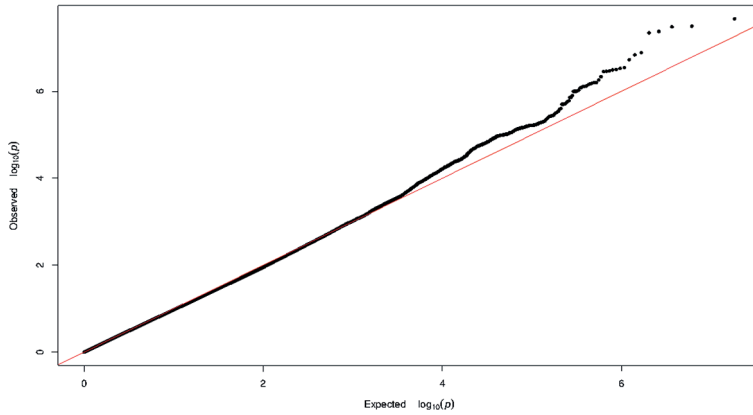

Figure 1. QQplot for the GWAS meta-analysis (N= 3,686) on benzodiazepine-related fall risk. The *p*-values were generated using logistic regression analysis.
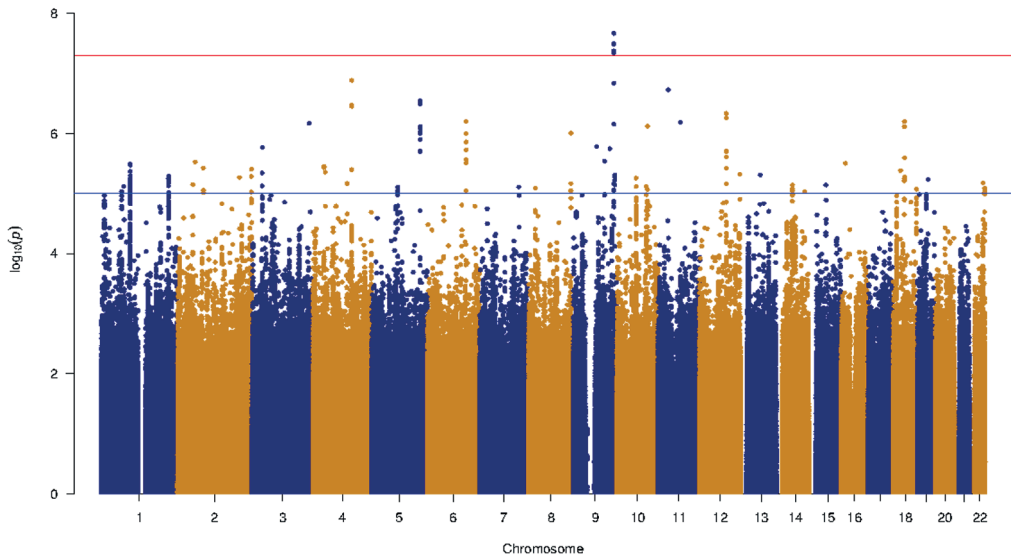

Figure 2. Manhattan plot for the GWAS meta-analysis (N= 3,686) on benzodiazepine-related fall risk. The *p*-values were generated using logistic regression analysis.
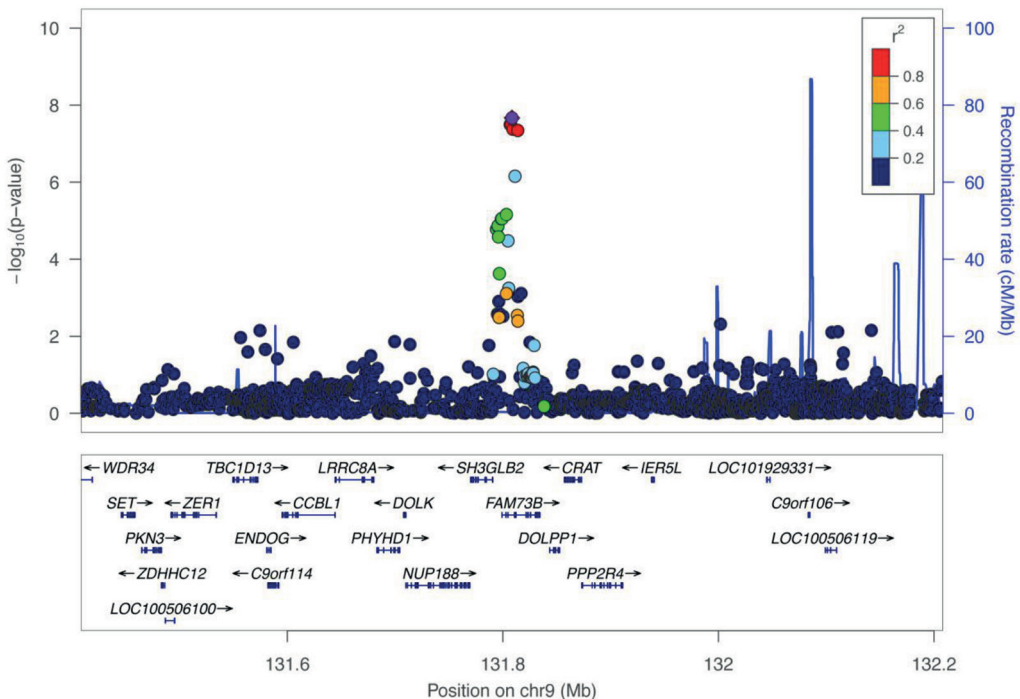
Figure 3. Regional association plot for the genome-wide significant association with benzodiazepine-related falls.

Table 2. Genome-wide association of the top hit (meta-analyses) and separate results for the Rotterdam Study and B-PROOF.

Study	Chr	All 1	All 2	MAF	Effect (β)	SE	OR (95% CI)	p-value	N
Rotterdam Study	9	t	c	0.036	-1.079	0.217	0.34 (0.22; 0.52)	6.56*10-7	3,388
B-PROOF	9	t	c	0.019	-2.208	0.731	0.11 (0.03; 0.46)	2.51*10-3	298
Combined	9	t	c	0.035	-1.171	0.209	0.31 (0.21; 0.47)	2.15*10-8	3,686

Chr= chromosome, All= allele, MAF= minor allele frequency, SE= standard error, OR= odds ratio, 95% CI = 95% confidence interval.

Within the GWAS catalog, 2 studies are reported within a ±500kb distance from the variant, though both studies did not observe genome wide significant hits. One study investigated response to amphetamines (N= 381, no replication),[53] the other systemic lupus erythematosus (N= 1,311 cases, N= 3,340 control with replication in N= 793 cases, N= 857 control).[54]

The genes near (±500kb) the variant were also reviewed for their function, which is presented in supplementary Table 2.

Discussion

The genome wide association analyses of benzodiazepine-related fall incidents in two independent study populations identified a less common variant located in FAM73B gene. This variant is associated with a 3-times lower risk of falling, OR= 0.31 (95% CI 0.21; 0.47), p= 2.15*10-8, MAF= 3%, N= 3,686 (N= 258 cases and N= 3,428 controls).

To our knowledge, we are the first to use a GWAS approach to identify genetic variants that modify benzodiazepine-related fall incidents. The variant, is an intronic variant located on chromosome 9 within the FAM73B gene. Based on the narrow peak in the region plot and the functions of the surrounding genes, the FAM73B gene is probably the most likely gene underlying the observed association. FAM73B gene encodes a protein involved in regulation of mitochondrial fusion, and is involved in bone development. Moreover, mitochondrial fusion has been linked to neurodegenerative disorders.[50] On the other hand, expression data from GTEX reports expression in a wide range of cell types and tissues,[43] which is plausible with respect to the basic function of the gene. Nevertheless, in normal human tissue the highest median expression was in brain, cerebellum. This part of the brain plays a key role in motor control,[55] thereby the FAM73B gene could be linked to our phenotype, benzodiazepine-related fall incidents. Benzodiazepines are thought to affect fall risk by inducing sedation, dizziness and balance problems, due to their effect on the central nervous system.[11,12] Although the FAM73B gene has been linked to neurodegenerative disorders and is expressed in brain tissue, other information points towards its involvement in bone. Data from mouse models indicate that the gene is associated with skeletal phenotypes, including decreased bone mineral content and density, and decreased bone strength.[51,52]

Our phenotype, benzodiazepine-related falls, has been differently defined in both studies. Within the Rotterdam Study, falls resulting in a hospital admission or facture – serious falls – were assessed. Thereby, a connection to bone phenotypes may be plausible, especially as our observed association was mainly driven by the Rotterdam Study. However, there are more potential pathways, as mouse models also indicate that FAM73B is associated with other phenotypes including, craniofacial, homeostasis/metabolism, and immune system.[51,52] Overall, these potential explanations of our observed association remain speculative, because it is as of yet unknown whether the variant affects the function of FAM73B. Another option might be that the variant affects another gene – yet unknown –, as in the example of the obesity-associated variant, which was located in the intron of one gene but appeared to affect another gene.[56]

This study has strengths and limitations. Its major strengths is the detailed assessment of the phenotype, because of the prospective design of the study and because medication use could be determined at the time of the fall incident. Nevertheless, the heterogeneity between the study results could be caused by the different effect sizes, the Rotterdam study: OR 0.34 (95% CI 0.22; 0.52), p= 6.56*10-7, and B-PROOF OR 0.11 (95% CI 0.03; 0.46) p= 2.51*10-3. Otherwise, the heterogeneity is possibly due to the difference in fall definition used in both studies. In a previous epidemiologic study regarding the association

between benzodiazepine use and fall incidents, we had no indication that difference in fall definition would substantially affect the association.[57] However, with respect to the genetic background of benzodiazepine-related falls, this may in fact have an effect and warrants further investigation. In this particular case, the variant might be more related to serious consequences of a fall, e.g., a fracture, than to the fall itself. However, within the large GWAS on bone mineral density and fracture loci from the GEFOS consortium this variant was not reported.[58] It should be noted that this is still in the light of the above reported speculative explanation of our finding. Overall our study is limited with respect to its sample size. Another point to consider is the difference in exposure definition between the cases and the control. In cases, exposure at the time of the fall could be determined, while in controls exposure was defined as having at least one benzodiazepine dispensing record during the follow-up. This could have affected our observed outcome.

For future studies, it might be interesting to perform a replication study for both phenotypes, benzodiazepine-related falls and benzodiazepine-related serious falls. Thereby, a potential difference in underlying genetic mechanisms might be disentangled.

In conclusion, our study indicates that a specific variant significantly modifies benzodiazepine-related fall incidents, in a population of older individuals. Replication and functional studies are warranted to verify and support our observed finding.

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3. MEDICATION, HOMOCYSTEINE AND BONE MINERAL DENSITY
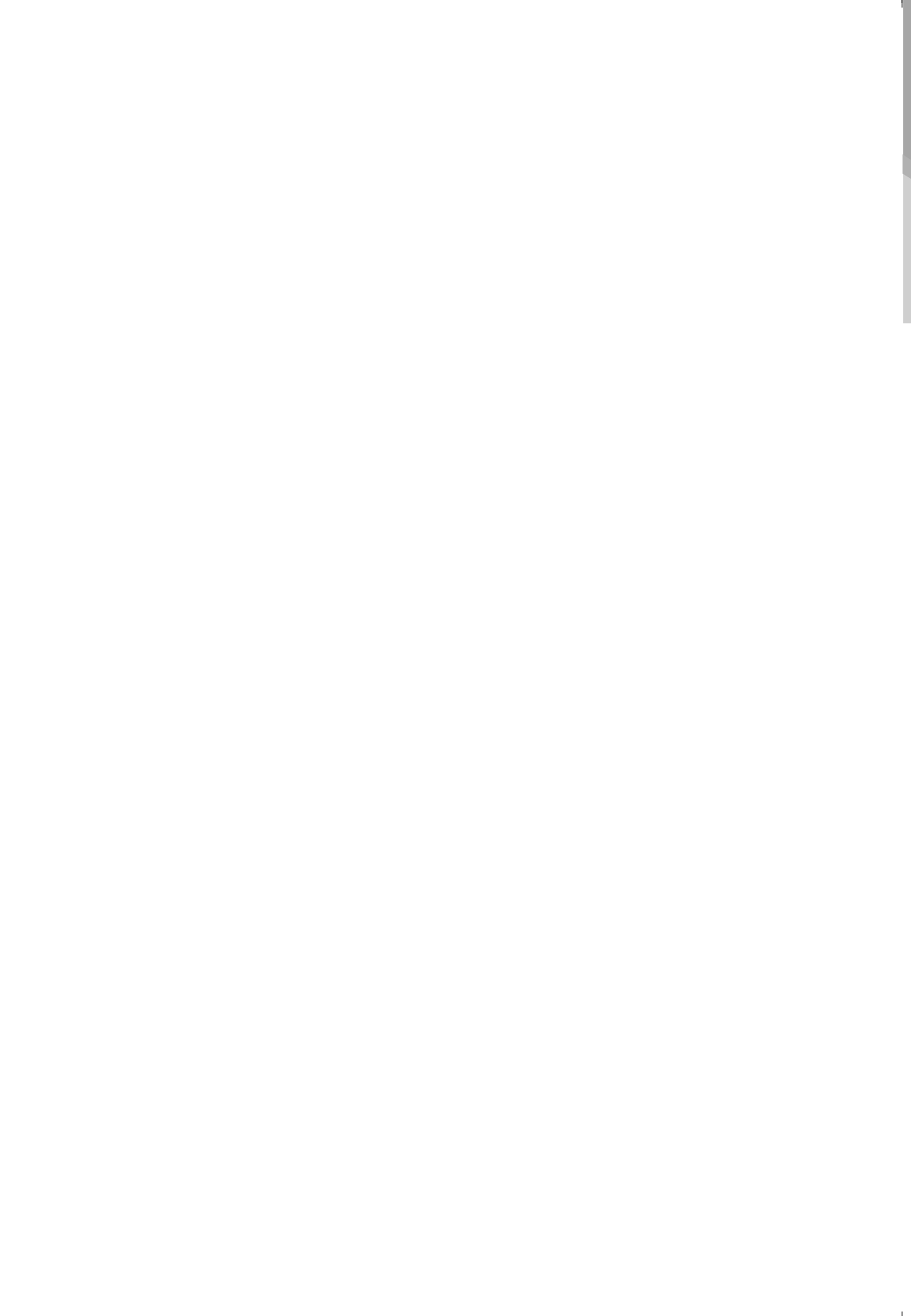
3.1

Associations between medication use and homocysteine levels in an older population, and potential mediation by vitamin B$_{12}$ and folate: data from the B-PROOF study

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Abstract

Background
Elevated homocysteine levels are a risk indicator for cardiovascular disease, fractures and cognitive decline. Previous studies indicated associations between homocysteine levels and medication use, including antihypertensive, lipid-lowering and antidiabetic medication. However, results were often contradictory and inconclusive. Our objective was to study the associations established previously in more detail by sub-classifying medication groups, and investigate the potential mediating role of vitamin B_{12} and folate status.

Materials and methods
Baseline data from the B-PROOF (B-vitamins for the PRevention Of Osteoporotic Fractures) study were used. We included 2,912 participants aged ≥65 years, with homocysteine levels of 12-50 µmol/L, creatinine levels ≤150 µmol/L, for whom self-reported medication data were available. We used multivariable linear regression models and analysis of covariance to assess the association between medication use and plasma homocysteine levels, and the potential mediation by serum vitamin B_{12} and folate.

Results
The mean age was 74 years (standard dieviation 6.5), 50% were women, and median homocysteine levels were 14 µmol/L [inter quartile range: 13-17 µmol/L]. Higher mean homocysteine levels were observed in users vs. non-users for diuretics (15.2 vs. 14.9, p= 0.043), high-ceiling sulphonamide diuretics (16.0 vs. 14.9, p <0.001), medication acting via the renin-angiotensin system (15.2 vs. 14.9, p= 0.029) and metformin (15.6 vs. 15.1, p= 0.006). Non-selective β-blocker use was associated with lower mean homocysteine levels (14.4 vs. 15.0, p= 0.019). Only this association was mediated by an underlying association with vitamin B_{12} and folate levels.

Conclusion
The associations between homocysteine levels and medication use appear to be fairly modest. Our results suggest that medication use is unlikely to contribute to clinically relevant changes in plasma homocysteine levels.

Key points

- In a population of mildly hyperhomocysteinemic persons aged ≥65 years, use of diuretics (and the subgroup high-ceiling sulphonamide diuretics), medication acting via the renin-angiotensin system, and metformin use was associated with higher plasma homocysteine levels.
- Non-selective β-blocker use was associated with lower homocysteine levels, and this was the only association that was characterized by an underlying association with vitamin B_{12} and folate levels.
- The differences in homocysteine levels between medication users and non-users were relatively modest (ranging from 0.3 to 1.1 μmol/L), suggesting that medication use is unlikely to contribute to clinically relevant changes in plasma homocysteine levels.

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Introduction

Hyperhomocysteinemia, >15 µmol/L, is prevalent in 10-30% of older Dutch individuals[1] and is one of the risk indicators of cardiovascular disease,[2,3] fractures,[4-6] and cognitive decline.[7,8] Homocysteine is an amino acid formed from methionine, an essential amino acid obtained from the diet.[9] Important causes for elevated homocysteine levels are reduced intake or absorption of vitamin B_{12} and/or folic acid.[9] Therefore, homocysteine levels can be lowered by supplementation of B vitamins, thereby potentially reducing the negative outcomes mentioned above. Nevertheless, trials assessing the effect of B vitamin supplementation were not able to show a benefit in preventing cardiovascular events,[10] whereas evidence for fractures[11] and cognitive decline[12,13] is still limited and inconclusive. In addition, homocysteine levels are affected by a genetic polymorphism in the methylenetetrahydrofolate reductase (MTHFR) gene, C677T. This polymorphism results in a reduced activity of the MTHFR enzyme that is required for the remethylation of homocysteine to methionine, thereby increasing homocysteine levels, especially in combination with low folate levels.[14] Besides B vitamin status and the MTHFR polymorphism, homocysteine levels may also be influenced unintentionally by several medications. This is particularly a problem in older individuals, because they are more prone to unintended medication effects as a result of their age-related changes in pharmacokinetics and pharmacodynamics.[15,16] Furthermore, the majority of older individuals have one or more chronic diseases and often multiple medicines are used concomitantly.[16-18] The use of antihypertensive, lipid-lowering, antidiabetic, antirheumatic, anticonvulsant and anti-Parkinson disease medication[19-21] has been associated with homocysteine levels (Table 1). Nevertheless, limited evidence is available per medication group and the studied subgroups were small. Additionally, outcomes are partly contradictory and inconclusive. Another issue that has as yet received little attention is the potential mediating role of vitamin B_{12} and folate. More insight into possible medication-related changes in homocysteine levels and the role of these vitamins could help to create clinical awareness, and might suggest the monitoring of these levels during the use of specific medications.

Overall, our objective is to study in more detail the associations suggested in the literature between medication use and homocysteine levels, by sub-categorising medication groups into therapeutic and chemical subgroups, in a large population of older community-dwelling individuals with mildly elevated homocysteine levels. By extending the models by adding vitamin B_{12} and folate levels, the potential mediation by these vitamins is also examined.

Methods and materials

Study design and population
For this cross-sectional study, we used baseline data from the B-PROOF study. B-PROOF is an acronym for 'B-vitamins for the PRevention Of Osteoporotic Fractures', its study design has been described elsewhere in more detail.[11] Briefly, it is a multi-centre, randomized, placebo-

controlled, double-blind intervention study investigating the efficacy of vitamin B_{12} and folic acid supplementation on the prevention of fracture incidence in persons aged ≥65 years with mildly elevated homocysteine levels. In total, 2,919 participants were included from the surroundings of the three Dutch University study centres in Wageningen, Rotterdam and Amsterdam. All participants had mildly elevated homocysteine levels (12-50 µmol/L), sufficient renal function (creatinine ≤150 µmol/L), and had not received intramuscular injections of vitamin B_{12} or folic acid supplementation (>300 µg daily). Additionally, they had not reported malignancies in the past 5 years. The Medical Ethics Committee of Wageningen University approved the study protocol, and the Medical Ethics Committees of Erasmus Medical Centre and VU University Medical Center gave approval for local feasibility. Before entering the study, all participants gave written informed consent.

Medication and supplement use

Self-reported medication and supplement use was gathered at the beginning of the study by a structured questionnaire filled out at home. Participants were categorised as users or non-users per medicine. Medicines were coded and grouped according to the Anatomical Therapeutic Chemical (ATC)-code system.[49] To investigate the main group effect we used ATC-2 levels (e.g. A01). To investigate the effect of therapeutic subgroups we used ATC-3 levels (e.g. A01A) and for class effects the chemical subgroups were used, i.e. ATC-4 levels (e.g. A01AA). Identical chemical substrates used for different indications have different ATC codes, such codes were combined because they can be expected to have the same therapeutic and adverse effects. Thus, for example, β-blockers used for cardiovascular treatment were combined with β-blockers used in eye treatment. The selected medication groups of interest were based on the literature (Table 1) and categorised according to the above-described method.

Laboratory measurements

At baseline, blood samples were collected in the morning when participants were either in a fasting state or had consumed a restricted breakfast. Plasma homocysteine levels were assessed from blood collected in EDTA tubes, which was stored on ice after blood collection and processed within 4h. To determine homocysteine, Wageningen University used high-performance liquid chromatography (intra assay coefficient of variation [CV]= 3.1%, inter assay CV= 5.9%), Erasmus MC used liquid chromatography-mass spectrometry/ mass spectrometry [LC-MS/MS] (intra assay CV= 5.5%, inter assay CV= 1.3%), and VU University Medical Center used the Architect i2000 RS analyzer (intra assay CV= 2%, inter assay CV= 4%). Cross calibration of the assays indicated no significant difference in outcome measures between the different methods.

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Table 1. A summary of previous studies regarding the association between medication use on homocysteine, vitamin B_{12} and folate levels.

Medication groups	Homocysteine	Vitamin B_{12}	Folate
Antihypertensive drugs			
Diuretics	↑[20,22]	↔[20]	↓[20]
Thiazides	↑[23,24]	↔[24]	↔[24]
β-blockers			
Selective β-blockers	↓[20,23]		
ACE inhibitors	↓[23] ↔[24,25]	↔[24]	↔[24]
Dihydropyridine derivatives	↔[23]		
Lipid-lowering drugs			
Statins	↑[26] ↓[27] ↔[20]		↑[26]
Fibric acid derivatives	↑[20]	↔[20]	↔[20]
Cholestyramine	↑[19]		
Antidiabetic drugs			
Biguanides	↑[28-31] ↔[32]	↓[28-33]	↓[29-31] ↔[32]
Sulphonylurea derivatives	↓[34,35]		
Rosiglitazone	↓[30]	↔[30]	↔[30]
Insulin	↓[19]		
Peptic ulcer and GORD drugs			
Proton Pump Inhibitors		↓[36,37] ↔[38]	↔[39]
H2-receptor antagonist		↓[37] ↔[36]	
Antirheumatic drugs			
Methotrexate	↑[19]		↓[19,40]
Sulfasalazine	↑[19]		↓[19]
Anticonvulsant drugs	↑[41,42]	↔[42]	↓[42] ↔[41]
Phenytoin	↑[42]	↔[42]	↓[42]
Carbamazepine	↑[43]	↔[42,43]	↓[43] ↔[42]
Valproic acid	↑[43]	↓[43] ↔[42]	↔[42,43]
Anti-Parkinson drugs			
L-dopa	↑[44]	↔[45,46]	↔[45,46]
Other drugs			
Oestrogens	↓[19]		
Tamoxifen/raloxifene	↓[19]		↑[19]
Theophylline	↑[19]		
Acetylcysteine	↓[19]		
Acetylsalicylic acid	↔[47]	↓[48]	

A positive association is indicated as ↑, and negative association as ↓. When no association was observed a ↔ is used, and an open space indicated no reported results.

Serum was used to determine folate, holotranscobalamin (HoloTC), methylmalonic acid (MMA) and creatinine. Folate was determined by immunoelectrochemiluminescence on a Roch Modular E170 (Roche, Almere, The Netherlands) (CV= 5.9% at 5.7 nmol/l and 2.8% at 23.4 nmol/l). A folate deficiency was defined as folate levels <10 nmol/L. HoloTC was measured using the AxSYM analyser (Abbott Diagnostics, Hoofddorp, The Netherlands) (C <8%) and MMA was measured using LC-MS/MS (CV <9%). HoloTC was used as measure of vitamin B_{12} status, because it has been shown to better reflect vitamin B_{12} status than serum total vitamin B_{12}.[50] A vitamin B_{12} deficiency was defined as both HoloTC levels <32 pmol/L and MMA levels >0.45 µmol/L.[50] Creatinine was measured using the enzymatic colorimetric Roche CREA plus assay (CV= 2%) on a Modular P analyser (Roch, Almere, The Netherlands). It was used to calculate the modification of diet in renal disease (MDRD) for men using the formula: $186 * (\text{serum creatinine (µmol/L)} / 88.4)^{-1.154} * \text{age}^{-0.203}$ (years) in ml/min/1.73m², and for women: $186 * (\text{serum creatinine (µmol/L)} / 88.4)^{-1.154} * \text{age}^{-0.203}$ (years) * 0.742 in ml/min/1.73m². The MDRD is an age-adjusted estimate of the glomerular filtration rate (GFR).[15]

Buffy coats were used to isolate DNA. The MTHFR genotypes, 677CC, 677CT or 677TT, were determined using the Illumina Omni-express array (Illumina Inc., San Diego, CA, USA).

Covariates
Demographic characteristics were ascertained using a questionnaire. The questions were on age, sex and health status variables, which included smoking habits, alcohol consumption, cardiovascular disease history, diabetes mellitus and hypercholesterolemia. A positive cardiovascular disease history was defined as having a history of at least one of the following disorders: heart problems (i.e. myocardial infarction, angina pectoris, heart failure, percutaneous coronary intervention), intermittent claudication, transient ischemic attack, stroke, thrombosis or embolism. During the baseline study visit, various characteristics were measured including weight, height and blood pressure.[11] Weight was measured with the participant wearing light garments. Height was measured with the participant standing erect, wearing no shoes. From weight and height, the body mass index (BMI, kg/m²) was calculated. Blood pressure was measured twice, using an Omron M1 plus device (Omron Healthcare Europe, Hoofddorp, The Netherlands), and the lowest diastolic and corresponding systolic blood pressure readings were included in the analyses. Hypertension was defined as systolic blood pressure >140 mmHg or diastolic blood pressure >90 mmHg.[51]

Statistical analyses
We assessed normal distribution for all variables, applying natural log transformation if necessary. Values were transformed back (e^x) and results were presented as geometric beta, 95% confidence intervals or means to facilitate the interpretation. Linear regression was used to determine associations between medication use and homocysteine levels (model 1, crude). When the number of users in a medication group was less than 1% of the overall population, only the crude association with homocysteine levels was determined and no

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further analyses were applied. Associations between covariates and homocysteine levels were assessed also using linear models. When a covariate had a *p*-value <0.2 it was considered as a potential confounder. Subsequently, age, sex and MDRD were added as fixed confounders while the other confounders were added using the forward-selection method (model 2). Medication use that was significantly associated with homocysteine levels according to the results of model 2, were added in model 3. Thereby we adjusted the associations for confounding effects of concomitantly used medication. To address the potential mediating effect of vitamin B_{12} and folate levels, these factors were subsequently added to model 3. When the point estimate of interest changed >10%, vitamin B_{12} and/ or folate levels were regarded as potential mediators. Thereafter, similarly as for homocysteine, models 1 and 2 were formed, but now using HoloTC or folate as the dependent variable. Confounders for these associations were also determined in the same way as for homocysteine.

To investigate whether the MTHFR genotype was an effect modifier, an interaction term was added to the fully adjusted model. When the p-value of the interaction term was <0.1, the results were stratified.

Analysis of covariance was used to calculate the estimated means for homocysteine levels in users and non-users, only for the significant findings based on model 3. Additionally, it was used to investigate whether there was an additive effect of combined use of significantly associated medication groups. Then, the estimated means for homocysteine levels were calculated for non-users and users of, respectively, one significantly associated medication group, two significantly associated medication groups and three or four significantly associated medication groups. Statistical analyses were done using the statistical software package SPSS version 21.0 (SPSS Inc., Chicago, IL, USA) and *p*-values <0.05 were considered to be statistically significant.

Results

Population characteristics
Self-reported medication use was available for 2,912 participants. The mean age of the study population was 74 years (± standard deviation 6.5) and 50% were women (Table 2). The median homocysteine level was 14 μmol/L [inter quartile range: 13-17] and a small percentage was deficient in vitamin B_{12} (4%) or folate (3%). A positive cardiovascular disease history was present in 38%, and 84% used at least one medicine.

Antihypertensive medication
The use of diuretics in general was positively associated with homocysteine levels (Table 3, model 3), users had a 0.3 μmol/L higher mean homocysteine than non-users , 15.2 vs. 14.9 μmol/L, *p*= 0.043 (Figure 1). Adding vitamin B_{12} and folate to the model changed the association slightly, resulting in mean homocysteine levels of 15.3 vs. 14.9 μmol/L, *p*= 0.004. Diuretic use was associated with both vitamin B_{12}, β= 5.58, *p*= 0.002 (adjusted

Table 2. Population characteristics.

	N= 2,912
Age (years)[a]	74 (6.5)
Sex (women, %)	50
BMI (kg/m^2)[a]	27 (4.0)
Smoking (%)	
Never	34
Former	56
Current	10
Alcohol consumption (%)	
Light	67
Moderate	29
Excessive	3
Very excessive	<1
Cardiovascular disease history (% yes)	38
Diabetic (% yes)	10
Vitamin B$_{12}$ supplement use (% yes)	15
Folate supplement use (% yes)	14
Hypertension	64
MDRD (ml/min/1.73m^2)[b]	74 [64-84]
Homocysteine (μmol/L)[b]	14 [13-17]
Vitamin B$_{12}$ (pmol/L)[b]	285 (116)
Folate (nmol/L)	21 (12)
MMA (μmol/L)[b]	0.23 [0.18-0.30]
HoloTC (pmol/L)[a]	72 (43)
Vitamin B$_{12}$ deficiency (%)[c]	4
Folate deficiency (%)[d]	3
MTHFR genotype(%)	
CC	45
CT	42
TT	13
Medication use (%)	84

[a] Presented as mean (± standard deviation). [b] Presented as median [inter quartile range]. [c 43as1] Defined as: HoloTC levels <32 pmol/L and MMA levels >0.45 μmol/L.[50] [d] Defined as: folate levels <10nmol/L.
BMI= body mass index, MDRD= modification of diet in renal disease, MMA= methylmalonic acid, HoloTC= holotranscobalamin, MTHFR= methylenetetrahydrofolate reductase.

for age, sex, MDRD and study centre), and folate levels, β= 1.06, *p*= 0.024 (adjusted for age, sex, MDRD, study centre, BMI, smoking and diabetes). High-ceiling sulphonamide diuretic use, i.e. furosemide or bumetanide, was also positively associated with homocysteine levels (Table 3, model 3). Users had a 1.1 μmol/L higher mean homocysteine than non-users,

16.0 vs. 14.9 µmol/L, p <0.001 (Figure 1). Extending the model with vitamin B_{12} and/or folate did not substantially alter the results. Furthermore, no association between high-ceiling sulphonamide use and vitamin B_{12} or folate was observed (data not shown). The use of other diuretic subgroups including thiazides, low-ceiling sulphonamides, aldosterone antagonists and potassium-sparing agents was not associated with homocysteine levels. Similarly, the use of β-blockers in general and selective β-blockers was not associated with homocysteine levels. In contrast, non-selective β-blocker use was associated with lower homocysteine levels, β= -1.04, p= 0.019 (Table 3, model 3, Figure. 1), but the association lost significance after adding vitamin B_{12} and folate to the model, β= -1.03, p= 0.115. Non-selective β-blocker use was associated with both vitamin B_{12}, β= 9.14, p= 0.011 (adjusted for age, sex, MDRD and study centre), and folate levels, β= 1.89, p= 0.035 (adjusted for age, sex, MDRD, study centre, BMI, smoking and diabetes). Use of medication acting via the renin-angiotensin system was positively associated with homocysteine levels (Table 3, model 3). Users had a 0.3 µmol/L higher mean homocysteine level than non-users, 15.2 vs. 14.9 µmol/L, p= 0.029 (Figure 1). Adding vitamin B_{12} to the model changed the association slightly, resulting in mean homocysteine levels of 15.2 vs. 14.8 µmol/L, p= 0.005, while adding folate had no effect. Use of medication acting via the renin-angiotensin system was positively associated with vitamin B_{12} levels, β= 5.44, p= 0.005 (adjusted for age, sex, MDRD, study centre and cardiovascular disease history). In contrast, the use of renin-angiotensin subgroup ACE inhibitors and angiotensin II inhibitors was not associated with homocysteine levels. Similarly, no association was seen between the use of calcium antagonists and, more specifically, dihydropyridine derivatives and homocysteine levels.

Lipid lowering medication

The use of statins was not associated with homocysteine levels (Table 3, model 2). None of the participants reported nicotinic acid use.

Peptic ulcer and gastro-oesophageal reflux disease medication

Neither proton pump inhibitor use nor histamine H_2-receptor antagonist use was associated with homocysteine levels (Table 3, model 2).

Antidiabetic mediation

Metformin, a biguanide, was positively associated with homocysteine levels (Table 3, model 3). Users had a 0.5 µmol/L higher mean homocysteine level than non-users, 15.6 vs. 15.1 µmol/L, p= 0.006 (Figure 1). Adding folate to the model changed the association slightly, resulting in mean homocysteine levels of 15.7 vs. 15.0 µmol/L, p=0.001. Metformin use was associated with folate levels, β= 1.76, p= 0.034 (adjusted for age, sex, MDRD, study centre, BMI and smoking). The use of sulphonylurea derivatives, and insulin was not associated with homocysteine levels (Table 3, model 3).

Table 3. Linear regression results for natural logarithm (ln) homocysteine per medication group, presented as geometric β (*p*-value).

	Number of users (%)	Model 1 Crude	Model 2	Model 3
Antihypertensive drugs				
Diuretics	774 (26.6)	1.05 (<0.001)	1.03 (0.001)[a*]	1.02 (0.043)[c*]
Thiazides	520 (17.9)	1.02 (0.047)	1.01 (0.175)[a]	
Low-ceiling sulphonamides	75 (2.6)	1.03 (0.216)	1.04 (0.156)[a]	
High-ceiling sulphonamides	146 (5.0)	1.14 (<0.001)	1.08 (<0.001)[a*]	1.07 (<0.001)[c*]
Aldosterone antagonist	50 (1.7)	1.09 (0.004)	1.03 (0.286)[a]	
Potassium-sparing agents	75 (2.6)	-1.00 (0.933)	-1.01 (0.689)[a]	
β-blockers	797 (27.4)	1.02 (0.089)	-1.00 (0.712)[a]	
Non-selective β-blockers	145 (5.0)	-1.03 (0.096)	-1.04 (0.032)[a*]	-1.04 (0.019)[c*]
Selective β-blockers	652 (22.4)	1.03 (0.011)	1.01 (0.428)[a]	
α & β blockers	20 (0.7)	1.00 (0.937)		
Renin-angiotensin agents	966 (33.2)	1.04 (<0.001)	1.02 (0.009)[a*]	1.02 (0.029)[c*]
ACE inhibitors	475 (16.3)	1.04 (0.021)	1.02 (0.023)[a*]	1.02 (0.083)[c]
Angiotensin II antagonist	507 (17.4)	1.03 (0.004)	1.01 (0.247)[a]	
Calcium antagonists	410 (14.1)	1.03 (0.014)	1.02 (0.176)[a]	
Dihydropyridine derivatives	334 (11.5)	1.03 (0.015)	1.02 (0.106)[a]	
Lipid-lowering drugs				
Statins	724 (24.9)	1.02 (0.033)	-1.00 (0.759)[a]	
Fibric acid derivatives	7 (0.2)	1.06 (0.472)		
Cholestyramine	3 (0.1)	-1.11 (0.365)		
Antidiabetic drugs				
Metformin	215 (7.4)	1.04 (0.002)	1.05 (0.001)[b*]	1.04 (0.006)[d*]
Sulphonylurea derivatives	104 (3.6)	1.06 (0.006)	1.04 (0.037)[b*]	1.01 (0.583)[d]
Thiazolidinediones	11 (0.4)	1.06 (0.365)		
Insulin	50 (1.7)	1.05 (0.101)	1.06 (0.034)[b*]	1.03 (0.276)[c]
Peptic ulcer and GORD drugs				
Proton Pump Inhibitors	612 (21)	1.01 (0.618)	-1.01 (0.617)[a]	
H2 antagonists	36 (1.2)	-1.03 (0.430)	-1.04 (0.241)[a]	
Antirheumatic drugs				
Selective immunosuppressants	2 (0.1)	1.41 (0.014)		
Other immunosuppressants	34 (1.2)	1.05 (0.149)	1.05 (0.165)[a]	
Anticonvulsant drugs	52 (1.8)	-1.02 (0.623)	-1.01 (0.753)[a]	
Barbiturates	2 (0.1)	-1.03 (0.847)		
Phenytoin	3 (0.1)	1.21 (0.095)		
Carbamazepine	4 (0.1)	-1.06 (0.549)		
Valproic acid	10 (0.3)	-1.07 (0.266)		

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Table 3. *(Continued)*

	Number of users (%)	Model 1 Crude	Model 2	Model 3
Anti-Parkinson drugs				
L-dopa & L-dopa derivatives	21 (0.7)	1.01 (0.896)		
Other drugs				
Oestrogens	26 (0.9)	-1.01 (0.735)		
Androgens	1 (<0.1)	1.20 (0.359)		
Raloxifene	7 (0.2)	-1.08 (0.321)		
Theophyline	49 (1.7)	1.45 (0.052)	1.20 (0.323)[a]	
Acetylcysteine	11 (0.4)	1.01 (0.811)		
Acetylsalicylic acid	726 (24.9)	1.02 (0.031)	-1.01 (0.355)[a]	

Model 1: crude model. [a] adjusted for age, sex, MDRD, smoking, diabetes. [b] adjusted for age, sex, MDRD, smoking. [c] confounders of [a], plus the other significantly associated drugs from model 2. [d] confounders of [b], plus the other significantly associated drugs from model 2. *P*-value <0.05.
GORD= gastro-oesophageal reflux disease, MDRD= modification of diet in renal disease.

Antirheumatic, anticonvulsant, anti-Parkinson disease and other medication

No association was observed between the use of the immunosuppressive agent methotrexate and homocysteine levels (Table 3, model 2). Methotrexate use was always combined with folate supplements; therefore, a correction for folate supplement use could not be made. We did not observe an association between the use of anticonvulsants and homocysteine levels (Table 3, model 2). Similarly, the use of acetylsalicylic acid and theophylline was not associated with homocysteine levels.

Additive effects and interaction with MTHFR

Medication groups that were positively associated with homocysteine levels were combined, and comprised of the use of diuretics in general (excluding high-ceiling sulphonamide diuretics), high-ceiling sulphonamide diuretics, medications acting via the renin-angiotensin system and metformin. Compared with those using none of these medicines (mean homocysteine level of 14.7 µmol/L), users of one of these medicines had a higher mean homocysteine level (15.2 µmol/L, *p*= 0.001), users of two medicines also had a higher mean homocysteine level (15.2 µmol/L, *p*= 0.003), and similarly those using three or four medicines had a higher mean homocysteine level (15.7 µmol/L, *p*= 0.011) (Figure 2). Because only six participants used all four medicines, this group was combined with the use of three medicines.

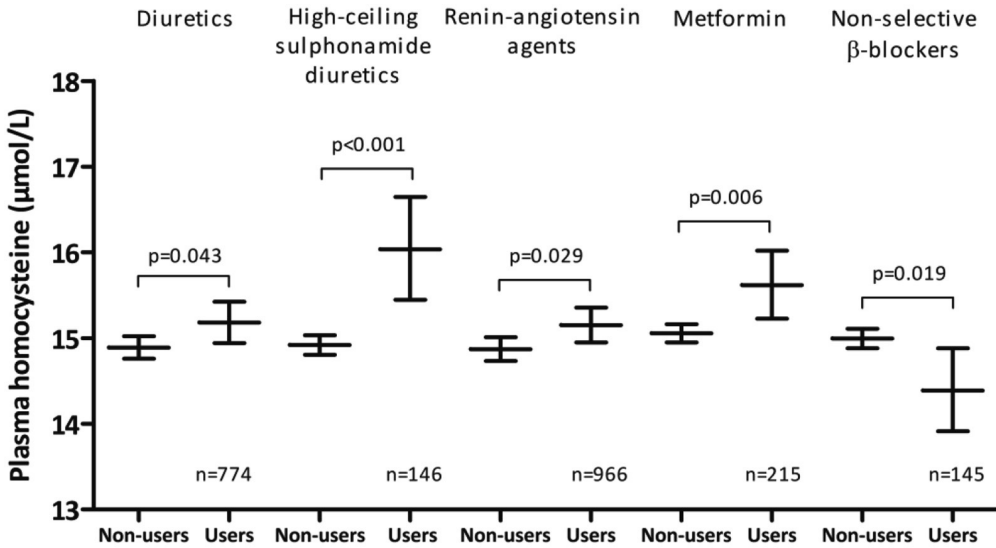   None of the interaction terms of a medication group with MTHFR was significant (data not shown).

Figure 1. Estimated geometrical mean (95% confidence interval) plasma homocysteine levels in users vs. non-users of diuretics, high-ceiling sulphonamide diuretics, renin-angiotensin agents, metformin, and non-selective β-blockers.
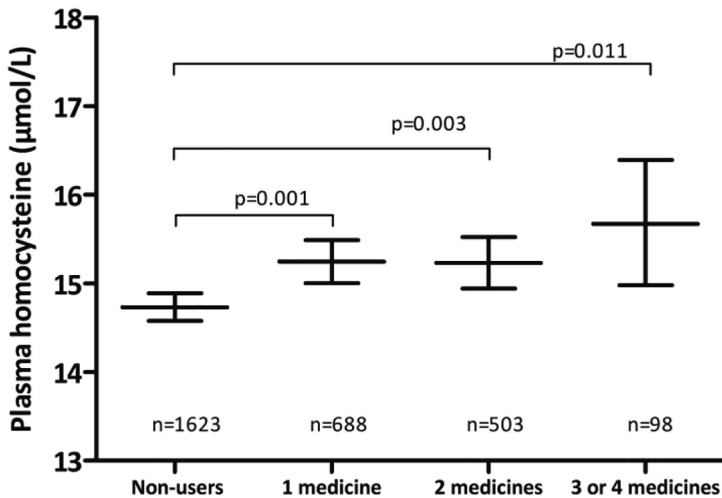

Figure 2. Estimated geometrical mean (95% confidence interval) plasma homocysteine levels in users of none, one, two, three or four of the significantly associated medication groups, which consisted of diuretics (excluding high-ceiling sulphonamide diuretics), high-ceiling sulphonamide diuretics, renin-angiotensin agents, metformin, and non-selective β-blockers.

Discussion

In this large population of older individuals, we demonstrated a small but significant positive association between plasma homocysteine levels and the use of diuretics in general, high-ceiling sulphonamide diuretics, agents acting via the renin-angiotensin system and metformin, which was largely independent of vitamin B_{12} and folate levels. When medication use of these drug groups was combined, no additive effect of the association was observed. In addition, an inverse significant association between non-selective β-blocker use and homocysteine levels was observed, which was characterised by an underlying association with vitamin B_{12} and folate levels. No association was observed between homocysteine levels and the use of thiazides, selective β-blockers, statins, sulphonylurea derivatives or anticonvulsants.

Diuretic use has previously been shown to be associated with higher homocysteine levels,[20,23] possibly through decreasing folate levels.[20] In our study, diuretic use in general was significantly associated with higher homocysteine levels, but this was independent of folate levels. From the diuretic subgroups, high-ceiling sulphonamides, i.e. furosemide or bumetanide, were strongly associated with higher homocysteine levels. To our knowledge, this association has not been reported before. Although the mechanism behind this elevation is unknown, high-ceiling sulphonamides are known as potent inhibitors of the reabsorption of electrolytes in the kidneys, resulting in reduced water reabsorption into the blood and thus increased water excretion.[52] This alteration in fluid status, or mild dehydration, might cause the observed relative increase of the plasma homocysteine level in the blood. This potential mechanism merits further investigation.

Other investigators observed a negative association between the use of β-blockers and homocysteine levels, especially metoprolol, which is a selective β-blocker.[20] However, we found no associations for selective β-blocker use, but did observe an association with homocysteine levels for non-selective β-blocker use, which depended on the mediating association with vitamin B_{12} and folate levels. How non-selective β-blockers may affect vitamin B_{12} and folate levels is still unknown.

The use of medication acting on the renin-angiotensin system was associated with slightly higher homocysteine levels. Previous studies only investigated the association with its subgroup ACE inhibitors, reporting a negative[23] or no[24,25] association with homocysteine levels. The mechanism behind our finding remains to be determined.

Our finding of higher homocysteine levels in metformin users is in agreement with findings of earlier studies.[28-31] It has been suggested that metformin reduces the absorption of vitamin B_{12}[21] and negatively affects folate status through an unknown mechanism.[28-31] However, this contradicts our finding of a relationship between metformin and homocysteine levels that was independent of vitamin B_{12} and which became slightly stronger after including folate levels into the model. This indicates that metformin might affect homocysteine levels through other mechanisms. Metformin increases insulin sensitivity,[21] and potentially insulin levels are involved. Nevertheless, the literature reports contradictory findings, as both insulin

sensitivity[53] as well as insulin resistance[54] were associated with higher homocysteine levels. More research is needed to elucidate the potential involvement of insulin levels.

A surprising result was the lack of association between peptic ulcer and gastro-oesophageal reflux disease (GORD) medication use and homocysteine levels. Because the literature consistently reports an association between lower levels of vitamin B_{12} and the use of peptic ulcer and GORD medication,[36,37,39,55] we hypothesised that users would also have higher homocysteine levels. It has been suggested that prolonged proton pump inhibitor use in particular results in lower vitamin B_{12} status and higher homocysteine levels.[36] The discrepancy in results might be caused by the fact that we were unable to distinguish between short- and long-term users, since our data were cross-sectional.

Although statin use has previously been associated with both an increase[26] and a decrease in homocysteine levels,[27] it is most frequently reported not to be associated.[20] Our results are in line with this absence of an association.

We did not observe a positive association between anticonvulsant drug use and homocysteine levels, while this has been consistently reported in the literature.[41-43] Anticonvulsant drugs may reduce folate levels and particularly in combination with the MTHFR TT genotype this may result in elevated homocysteine levels.[56,57] The discrepancy between our findings and other studies may be explained by the non-significant interaction with the MTHFR polymorphism and the low frequency of folate deficiency in our population.

The anti-Parkinson disease medication L-dopa has also been associated with increased levels of homocysteine;[44] however, because of the low frequency of users we could not test this association and a potential association therefore cannot be ruled out.

When the use of medication groups that were significantly associated with a higher homocysteine level was combined, no additive effect of the association was observed. To our knowledge, there is no literature regarding a potential additive effect of a combination of medication use on homocysteine levels. Nevertheless, because of multiple drug use, older individuals are more prone to unintended drug effects[16,18] of which increased homocysteine levels might have been one.

This study has some limitations. First, all participants had mildly elevated homocysteine levels, which may have reduced the contrast of the effect, because of the smaller range in homocysteine levels. Conversely, the observed associations may result in even greater differences in the overall population. Furthermore, despite the homocysteine cut-off value of 12 μmol/L, our study population represents approximately 30% of the general Dutch older population, as this percentage is known to have an increased homocysteine level.[1] Second, self-reported medication data were used, for which accuracy relies on the memory of the participant and recall bias might subsequently result in misclassification of exposure. In that respect, pharmacy supplied reports would have been a possible solution and would also have provided information about dose and duration of use, although they represent prescription data and not actual use, and over-the-counter medication still may be missed. Nevertheless, none of these two methods has been considered to be the 'gold standard' for measuring medication use,[58] and various studies have reported a high agreement

between both methods.[58-61] Third, pharmaco-epidemiological studies in general are prone to confounding by indication. We, however, do not think this would explain our significant results, as in addition to associations with diuretics in general and more specifically high-ceiling sulphonamides, and metformin use, associations with the use of other diuretic subgroups and diabetic medication would have also been expected, while these were not observed. Fourth, based on the number of medication groups investigated, a chance finding could have occurred. Finally, because this is a cross-sectional study, we cannot draw any conclusions about cause or effect. Despite the limitations, our study also has its strengths; it was conducted in a large population and addressed hyperhomocysteinemia as an unintended drug effect in an important target group, namely older persons. We also investigated whether the association between medication use and homocysteine levels was mediated by an underlying association with vitamin B_{12} and/or folate, as levels of these vitamins are key determinants for homocysteine levels.[62]

Conclusion

In a population of mildly hyperhomocysteinemic older persons, we have confirmed that diuretic use in general and metformin use is indeed associated with higher homocysteine levels. In addition, we have shown that users of the diuretic subgroup high-ceiling sulphonamides, and users of agents acting via the renin-angiotensin system had higher homocysteine levels than non-users. The difference in homocysteine levels between users and non-users had a range of 0.3-1.1 µmol/L. However, the question is whether these small differences are clinically relevant. Non-selective β-blocker users had lower homocysteine levels than non-users. Only the association with non-selective β-blocker use was mediated by an underlying association with vitamin B_{12} and folate levels, so additional research is needed to elucidate underlying mechanisms for metformin, diuretics and medication acting via the renin-angiotensin system. Other medications, belonging to the group of antihypertensives, lipid lowering agents, antidiabetic drugs, peptic ulcer and GORD medications, antirheumatic drugs, anticonvulsant drugs and anti-Parkinson medication were not associated with homocysteine levels.

Taken together, in this study, the associations between medication use and homocysteine levels appear to be fairly modest, suggesting that medication use is unlikely to contribute to clinically relevant changes in plasma homocysteine levels.

Acknowledgments

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3.2

Use of SSRIs and bone mineral density change: a population based longitudinal study in middle aged an elderly individuals

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Abstract

Background

Longitudinal studies showed conflicting results regarding the association between use of SSRIs and bone mineral density (BMD). Therefore, we investigate the association between – duration of – SSRI use and BMD, and change in BMD.

Methods

Data from the population-based Rotterdam Study cohort (1991-2008) were used. In total, 4,915 men and 5,831 post-menopausal women, aged ≥45 years were included, having measurement visits at 4-5 year intervals. Multivariable linear mixed models were applied to examine the association between SSRI use, based on pharmacy records, duration of SSRI use, and repeated measures of BMD, and changes in BMD, compared to non-use. Femoral neck BMD (g/cm^2) was measured at 4 visits, comprising 19,861 BMD measurements. Three delta BMD periods were examined, comprising 7,897 delta BMD values. Delta BMD was expressed in the annual percentage change in BMD between 2 consecutive visits.

Results

In men and women, we observed no association between SSRI and BMD when compared to non-use (women: mean difference 0.007 g/cm^2, 95% CI -0.002; 0.017, p-value= 0.120). We did not find an association between duration of SSRI use and change in BMD (women: annual percentage change -0.081, 95% CI -0.196; 0.033, p-value= 0.164).

Conclusion

In conclusion, use of SSRIs is not associated with BMD or change in BMD – after taking duration of treatment into account – in middle-aged and elderly individuals. Therefore, our results question previously raised concerns on the adverse effects of SSRIs on BMD.

Introduction

Loss of bone mass is associated with an increased risk of osteoporosis and fractures, and is a major problem in older individuals, particularly in women. It has serious consequences for quality of life, health outcomes and related costs.[1,2] Important risk factors for loss of bone mass include advanced aging, low body weight, reduced physical activity, poor nutritional status, smoking and alcohol use.[3] In addition, multiple drugs are suspected to negatively affect bone mass (e.g., glucocorticoids and thyroid hormones).[4-6] Selective serotonin reuptake inhibitors (SSRIs), a frequently used drug group in elderly, are also suspected to have a direct negative effect on bone health.[3,7,8] SSRIs modulate serotonin levels, and major bone cell types such as osteoblasts, osteoclasts and osteocytes carry serotonin receptors and transporters, therefore, SSRIs could play a regulatory role in bone turnover.[7,8] Peripheral serotonin could reduce osteoblast proliferation, and consequently negatively affect bone formation.[8]

The association between SSRIs and bone mineral density (BMD) has been investigated in various observational studies. Cross-sectional studies almost consistently reported an association between use of SSRIs and low BMD.[9-14] However, these results are not supported by longitudinal observational studies, conducted in women only, which reported conflicting results.[15-17] Moreover, in previous cross-sectional and longitudinal studies, use of SSRIs was assessed through interview data and limited information was available regarding duration of use at the study visit or in-between study visits.[9-17] Since bone remodeling is a slow process,[18] we expect that longer duration of SSRI use is an important risk factor for low BMD.

The present study aims to investigate the association between use of SSRIs (yes/no) and duration of treatment – assessed with pharmacy records over 4 year periods – with BMD, and change in BMD in a population-based cohort of middle-aged and older men and postmenopausal women.

Methods

Study setting
The Rotterdam Study is a prospective population-based cohort study, its design, objectives and methods have been described in more detail,[19] In short, the study was initiated in 1989 and in total 7,983 participants, aged ≥55 years, were included between 1989-1993 (78% response rate, cohort I). In 2000, the study was extended with a second cohort of 3,011 participants, aged ≥55 years (67% response rate, cohort II). Additionally, in 2006 a second extension of the cohort was initiated, including 3,923 participants aged 45 years or older (65% response rate, cohort III). From baseline onwards, follow-up examinations were conducted every 4-5 years including interviews and an extensive set of examinations.

The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus MC and by the Ministry of Health, Welfare and Sport of the Netherlands, implementing the

"Wet Bevolkingsonderzoek: ERGO (Population Studies Act: Rotterdam Study)". All participants provided informed consent to participate in the study and to obtain information from their treating physicians.

Study population

We included participants with available pharmacy dispensing data and at least 1 BMD measurement during the follow-up period between 1991 and 2008. We restricted our population to men and postmenopausal women, thereby excluding measurements of pre- and peri-menopausal women, because of increased BMD loss around menopause.[1,2] Postmenopausal state was defined as previously described, in short: natural menopause – after 12 continuous months or amenorrhea –, surgical menopause, or assumed menopause after the age of 65 if detailed data were missing.[20] To avoid potential misclassification of exposure, we ensured that all participants had pharmacy dispensing records available for at least 5 months prior to their baseline measurement. Moreover, we excluded measurements of participants who used or initiated use of drugs affecting bone structure and mineralization, notably bisphosphonates (Anatomical Therapeutic Chemical [ATC]-code: 'M05B'), or used other antidepressant drugs than SSRIs or tricyclic antidepressants (TCAs) in six months before the BMD measurement or at time of the BMD measurement.

Exposure assessment

Pharmacy dispensing records from regional pharmacies were available from January 1st 1991 onwards. More than 95% of the participants fill their drug prescriptions at one of these pharmacies. Dispensing records include date of dispensing, total amount of drug units per dispensing, prescribed daily number of units, product name of the drug and corresponding Anatomical Therapeutic Chemical (ATC) code. Duration of a dispensing was calculated by dividing the total number of dispensed units by the prescribed daily number of units. The average prescribed daily dose was expressed in number of standardized defined daily doses (DDDs).

SSRIs were defined based on the 4th-level (chemical subgroup) ATC-code: 'N06AB'. To consider potential confounding by indication (i.e. depression), TCAs (ATC-code: 'N06AA') were also studied as exposure of interest. SSRI and TCA use was defined in multiple ways (Figure 1). First, current use (yes/no) was defined as a SSRI or TCA dispensing in the six months prior to the BMD measurement or at time of the BMD measurement. Non-use in the six months before and at the measurement round was considered as reference population. In addition, duration of treatment between two measurement rounds was taken into account to categorize current users in 1-364 days and 365 days or longer of current antidepressant use. Second, for the longitudinal analyses, duration of SSRI and TCA exposure was defined based on the sum of all prescription lengths (in years) between two consecutive BMD measurements. This exposure definition includes all use of SSRIs or TCAs between the measurement visits if there was at least one dispensing in the half year prior to or at the BMD measurement, otherwise exposure was coded as non-use. Our assumption was that any effect on BMD by SSRI would

vanish in 6 months after discontinuation of intake. Therefore, we assessed use of SSRI in the 6 months before BMD measurement in the cross-sectional assessment and calculated cumulative exposure to SSRI between two BMD measurements only for those patients who had used SSRI within 6 months before the second BMD measurement in the delta BMD assessment.

Outcome

BMD (g/cm^2) of the right proximal femur neck was assessed with Dual-energy X-ray absorptiometry (DXA) scanning.[21] Per participant one to four BMD measurements were available (between 1991 – 2008, Figure 1). On the first three BMD measurement visits – i.e. the first 3 visits of the first cohort and the first visit of the second cohort – a Lunar DPX-L densitometer was used, while from 2002 and onwards a Lunar Prodigy densitometer was used (Lunar Radiation Corp., Madison, WI, USA). Cross-calibration between the two devices showed no significant differences in BMD levels with a negligible slope.[22] We used femoral neck (FN) BMD instead of lumbar spine BMD, as this measurement is not affected by degenerative changes (i.e. osteophytes) increasingly seen with aging.[21] Our outcome measures are the BMD values (g/cm^2) at the visits, and the annual percentage change in BMD between two consecutive visits. The percentage change in BMD (ΔBMD) was calculated by subtracting the previous BMD value from its subsequent BMD value, divided by the previous BMD value and multiplied by 100. The percentage change was further divided by the number of years between the two consecutive BMD measurements resulting in the annual percentage change in BMD. In formula: $(((BMD_{i+1} - BMD_i)/BMD_i)*100)/$ (date BMD_{i+1} - date BMD_i in years). Up to three ΔBMD measurements were available per participant (Figure 1).

Covariables

We considered several covariables that could confound the association between use of SSRIs or TCAs and BMD.[2,4,23,24] These were; age, body mass index (BMI), lower-limb disability, depressive symptoms, smoking, alcohol intake, cognitive status, age at menopause, hypertension, diabetes mellitus and drugs known to be associated with BMD (i.e. thiazides, statins, glucocorticoids, thyroids, sex hormones, anticonvulsants, psycholeptics, and calcium and vitamin D supplements).[2,4,23,24] All covariables were time-dependent and determined at the time of the BMD measurement. Change in BMI and change in lower-limb disability over time were also considered as potential confounders in the analyses regarding delta BMD. BMI was defined as weight (in kilograms) divided by height (in meters squared). Lower-limb disability was assessed using a modified version of the Stanford Health Assessment Questionnaire. The score was based on answers to questions regarding rising, walking, bending, and getting in and out of a car.[25] Disability was defined as a score of 3 or higher.[25] The Center for Epidemiological Studies Depression Scale (CES-D),[26] and the Hospital Anxiety and Depression Scale (HADS-D)[27] in a subsample of the population, were used to screen for depressive symptoms. On both questionnaires, higher scores indicate more depressed feelings. The CES-D results in a score ranging from 0 to 60, with a score of 16 or higher being
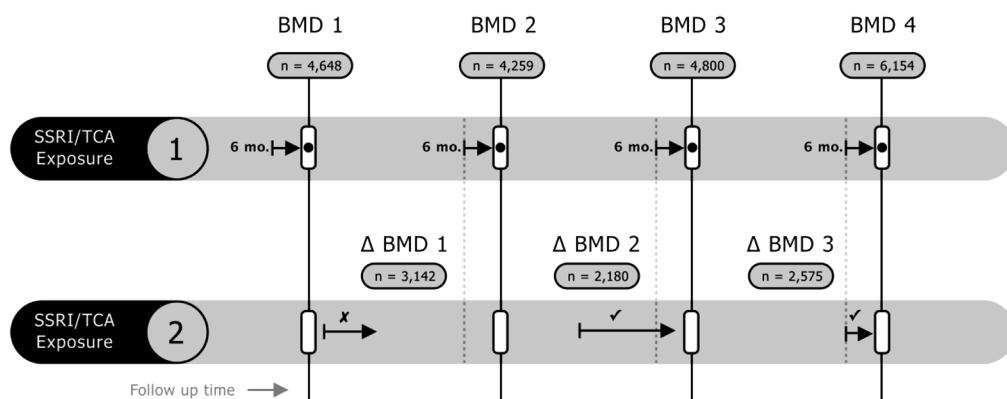
Figure 1. Definitions of antidepressant exposure (e.g. selective serotonin reuptake inhibitors and tricyclic antidepressants) and bone mineral density or change in bone mineral density.

1 = current antidepressant use – in the six months before or at time of – the bone mineral density measurement.

2 = duration of antidepressant use (in years) assessed between two consecutive bone mineral density measurements. This exposure definition includes all use of SSRIs or TCAs between the measurement visits if there was at least one dispensing in the half year prior to or at the BMD measurement, otherwise exposure was coded as non-use.

Three examples of duration of antidepressant use are given in the form of an arrow. The arrow with a 'x' represents a duration of antidepressant exposure that was coded as non-use. The other two arrows, with a '✓', represent duration periods that are included in 'duration of antidepressant use'.

BMD 1: Participants from cohort I, visit 1
BMD 2: Participants from cohort I, visit 2
BMD 3: Participants from cohort I, visit 3 and cohort II, visit 1
BMD 4: Participants from cohort I, visit 4, cohort II visit 2 and cohort III, visit 1
Abbreviations: BMD = bone mineral density, ΔBMD = change in bone mineral density

indicative of clinically-relevant depressive symptoms. The HADS-D consists of 7 items, and its score ranges from 0 to 21, with 9 points or higher being indicative of depressive symptoms. These cut-offs have a high sensitivity for detecting relevant depressive symptoms in the general and older adult population.[28,29] Cognitive status was assessed with the Mini Mental State Examination (MMSE).[30] Smoking behavior (current, former or never) was determined based on interview data. When data on a round was missing, the results from the preceding round were imputed to minimize the number of missing values. Alcohol intake was assessed based on interview or food frequency questionnaire data.[31] Age at menopause was also based on interview data, and validated as previously described.[20] Hypertension was defined as a systolic blood pressure of 140 mmHg or higher, a diastolic blood pressure of 90 mmHg or higher, or the use of blood pressure lowering medication for the indication hypertension.

Diabetes mellitus status was based on the use of any glucose lowering drugs (ATC-code= 'A10'). Other potential confounding drug use was assessed with pharmacy records: thiazides ('C03A', 'C03EA'), statins ('C10A'), sex hormones ('GO3'), glucocorticoids ('H02AB' or 'R03BA'), thyroid therapy ('H03'), anticonvulsants ('N03A') and psycholeptics ('N05'). Besides, calcium and vitamin D supplementation use ('A12') was assessed at home interview. Here, if data on a round was missing, the results from the preceding round were imputed to minimize the number of missing values.

Statistical analyses

Baseline characteristics were defined based on data from the first eligible BMD measurement. We used linear mixed models to examine the association between current use of SSRIs or TCAs (yes/no) and repeated measurements of BMD. Linear mixed models take the within-person correlation between multiple visits into account. We selected the unstructured correlation matrix based on the lowest Akaike's Information Criterion (AIC).[32] Additionally, current use was stratified on duration of use (1-364, ≥365 days) between the measurements of BMD. Furthermore, we studied a possible dose-response relationship between the average number of prescribed DDDs, split on the median prescribed DDD, and BMD. Non-use of any antidepressant was the reference in all abovementioned analyses. Linear mixed models were also used to study the association between duration of SSRI and TCA exposure between 2 visits and repeated measurements of the change in BMD. All analyses were stratified by sex, and adjusted for age (model 1). With aging the decline in BMD differs between men and women,[1,2] and SSRIs could have different effects in men and women.[33] The full model was additionally adjusted for covariables that were associated with BMD, and changed the age-adjusted point estimate of the association between SSRIs and BMD by more than 10% (model 2). Menopausal age was added as an extra covariable to the full model in the postmenopausal female population. In additional analyses, we studied potential confounding by presence of depressive symptoms (model 3). These analyses were conducted in a subsample of the population since depressive symptoms were only assessed from the second measurement round onwards.

Moreover, additional sensitivity analyses were adjusted instead of stratified for sex and conducted in the complete study population to investigate overall associations. Furthermore, we defined current use as a dispensing at the time of the BMD measurement. SSRI or TCA use in the six months before the BMD measurement was classified as non-use.

All statistical analyses were performed with IBM SPSS statistics (version 21.0, IBM Corp., Somers, NY, USA). A two-sided p-value below 0.05 was considered statistically significant.

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Results

Baseline characteristics

The flowchart in Figure 2 depicts the selection of our study population. The study population included 10,746 participants, with a total of 19,861 BMD measurements with a median of 1 BMD measurement per participant, 5,291 participants (49.2%) had more than 1 BMD measurement. There were 4,926 participants with a delta BMD measurement, as not all BMD measurements were in a consecutive order. In total, there were 7,897 delta BMD measurements during follow-up, with a median period of 4.0 years (interquartile range: 1.95-4.6) between two consecutive visits. Of these 4,926 participants, 1,913 (38.8%) had more than 1 delta BMD measurement. Baseline characteristics, using data from the first eligible BMD measurement, are depicted in Table 1. In short, the mean age of the study population was 64.1 years (standard deviation [SD] 8.7) in men, and 65.8 years (SD 8.7) in women.

Total number of BMD measurements

N= 22,122 measurements

N= 11,698 participants

N= 9,081 measurements delta BMD

N= 5,452 participants

Exclusion criteria and number of (BMD / delta BMD) measurements:

– less than 6 months of pharmacy records available

  (N=1,057 / N= 759)

– pre- or perimenopausal women

  (N=594 / N= 62)

– use of drugs that affect the bone structure and mineralization

  (N= 534 / N= 296)

– other antidepressant drug use

  (N= 76 / N= 67)

Final number of participants and BMD measurements

N= 19,861 measurements BMD

N=10,746 participants

N= 7,897 measurements delta BMD
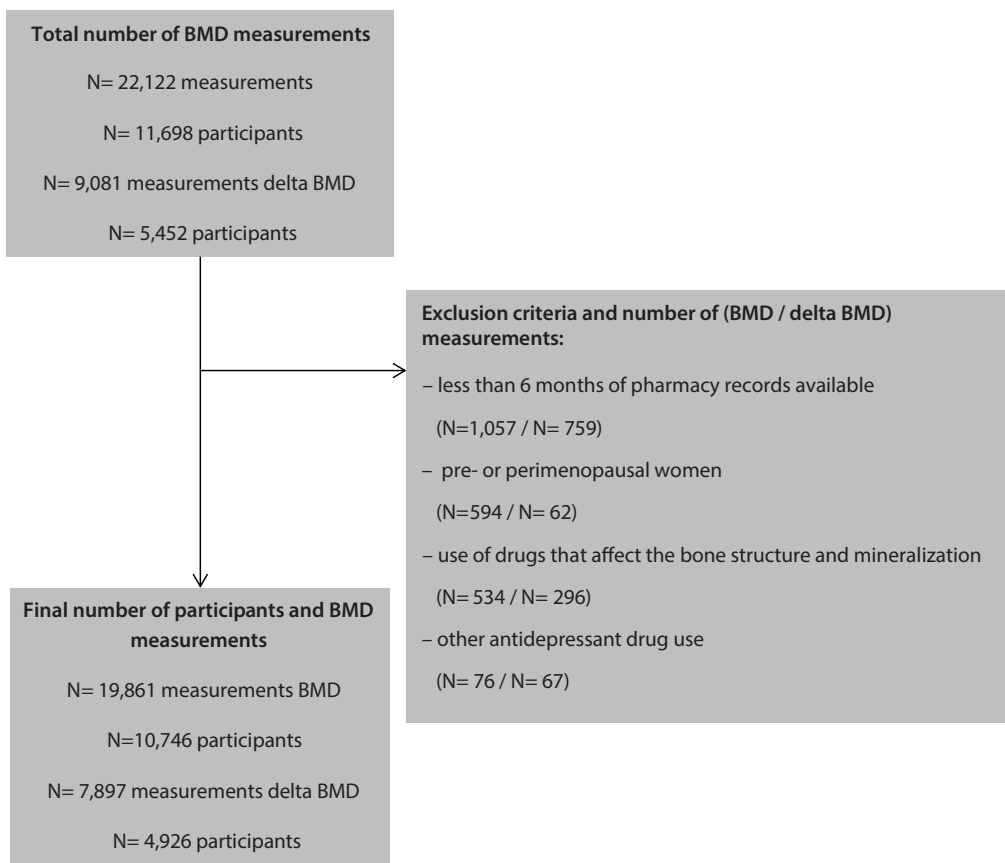
N= 4,926 participants

Figure 2. Flow-chart of the study population. *Abbreviations:* BMD = bone mineral density.

Antidepressants and BMD

Current SSRI and TCA use was not associated with BMD, when compared to non-use, in either men or women (in women, SSRI: mean difference 0.007 g/cm^2, 95% CI -0.002; 0.017, TCA: mean difference 0.003 g/cm^2, 95% CI: -0.007; 0.012). Additional adjustment for BMI, MMSE score, hypertension, and age at menopause (in women only) did not significantly change the results (Table 2). When categorized by duration of current use, neither short nor long term SSRI or TCA use between the visits was associated with BMD (Supplemental Table 1a and 1b). Additionally, we observed no dose-response relationship between the average number of prescribed DDDs of SSRIs or TCAs and BMD in men and women (data not shown). Additional

Table 1. Baseline characteristics of the study population.

	Men (N= 4,915)	Women (N= 5,831)
Age (years)	64.1 (8.7)	65.8 (8.7)
Body mass index (kg/m^2)	26.6 (3.4)	27.1 (4.3)
Lower-limb disability score	0 [0-1]	1 [0-3]
Lower-limb disabled[a]	656 (14.5)	1,418 (26.4)
Presence of depressive symptoms[b]	153 (5.5)	389 (13.0)
Alcohol intake (g/day)	16.5 [1.7-25.6]	6.9 [0.0-10.3]
Smoking status		
Never	787 (16.1)	2,676 (46.1)
Current	1,247 (25.5)	1,183 (20.4)
Former	2,864 (58.5)	1,951 (33.6)
Hypertension[c]	2,776 (57.2)	3,369 (58.4)
Number of BMD measurements	1 [1-2]	1 [1-2]
Number of delta BMD measurements	1 [1-2]	1 [1-2]
Drug use		
Glucose lowering drugs	244 (5.0)	234 (4.0)
Thiazides	243 (4.9)	530 (9.1)
Statins	445 (9.1)	365 (6.3)
Sex hormones	11 (0.2)	233 (4.0)
Glucocorticoids	241 (4.9)	231 (4.0)
Thyroid therapy	26 (0.5)	220 (3.8)
Antiepileptics	54 (1.1)	65 (1.1)
Psycholeptics	385 (7.8)	1,082 (18.6)
Calcium and vitamin D supplements	46 (0.9)	249 (4.3)

Characteristics are presented as mean (standard deviation), median [interquartile range] or number (valid percentage). Abbreviations: BMD = bone mineral density. [a] Disability was defined as a score of 3 or higher based on a modified version of the Stanford Health Assessment Questionnaire.[25] [b] Number and percentages were defined at the second measurement round, and depressive symptoms were based on a Center for Epidemiological Studies Depression Scale (CES-D) score of 16 or higher,[26] or a Hospital Anxiety and Depression Scale (HADS-D) score of 9 or higher.[27] [c] Hypertension was defined as a systolic blood pressure of 140 mmHg or higher, a diastolic blood pressure of 90 mmHg or higher, or the use of blood pressure lowering medication for the indication hypertension.

Table 2. Association between current SSRI and TCA use and mean femoral neck bone mineral density in men and postmenopausal women.

	Model 1				Model 2				Model 3[c]			
	N[a]	Mean difference (g/cm²)	95% CI	p-value[b]	N[a]	Mean difference (g/cm²)	95% CI	p-value[b]	N[a]	Mean difference (g/cm²)	95% CI	p-value[b]
Men												
Non use	8,778	ref			8,527	ref			6,310	ref		
SSRI use	130	-0.001	-0.014; 0.013	0.927	126	-0.001	-0.014; 0.013	0.904	111	0.005	-0.012; 0.21	0.582
TCA use	131	0.008	-0.005; 0.022	0.204	126	0.010	-0.004; 0.023	0.148	98	0.002	-0.015; 0.019	0.834
Women												
Non use	10,291	ref			9,756	ref			6,811	ref		
SSRI use	286	0.007	-0.002; 0.017	0.120	266	0.005	-0.004; 0.015	0.288	237	0.007	-0.004; 0.018	0.215
TCA use	266	0.003	-0.007; 0.012	0.569	254	0.0003	-0.009; 0.010	0.946	185	0.005	-0.007; 0.017	0.430

N[a] represents number of measurements and not number of unique participants. Number of measurements are different for the different models as we performed a complete caseset analysis. [b] Significance level of the association between SSRI or TCA use compared to non-use. [c] In a subsample of the population with information available regarding presence of depressive symptoms. Model 1: adjusted for age. Model 2: adjusted for age, body mass index, Mini-Mental State Examination score, hypertension, and age at menopause (in women only). Model 3: adjusted for age, body mass index, Mini-Mental State Examination score, hypertension, age at menopause (in women only), and presence of depressive symptoms.

Abbreviations: SSRI= selective serotonin reuptake inhibitor, TCA= tricyclic antidepressant, 95% CI= 95% confidence interval

Table 3. Association between total duration of SSRI and TCA exposure – between 2 consecutive visits – and annual percentage change in femoral neck bone mineral density in men and postmenopausal women.

	Model 1			Model 2			Model 3[b]		
	Change in BMD[a]	95% CI	p-value	Change in BMD[a]	95% CI	p-value[d]	Change in BMD[a]	95% CI	p-value
Men									
SSRI exposure in years	-0.050	-0.239; 0.138	0.601	-0.037	-0.251; 0.176	0.731	0.005	-0.209; 0.219	0.965
TCA exposure in years	0.019	-0.106; 0.145	0.761	-0.053	-0.194; 0.088	0.464	-0.088	-0.252; 0.077	0.298
Women									
SSRI exposure in years	-0.081	-0.196; 0.033	0.164	-0.109	-0.246; 0.028	0.118	-0.081	-0.229; 0.067	0.285
TCA exposure in years	0.003	-0.113; 0.119	0.954	0.111	-0.020; 0.241	0.096	0.051	-0.087; 0.189	0.470

[a] is expressed in annual percentage change in BMD per years of antidepressant exposure. [b] In a subsample of the population with information available regarding presence of depressive symptoms. Model 1: adjusted for age. Model 2: adjusted for age, body mass index, delta body mass index, delta lower-limb disability score, alcohol intake, hypertension, and age at menopause (in women only). Model 3: adjusted for age, body mass index, delta body mass index, delta lower limb disability score, alcohol intake, hypertension, age at menopause (in women only), and presence of depressive symptoms.
Abbreviations: SSRI= selective serotonin reuptake inhibitor, TCA= tricyclic antidepressant, BMD= bone mineral density, 95% CI= 95% confidence interval.

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adjustment for depressive symptoms did not substantially change the associations between current use of SSRIs or TCAs, and BMD in men and women (in women, SSRI: mean difference 0.007 g/cm², 95% CI: -0.004; 0.018, TCA: mean difference 0.005 g/cm², 95% CI -0.007; 0.017).

Antidepressants and change in BMD (Δ BMD)

Duration of SSRI or TCA use between two consecutive visits was not associated with the annual percentage change in BMD over time in men and women (in women, SSRI: -0.081, 95% CI -0.196; 0.033, TCA: 0.003, 95% CI -0.113; 0.119, Table 3). Additional adjustment for other covariates and, in a subsample of the population for depressive symptoms, did not result in different results (Table 3, model 2 and 3). In men, the median duration period of SSRI use was 1.05 year [IQR: 0.32-3.07], and of TCAs 0.95 year [0.21-3.82]. In women, the median duration period of SSRI use was 1.59 year [0.35-3.34], and of TCAs 1.18 year [0.32-2.97].

Sensitivity analyses

Results on the association between SSRI or TCA use and BMD or delta BMD were not different when data from men and women were combined (results not shown). Moreover, restriction of the definition of current use – to dispensings at time of the BMD measurement – did not substantially change our results (results not shown).

Discussion

Our results indicate that use of SSRIs was neither associated with a lower BMD, nor with change in BMD, in a population-based cohort of middle-aged and older men and postmenopausal women.

Our main results are in line with findings reported by Spangler et al.[16] and Diem et al.[17] who did not observe an association between use of SSRIs and change in BMD. Contrarily, another study by Diem and colleagues reported an association between SSRIs and reduction in BMD in an older female population.[15] However, they observed similar effects in bone loss for intermittent and continuous SSRI users (interval of 4.9 years), which does not support a real drug effect or might indicate lack of power. Antidepressant use was based on interview data and limited information regarding duration of use between the study visits was available.[15-17] This could have resulted in misclassification of exposure. A potential association between SSRIs and change in BMD is unlikely to be the result of an acute effect of SSRIs, as bone remodeling is a relative slow process.[18] In our study, pharmacy records were available on a day-to-day basis so we took duration of treatment between visits over a four year period into account. Nevertheless, we did not observe an association between duration of SSRI use between the visits and decline in BMD.

Furthermore, we did not observe an association between SSRIs and repeated cross-sectional measures of BMD, while most previous cross-sectional studies did.[9-14] The main differences between these studies and the present study are the design and exposure

assessment. We assessed SSRI exposure and BMD at multiple points in time and took duration of SSRI use into account. Also, we did not observe an association between TCAs and BMD, or decline in BMD. TCAs can also to some extent modulate serotonin levels,[34] and thereby potentially influence BMD. However, the expected effects would be smaller than with SSRIs. Therefore, we included TCAs in our analyses as a negative control and additionally to rule out possible confounding by indication.

It is hypothesised that SSRIs influence bone health via modulation of serotonin levels.[3,7,35] The major part of serotonin is synthesized in the gut (>95%), and the remainder is synthesized in the brainstem.[8] Since serotonin cannot cross the blood-brain barrier, it mainly exhibits local functions. Both the peripherally and centrally acting serotonin appear to modulate bone, although in an opposing manner. Peripheral serotonin can reduce osteoblast proliferation and thereby negatively affect bone formation.[3,7,8] On the other hand, central serotonin can increase bone mass, by decreasing the sympathetic output, which is an inhibitor of bone mass accumulation.[3,7,8] In the majority of rodent and *in-vitro* studies, but not consistently, the biological mechanism of the peripheral serotonin mechanism appears to surpass the central acting mechanism.[3,7,36-38] In addition, results from human studies investigating the effect of serotonin reuptake inhibitors on bone turnover markers – which may give insight into the effect on bone resorption or bone formation – are not uniform and only encompass two studies.[35] So, the exact role of serotonin on bone remains unclear, but our data suggests that use of SSRIs has no strong effects on BMD in the middle-aged and elderly.

Our results are important in the context of fracture risk. SSRI use has been associated with fracture risk, including within this study population of the Rotterdam Study,[39] and a potential association between SSRIs and BMD has been proposed as an underlying mechanism.[3,7,35] Nevertheless, our results do not confirm this potential underlying mechanism. This is in line with the finding of Spangler et al.,[16] as they did not observed an association between SSRI use and change in BMD, but did observe an association with fracture risk. The association between SSRIs and an increased fracture risk might by related to other aspects, such as sedative properties of SSRIs that might increase fall risk, or changes in other markers of bone quality.[3,7,35]

This study has strengths and limitations. Strengths of our study are the population-based character, availability of pharmacy dispensing records, time-dependent covariables, and repeated measurements of BMD. A limitation may be confounding by indication because depression itself has been associated with bone loss.[35] However, we did not observe an association with BMD, and additional adjustment for depressive symptoms, based on the CES-D or HADS-D, did not alter our results substantially. Therefore, it is unlikely that our negative results are the result of confounding by indication for use. Another important consideration is the use of two densitometers (and accompanied change in software) during follow-up, which may have an effect on the degree of change in BMD that can be assessed in our study setting. Within our study, the precision error and least significant change (LSC) have not been determined. Yet, assuming similar precision errors to those reported in the literature,[40] we can estimate the precision of the DPX-L and Prodigy devices in our study

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to be 0.028 g/cm^2 and 0.009 g/cm^2, respectively, and the mean expected LSC value for both densitometers is calculated to be ~0.0513 g/cm^2 (DPX-L: 0.028*2.77= 0.0776 g/cm^2 and Prodigy 0.009*2.77= 0.0249 g/cm^2).[40] This way, the small and non-significant change in BMD observed across duration of SSRI use does not exceed the calculated LSC. Our study had a sufficient long follow-up to detect effects on BMD change greater than the LSC.[40] Yet, minimal true effects of drug use on change in BMD (which are smaller than the LSC) might still go unnoticed. Moreover, we also lacked serum markers of bone resorption, which could be more sensitive to detect changes associated with SSRIs use than those observed on BMD levels. Nevertheless, we do not expect big differences compared to the analysis of BMD levels, considering the long follow-up with multiple BMD assessments in time. Furthermore, drug exposure was based on dispensing records over four year periods between the BMD measurements rounds. As in this way long term users (>4 years) were classified as being exposed for a maximum of 4 years, this may have introduced slight random misclassification of exposure. Nevertheless, we think the effect will be minimal as the number of such long-term users was low compared to the complete study population. Finally, drug exposure was based on dispensing records, and not on actual intake. However, non-adherence to antidepressant use would probably not be different for users with a low or high BMD, and therefore misclassification of exposure would probably be random in this setting.

In conclusion, our results suggest that use of SSRIs is not associated with lower BMD or a greater loss in BMD in men and postmenopausal women, or if there is an actual effect on BMD, it is minimal and unlikely to be clinically relevant. Although previous studies hinted at a potential role of serotonin in bone metabolism, its exact function remains to be demonstrated.

Acknowledgments

Supplementary material

Table 1a. Association between duration of current SSRI and TCA use and mean femoral neck bone mineral density in **men**.

	Model 1				Model 2			
	N^a	Mean difference (g/cm²)	95% CI	*p*-value[b]	N^a	Mean difference (g/cm²)	95% CI	*p*-value[b]
Non-use	8,748	ref			8,498	ref		
SSRI use								
1-364 days	51	-0.007	-0.024; 0.010	0.407	50	-0.005	-0.022; 0.012	0.543
≥365 days	79	0.008	-0.011; 0.028	0.395	76	0.005	-0.015; 0.025	0.596
TCA use								
1-364 days	72	0.006	-0.008; 0.020	0.418	69	0.007	-0.008; 0.022	0.341
≥365 days	59	0.016	-0.007; 0.039	0.164	57	0.017	-0.005; 0.039	0.136

N^a represents number of measurement and not number of unique participants. Number of measurements are different for the different models as we performed a complete caseset analysis. [b] Significance level of the association between SSRI or TCA use compared to non-use. Model 1: adjusted for age. Model 2: adjusted for age, body mass index, Mini-Mental State Examination score and hypertension.
Abbreviations: SSRI= selective serotonin reuptake inhibitor, TCA= tricyclic antidepressant, 95% CI= 95% confidence interval.

Table 1b. Association between duration of current SSRI and TCA use and mean femoral neck bone mineral density in **women**.

	Model 1				Model 2			
	N^a	Mean difference (g/cm²)	95% CI	*p*-value[b]	N^a	Mean difference (g/cm²)	95% CI	*p*-value[b]
Non-use	10,250	ref			9,719	ref		
SSRI use								
1-364 days	96	0.010	-0.003; 0.022	0.125	90	0.012	-0.001; 0.025	0.071
≥365 days	190	0.005	-0.008; 0.018	0.462	176	-0.002	-0.015; 0.011	0.785
TCA use								
1-364 days	116	<0.0001	-0.011; 0.011	0.989	107	0.0001	-0.011; 0.012	0.984
≥365 days	150	0.007	-0.007; 0.021	0.323	147	0.001	-0.013; 0.014	0.930

N^a represents number of measurement and not number of unique participants. Number of measurements are different for the different models as we performed a complete caseset analysis. [b] Significance level of the association between SSRI or TCA use compared to non-use. Model 1: adjusted for age. Model 2: adjusted for age, body mass index, Mini-Mental State Examination score, hypertension, and age at menopause.
Abbreviations: SSRI= selective serotonin reuptake inhibitor, TCA= tricyclic antidepressant, 95% CI= 95% confidence interval.

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3

4. GENERAL DISCUSSION

Fall incidents are an important health problem in older individuals, which has multiple risk factors including; a history of falling, muscle weakness, impaired balance, gait, vision and cognition, but also hazards in the environment, and medication use.[1-3] In this thesis, we addressed the association between medication use and fall incidents, and their potential underlying pathways. For this, we examined the role of genetic variants, homocysteine and bone mineral density. We focused on the following objectives:

Objective 1: Which medication is associated with fall risk in the B-PROOF study population? (*Chapter 2.1*)
Objective 2: Are there genetic variants that modify the association between medication use and fall risk? (*Chapter 2.2, 2.3* and *2.4*)
Objective 3: Is medication use associated with homocysteine levels? (*Chapter 3.1*)
Objective 4: Is use of SSRIs associated with BMD and change in BMD over time? (*Chapter 3.2*)

In this last chapter, we first discuss our main findings, thereafter we outline methodological considerations that are of importance in our research, and finally we discuss medication-related falls in a broader perspective, ending with suggestions for clinical implications and future directions of research.

Main findings of this thesis

Benzodiazepine-related falls

In chapter 2.1 we observed an association between benzodiazepine use and an increased fall risk, which is in line with previous literature where this association has consistently been reported.[4-7] We further investigated this association in chapter 2.2 and 2.4. Benzodiazepines are a group of medications mainly used for anxiolytic and hypnotic purposes, and sometimes as anticonvulsants.[8-10] In chapter 2.2, it appeared that the association seemed to be driven by hypnotic use. Although the various benzodiazepines are chemically very much alike, their pharmacodynamics effects can differ. Onset and duration of effects depend, among others, on the lipophilic properties and half-life.[8-10] Additionally, the dosing regimens for anxiolytic and hypnotic properties differ, as for anxiolytic purposes benzodiazepines are often used multiple times per day to induce and sustain a stable plasma level. Moreover, they may be used for a longer period of time, as tolerance to the anxiolytic effect develops more slowly than tolerance to the hypnotic effects.[10] Benzodiazepines for hypnotic purposes are mainly used in the evening and its use is only recommended for a short period,[8,9,11] although this is not always the case in clinical practice. Whether these differences may contribute to a potential difference in fall risk is unclear, but it might be possible that differences in clinical effects are also reflected in adverse effects. On the other hand, there is also a study indicating that both anxiolytic and hypnotic use increased the risk of a hip fracture, which is in majority caused by a fall[12] and literature suggest that the adverse reaction profile is grossly similar.[13]

Taken together, anxiolytic and hypnotic benzodiazepines may have a different frequency and magnitude of (adverse) effects, but more research is needed to quantify these effects and their underlying mechanisms.

Another aspect we examined is the role of genetic variants on benzodiazepine-related falls, by applying a candidate gene study and a genome wide association study. In chapter 2.2, we observed that the association between benzodiazepine use and fall incidents was modified by CYP2C9*2 and *3 allele variants. Participants using benzodiazepines and having reduced CYP2C9 enzyme activity – based on their genotype – had an increased fall risk. So, by having a reduced CYP2C9 enzyme activity, the benzodiazepine metabolism is reduced, potentially leading to increased blood and tissue levels and thereby more adverse effects. This is an interesting finding, because up to now only limited evidence indicated that CYP2C9 plays a role in the metabolism of benzodiazepines, as CYP3A enzymes and 2C19 enzymes are currently thought to be the main enzymes involved in benzodiazepine biotransformation.[9,14-21] In future research, the role of CYP2C9 has to be elucidated and our findings should be verified. Furthermore, it would be interesting to separately investigate anxiolytics and hypnotics, and also take into account benzodiazepines with pharmacologically active metabolites after biotransformation. Currently, we could not account for all these factors because of our limited sample size.

In addition to CYP2C9 genotypes, we identified genetic variants that influence the association between benzodiazepine use and fall risk in chapter 2.4. In the discovery cohort of 2 studies, N= 3,686 (N= 258 cases and N= 3,428 controls), we identified a less common variant, having a minor allele frequency of ~3% and an effect size of OR= 0.31 (95% CI 0.21; 0.47), p= 2.15*10-8. This intronic variant is located on chromosome 9 within the FAM73B gene. This gene encodes a protein involved in mitochondrial fusion, while dysregulation of mitochondrial fusion has been related to neurodegenerative disorders in humans.[22] Furthermore, expression data reports the highest median expression in brain- cerebellum.[23] This part of the brain plays a key role in motor control,[24] thereby the FAM73B gene could be linked to our phenotype, benzodiazepine-related fall incidents. On the other hand, FAM73B gene has been associated with bone phenotypes in mice.[25,26] Overall, these potential explanations of our observed association remain speculative, because it is still unknown whether the genetic variant affects the function of FAM73B. Therefore, additional replication and functional studies are needed to verify and support our finding.

Taken together, use of benzodiazepines is associated with fall incidents and potentially there are class or individual drug effects. Moreover, genetic variants can influence this association and more research is needed to verify our findings and identify other variants and their function. Thereafter, research should evaluate the additional value of genotyping as a prognostic factor for prescribing benzodiazepines to older individuals with respect to fall risk.

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Key points: future directions

- Investigate the role of CYP2C9 and its genetic variants in benzodiazepine metabolism, and accordingly associate it with benzodiazepine (adverse) effects.
  - *How?* In vitro studies, using human liver microsomes to investigate if and to what extent CYP2C9 plays a role in the metabolism of various benzodiazepines. In addition, in vivo studies can be used to measure benzodiazepine/metabolite levels across genotypes. Furthermore, with population-based studies (adverse) effects of genetic variants in CYP2C9 can be investigated.
- Replicate the association between our observed genetic variant and benzodiazepine-related falls, and accordingly further investigate the functionality of the genetic variant.
  - *How?* Replication in population-based studies where falls and medication use are prospectively gathered. Functionality, could be investigated with in animal models, such as knockout mouse models, and in vitro studies.

Beta-blocker-related falls

Use of beta-blockers has been associated with fall risk, but literature is contradictory.[4,27-29] Previous studies investigated all beta-blockers as one medication group and did not distinguish between different characteristic of beta-blockers. We hypothesised that characteristics of beta-blockers regarding their selectivity for adrenergic receptors, lipid solubility, intrinsic sympathetic activity (ISA), and their elimination route, differed in their association with fall incidents. In chapter 2.3, we observed that non-selective beta-blockers in specific were associated with an increased fall risk, while selective or lipophilic beta-blockers were not. Additionally, we did not observe an association between beta-blocker use and fall risk across genotypes of CYP2D6. CYP2D6 is an enzyme, which is of major importance in the metabolism of some beta-blockers, such as metoprolol.[30,31] Although no significant association between selective beta-blocker use and fall risk was observed, our results point towards a slightly decreased fall risk (HR= 0.92, 95% CI 0.83; 1.01). These apparently contradictory results – a potentially decreased fall risk for selective beta-blockers and an increased fall risk for non-selective beta-blockers – are noteworthy. We have no clear explanation for this other than their differential binding to β1-, β2- and α-receptors and resulting clinical and potential adverse effects,[32-34] as discussed in chapter 2.3. In comparison to selective beta-blockers, non-selective beta-blockers not only reduce heart rate and contractility, they also induce peripheral vasoconstriction, including in afferent blood vessels in skeletal muscle.[32,33] Nevertheless, a drug class review on the differences in effectiveness and safety for various indications, reported no substantial differences in adverse effects between various beta-blockers, all with their own characteristics.[35] Thus, potential differences in adverse effects are plausible based on their working mechanism, though they are not uniform in literature. In addition, beta-blockers have been shown effective for a variety of cardiovascular diseases.[35] So, with respect to fall risk, additional research is needed to confirm a potential positive effect for selective beta-blockers, and negative effect of non-selective beta-blockers. For

clinical practice, this stresses the importance of a well-balanced decision – based on patient characteristics and indication – regarding beta-blocker prescriptions.

Key points: future directions
- Further investigate the association between beta-blocker selectivity and fall risk, and accordingly formulate clinical recommendations.
  - *How?* A similar (epidemiologic) study for verification of our finding, potentially also investigating new users and the effect of duration of use.

Medication, bone mineral density and fractures

SSRI use has been associated with fracture risk, including in the population-based cohort of the Rotterdam Study.[36-39] This has been attributed to an increase in fall risk and/or a potential direct effect on bone.[37-39] In chapter 3.1, we did not observe an association with BMD or change in BMD when taking into account duration of treatment. So, our study did not confirm an association between SSRI use and BMD, and the exact role of serotonin on bone remains unclear.[37,38,40] Furthermore, literature evidently indicates an association of SSRIs with fall risk.[41-43] Thereby the most probable mechanism underlying the association between SSRIs and fracture risk appears to be their association with fall incidents rather than a potential effect on bone, as measured with BMD. However, SSRIs may affect bone in another way than is reflected in BMD, as other determinants of bone strength, bone remodeling or bone matrix/geometry might be affected.[44,45]

Key points: future directions
- Investigate the role of serotonin in bone, and accordingly investigate the association between use of SSRIs and bone characteristics, e.g. bone strength (other than BMD), bone geometry, and bone markers.
  - *How?* Animal and in vitro studies to investigate the mechanism, and population-based studies to investigate the association between use of SSRIs and bone characteristics.

4

Methodological considerations

Overall, we observed some medication groups to be associated with fall risk, but in our study many other groups which have been suggested to increase that risk were not associated with it. Why did we not observe an association? The exact answer is obviously unknown, but it can be distinguished into the following 2 options: 1) there may be a true association, but we did not observe it, or 2) the association is false, despite results from other studies. Considering option 1, there are multiple factors playing a role that probably all relate to methodological issues, including study design, study population, assessment of outcome, exposure and covariables, and sample size, separately or in combination. These factors are separately discussed in more detail below.

Study population and design

Data from three population-based studies were used, B-PROOF,[46] The Rotterdam Study[47] and LASA,[47] though for some research questions only one or two of these study populations were used. Although all studies included community dwelling older individuals, B-PROOF concerned a more selected population. Participants were primarily included on the basis of their homocysteine levels, thereby results from this study are not completely generalizable to the general population. Nevertheless, the generalizability of study results also depends on whether the studied association is related to homocysteine levels. With respect to medication-related falls, we are not aware of a mechanism whereby homocysteine could be expected to affect this association. Additionally, the B-PROOF study population was subjected to an intervention with vitamin B_{12}, folic acid and vitamin D, or only vitamin D (placebo). However, the intervention did not affect the time to the first or second fall, and the total number of incident falls.[48] Thus, for the B-PROOF study population, we do not expect that the inclusion based on homocysteine levels or the intervention affected the generalizability of the results – regarding medication-related falls – described in this thesis. Nevertheless, the B-PROOF study population is used as a cohort, and cohort studies also do not completely represent the general population, as the healthy volunteer effect and differential loss to follow-up are inevitable.[49,50] These limitations are, however, inherent to epidemiological studies and should be kept in mind when translating study results to clinical practice.

Measuring the outcome: falls

Fall data can be gathered in various ways. In this thesis, we used prospectively gathered fall data, which better reflect the actual fall incidents than retrospective fall data, as they are not subjected to 'recall bias'. Fall incidents were self-reported (B-PROOF and LASA) or defined as 'serious falls' (the Rotterdam Study). Self-reported falls were gathered using a fall-calendar,[51,52] and defined as 'an unintentional change in position resulting in coming to a rest at a lower level or on the ground'.[53] Serious fall data were obtained from computerized reporting systems and are defined as 'a fall leading to a hospital admission or leading to a fracture'.[54] By definition – falls and serious falls – represent a somewhat different type of outcome, that may affect the generalizability of the study results. Nevertheless, the effect sizes observed for benzodiazepine-related falls and beta-blocker related falls in B-PROOF, the Rotterdam Study and LASA were similar (chapter 2.2 and 2.3). Indicating that these medications are similarly associated with falls and serious falls.

Measuring the exposure: medication use

Like fall data, medication use can be assessed in various ways. In this thesis, we made use of self-reported data and pharmacy dispensing records.[46,47,55] Unfortunately there is no 'gold standard' for assessing medication use in observational studies and both methods have pro's and con's.[56,57] Self-reported medication data could be relatively easily assessed using a questionnaire and or a medication interview to gather information regarding prescribed and over the counter (OTC) medications. However, the accuracy relies on the memory of

the participant and recall bias might subsequently result in misclassification of exposure.[56-59] Obtaining pharmacy dispensing records in a population-based setting is logistically more complex and cumbersome. However, when possible, a large amount of detailed data, regarding dose, frequency and duration information, can be obtained. Nevertheless these data will not include OTC's and does not necessarily imply actual use, and could thereby also result in misclassification of the exposure.[57,59,60] Both types of misclassification are most likely not random, and may accordingly affect the outcome. Overall, the applicability of a method depends on the research question and the resources available, though the limitations of the method should be taken into account. For the study of falls, pharmacy dispensing records are probably favored, as it is important to determine which medication is used at the time of the fall or prior to the fall.

Covariables and confounding factors

Although, the outcome and the exposure – when pharmacy dispensing records were used – were assessed at a certain point in time, covariables – including potential confounders – were not, and thereby we rely on priory assessed determinants. These determinants will not always capture the health status at the time of the outcome and consequently could have affected the risk estimate. This may have led to under- or overestimation of the association.

Another important item is confounding by indication, which refers to the phenomenon that the reason for use itself or the severity of the disease is independently associated with the outcome instead of the exposure.[61] In this thesis, we used various ways to investigate whether confounding by indication may have played a role. We examined associations in past users of certain medications (Chapter 2.1, 2.2, 2.3), we examined the association in users of certain medications (Chapter 2.2), we investigated dose-response relations (Chapter 2.1, 2.2, 2.3, and 3.2), and we examined the association with another medication group, used for the same indication (Chapter 3.2).

Our observed association between antidepressant use and fall incidents (Chapter 2.1), could have been confounded by indication, as both depression and antidepressant use has been associated with falls.[41,42,62] Therefore, we also examined the association in past users (who of course had had the same indication), which did not indicate a significant association. However, this does not completely rule out potential confounding by indication, as the indication depression varies over time, and because it is difficult to separate the effect of antidepressants from depression itself. Though, two self-controlled case-series studies also reported an association between antidepressant use and fall risk, and this method is less subject to confounding by indication,[42,63] unless the indication varies over time. Furthermore, a cohort study investigating the association with both depression and antidepressants concluded that both aspects contributed to fall risk.[41] So, our observed association may be confounded by indication, but based on literature it is likely that there is also a true association between antidepressant use and fall incidents.

In addition, it may be possible that factors related to benzodiazepine use influenced the modifying effect of CYP2C9 genotype on benzodiazepine-related falls (Chapter 2.2).

However, to investigate whether this modifying effect was 'truly' due to CYP2C9 genotypes we examined an allele-dose association. This indicated that homozygous *2 or *3 versus heterozygous allele carriers have a higher increased fall risk. Furthermore, we assessed the association between CYP2C9 genotype and falls within benzodiazepine users. This analysis showed that also within benzodiazepine users carrying a CYP2C9*2 or *3 allele was significantly associated with an increased fall risk. Moreover, an allele-dose association was observed. Overall, these results indicated that factors, which are potentially related to benzodiazepine use, did not substantially influence the modifying effect of CYP2C9 genotype on the association between benzodiazepine use and falls.

Regarding dose-response relations, when an association is potentially caused by the use of a certain medicine, one would expect a dose-response relation. Only for the use of hypnotics we observed a dose-response relation (Chapter 2.2). Nevertheless, for benzodiazepines overall, literature reports a dose-response relation for fall risk and/or hip fractures.[12,64-67]

A potential association between use of SSRIs and BMD may also be confounded by indication, as depression has been associated with BMD.[37,39] Within the analyses we adjusted the models for presence of depressive symptoms, which did not change the results substantially. Furthermore, we investigated the associations between tricyclic antidepressant (TCA) use and BMD, as TCAs are also used for depressive symptoms. However, like SSRIs, TCAs can modulate serotonin levels, though to a lesser extent, thereby TCAs may also be related to BMD. Nevertheless, a potential effect would be smaller than with SSRI use. Overall, we did not observe an association between use of SSRIs or TCA's and BMD. This suggests that confounding by indication did not substantially affect our results.

Sample size and exposure window
To study adverse effects of medication use, such as falls, large populations are needed. A fall incident is relatively common, as one third of those ≥65 years encounters a fall every year.[68] In an older population medication is also frequently used, although use varies between medication groups. Benzodiazepines use varied between 4-15% (The Rotterdam Study, B-PROOF and LASA), and beta-blocker use, 13% and 26% (The Rotterdam Study, B-PROOF). However, the combination of exposure and outcome is less frequent, and therefore we could not study all medication (sub)groups in for example chapter 2.1. For genetic studies sample size is even more important.[69-72] Although we investigated 'common' genetic variants – those with a minor allele frequency ≥5%[72] – large populations are needed to study the combination of exposure and a genetic variant on an outcome.

Another important factor when studying adverse effects is timing of exposure. Within our studies we used prevalent users, which may be those who tolerate the drug well and are less susceptible to adverse effects. New users, might be better to study,[73,74] however it also depends on the type of adverse effect. With respect to the association between antihypertensive medication and fall risk, initiation of antihypertensive medication is thought to increase fall risk by inducing a hypotensive effect that stabilizes over time.[28,75] Therefore it might be better to study this association within new users. On the other hand,

antihypertensive are often used for many years and it is of clinical value to examine if use – prevalent use – is associated with fall incidents.[76] For an association between SSRI use and BMD, prevalent users are probably very suitable to study, because we hypothesized that a longer duration of use would affect BMD, as bone remodeling is a slow process.[77] For both initiation and a longer duration of use, large sample sizes are needed. Therefore, we were not able to properly study initiation of use, and as the number of antidepressant users was limited, duration of SSRI use could not be studied in B-PROOF.

Implications and future directions

Personalized medicine and medication-related falls

Personalized medicine, precision medicine or stratified medicine,[71,78] irrespective of the name, have the common goal to optimize medication effects – efficacy and toxicity – based on patient characteristics, including age, gender, health status, lifestyle and genetic factors. With respect to genetic factors, it is probably too early to incorporate genetic testing to reduce medication-related fall incidents. Because, there are only few studies done,[79,80] and their results need to be replicated, and their underlying mechanism need to be elucidated (chapter 2.2 and 2.4). However, these studies do indicate that genetic variants play a role in medication-related falls, and may form a first step towards pharmacogenetics testing in clinical practice. More research is needed to identify additional genetic variants that may play a role in medication-related falls. Thereby, it may be interesting to focus on medication groups that have a well-established relation with fall incidents, such as benzodiazepines and antidepressants. For antidepressants there are various pharmacogenetics studies,[14,81] so it would be interesting to investigate whether variants identified in those studies also influence fall incidents. On the other hand, one could also investigate medication groups of which the relation with fall incidents is less certain. It might be that only those with a specific genetic predisposition are at increased risk, while others are not.

Medication-related falls is a complex outcome for the determination of genetic factors involved, as falls by itself is multifactorial. Therefore, it could be of value to also investigate the role of pharmacogenetics in the pathways associated with medication-related fall, such as muscle weakness, balance and dizziness, although these are also outcomes in which multiple factors are involved.

More information on the role of genetics in medication-related falls, and their underlying path-ways could help discriminate those persons at (increased) risk and provide clinicians with valuable information that help them with appropriate prescribing of medication.

Medication use and fall risk from a broader perspective

In this thesis we investigated medication-related falls, as medication use is a potentially modifiable risk factor. However, stopping or adjusting medication use has been shown to be very difficult. Studies have tried to reduce fall risk and fall rate, but results are not uniform.[79,82-84]

So, can we prevent falls by withdrawing or reducing the dose of fall-risk increasing drug, and/or should we focus on other fall-risk factors? Fall incidents have multiple risk factors, including fall history, muscle weakness, impaired balance, gait, vision and cognition, and also environmental hazards such as uneven surfaces or slippery floors. In older people, especially those with multiple morbidities, it is most likely that a combination of factors contributes to their fall risk. There are various fall prevention guidelines and literature reviews[6,83,85-88] and overall they recommend an individualized multifactorial approach, focusing on fall risk assessment and accordingly on intervention to reduce fall risk and fall rate. All assessing and targeting factors including, fall history, medical history (osteoporosis, depression, cognitive disorders and cardiovascular disease), physical and functional disabilities (strength, balance, gait, vision, urinary function, fear of falling), environmental factors, and medication review (psychoactive drugs and polypharmacy).[6,83,86-88] However, these fall prevention guidelines might benefit from well-established fall risk prediction tools that help to better identify individuals at risk and the most important risk factors per individual. Accordingly a more personalized and effective multifactorial approach could be initiated.[89,90] However, although various fall risk prediction tools have been developed, most have not been validated and their predictive accuracy is only modest.[90-92] So, in further research, fall risk prediction tools should be investigated, in which the complex interaction of different risk factors and covariables are taken into account, as well as the subsequent interventions to reduce fall risk. With respect to the results of this thesis, we would recommend to include medication use in the prediction tools and especially benzodiazepine and antidepressant use. Regarding cardiovascular medication, we only observed an increased fall risk for non-selective beta-blockers, but this association needs verification. Furthermore, antihypertensive medication has been associated with fall risk,[4,28,76] though not consistently.[27,93,94] So, this medication group also needs further investigation and should be evaluated in risk prediction tools. For studies investigating these tools it is of importance to use prospectively gathered fall and medication data. Furthermore, when the role of genetic variants in medication-related falls is evident, these genetic variants should also be evaluated in prediction tools. For now, for clinical practice, medication reviews are important and we would recommend to give special attention to use of antidepressants and/or benzodiazepines, based on our results[27,95] and those from literature.[4-7,41-43] There are various tools that can help a clinician to judge whether a prescription is appropriate.[96] The key points in all are that it should be well evaluated if prescription is necessary, contraindicated, has the right doses, whether the benefits outweigh the potential negative effects, and if there are better substitutes.[96]

Key points: clinical implications of the key findings
- Use of benzodiazepines, antidepressants, anti-arrhytmics and non-selective beta-blockers was associated with increased fall risk.
- Clinicians should prescribe medication associated with fall risk with caution and if possible choose safer alternatives for older patients.

- The differences in homocysteine levels between medication users and non-users were relatively modest, suggesting that medication use is unlikely to contribute to clinically relevant changes in plasma homocysteine levels in our population.

4

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5. APPENDICES

5.1　Summary

Fall incidents are a major problem in older individuals, because approximately one third encounters at least one fall yearly. Of all falls, 5 to 12% result in serious injuries or fractures requiring medical attention, which lead to reduced quality of life and substantial health care costs. Medication use is one of the risk factors for falling, however literature is inconclusive on the medication groups that are associated with an increased fall risk. Moreover, the pathways underlying the association between medication use and fall incidents need to be further examined. In this thesis we addressed the association between medication use and fall incidents, and their potential underlying pathways. For this, we examined the role of genetic variants, homocysteine and bone mineral density (BMD).

In chapter 2.1, using the B-PROOF study population, we observed several medications to be associated with an increased fall risk, namely; benzodiazepines, antidepressants, non-selective beta-blockers, and anti-arrhythmics. On the other hand, statin use was associated with a lower fall risk. We further investigated the potential underlying pathways of the associations between benzodiazepine and beta-blocker use, and fall incidents. For benzodiazepine-related falls we examined the role of genetic variants, by applying a candidate gene study and a genome wide association study. We observed that the association between benzodiazepine use and fall incidents was modified by CYP2C9*2 and *3 allele variants (chapter 2.2). Individuals using benzodiazepines and having reduced CYP2C9 enzyme activity – based on their genotype – were at increased fall risk. So it seems that by having a reduced CYP2C9 enzyme activity, the benzodiazepine metabolism is reduced, potentially leading to increased blood levels and thereby more adverse effects. However, it should be noted that the exact role of CYP2C9 in benzodiazepine metabolism is still unclear. In future research the role of CYP2C9 has to be elucidated and our findings should be verified.

In addition to CYP2C9 genotypes, we identified a genetic variant that influenced the association between benzodiazepine use and fall risk in chapter 2.4. We identified a less common variant within the discovery cohort of 2 studies, N= 3,686 (N= 258 cases and N= 3,428 controls). The variant has a minor allele frequency of ~3% and an effect size of OR= 0.31 (95% CI 0.21; 0.47), p= 2.15*10-8. It is an intronic variant – with unknown effect on protein – located on chromosome 9 within the FAM73B gene. This gene encodes a protein involved in mitochondrial fusion, and dysregulation of mitochondrial fusion has been related to neurodegenerative disorders in humans. Furthermore, FAM73B gene has been associated with bone phenotypes in mice. Overall, the effect of the variant on the gene is unknown and therefore additional replication and functional studies are needed to verify and support our finding.

Use of beta-blockers overall was not associated with fall risk (chapter 2.3). Interestingly use of non-selective beta-blockers was associated with an increased fall risk, while selective or lipophilic beta-blockers were not. Additionally, we did not observe an association between beta-blocker use and fall risk across genotypes of CYP2D6. The CYP2D6 enzyme is of major

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importance in the metabolizing pathway of some beta-blockers, such as metoprolol. A potential explanation for the contradictory findings between selective and non-selective beta-blockers is their differential binding to β1-, β2- and α-receptors and the resulting differences in clinical and potential adverse effects. In comparison to selective beta-blockers, non-selective beta-blockers not only reduce heart rate and contractility, they also induce peripheral vasoconstriction, including in blood vessels towards and in skeletal muscle. Nevertheless, literature does not report substantial differences in adverse effects between various beta-blockers. Therefore, additional research is needed to confirm a negative effect of non-selective beta-blockers use on fall risk.

We investigated the relation between medication use and homocysteine levels in chapter 3.2. Our results indicated that users of diuretics in general, high-ceiling sulphonamide diuretics, agents acting via the renin-angiotensin system and metformin had slightly higher homocysteine levels. The association were, however, fairly modest suggesting that medication use is unlikely to contribute to clinically relevant changes in plasma homocysteine levels within our study population.

Selective serotonin reuptake inhibitor (SSRI) use has been associated with fracture risk in several studies, including the Rotterdam Study. This has been attributed to an increase in fall risk and/or a potential direct effect on bone. However, we did not observe an association with BMD or change in BMD – when taking into account duration of treatment – (chapter 3.3). So, our study did not indicate an association between SSRI use and BMD, and the exact role of serotonin on bone remains unclear. Furthermore, literature evidently indicates an association with fall risk. Thereby the most probable mechanism underlying the association between SSRIs and fracture risk appears to the be their association with fall incidents. However, SSRIs may affect bone in another way than is reflected in BMD, as other determinants of bone strength, bone remodeling or bone geometry might be affected.

In the discussion (chapter 4) we conclude that based on our results and those form literature, benzodiazepines and antidepressants are two of the most important medications groups that are associated with increased fall risk. When evaluating fall risk in older persons, clinicians should give special attentions to these groups during the medication review. In addition, genetics variants appeared to play a role in benzodiazepine-related fall risk, but further research is needed to elucidate its exact role.

5.2 Dutch summary – samenvatting

Voor oudere mensen zijn valincidenten een groot probleem, aangezien ongeveer één derde van de ouderen op zijn minst één keer per jaar valt. Van alle vallen resulteert 5 tot 12% in ernstig letsel of fracturen, wat leidt tot een verminderde kwaliteit van leven en hoge kosten voor de gezondheidszorg. Medicijngebruik is één van de risicofactoren voor vallen, alhoewel de literatuur niet eenduidig is over welke medicatiegroepen gerelateerd zijn aan valrisico. Verder moeten de mechanismes, onderliggend aan de relatie tussen medicatiegebruik en vallen, verder onderzocht worden. In dit proefschrift kijken we naar de relatie tussen medicatiegebruik en vallen. Tevens kijken we naar de onderliggende mechanismen, hiervoor onderzoeken we de rol van genetische variatie, homocysteïne en botmineraaldichtheid (BMD).

In hoofdstuk 2.1, in de B-PROOF studiepopulatie, vonden we een relatie tussen het gebruik van verschillende medicijnen en een verhoogd valrisico, namelijk voor benzodiazepines, antidepressiva, niet-selectieve bètablokkers en anti-aritmica. Daarentegen was het gebruik van statines gerelateerd aan een verlaagd valrisico. De relatie tussen benzodiazepines, niet-selectieve bètablokkers en valrisico hebben we verder onderzocht. Voor benzodiazepine-gerelateerde valincidenten onderzochten we de rol van genetische varianten door middel van een kandidaatgen studie en een genoomwijde associatiestudie. Daarbij vonden we dat de relatie tussen benzodiazepines en valincidenten gemodificeerd werd door CYP2C9*2 en *3 allel varianten (hoofdstuk 2.2). Personen die benzodiazepines gebruikten én een verminderde CYP2C9 enzymactiviteit hadden, gebaseerd op hun genotype, hadden een verhoogd valrisico. Dit zou kunnen impliceren dat door middel van een verminderde CYP2C9 enzymactiviteit het benzodiazepinemetabolisme verminderd is, wat mogelijk kan leiden tot verhoogde bloedspiegels en daardoor meer bijwerkingen veroorzaakt. Hierbij moet wel in gedachten worden gehouden dat de precieze rol van CYP2C9 in het benzodiazepinemetabolisme nog niet geheel duidelijk is. In vervolgonderzoek moet de precieze rol opgehelderd worden en onze resultaten bevestigd worden.

Naast de CYP2C9 genotypen identificeerden we een genetische variant die de associatie tussen benzodiazepinegebruik en valincidenten beïnvloede (hoofdstuk 2.4). Deze niet veel voorkomende variant vonden we in een begin-cohort bestaande uit twee studies, N= 3,686 (N= 258 cases en N= 3,428 controles). De variant heeft een minor allelfrequentie van ~3% en een effectgrootte van OR= 0.31 (95%BI 0.21; 0.47), *p*= 2.15*10-8. Het betreft een intron variant, met een onbekend effect op het eiwit, op chromosoom 9 in het FAM73B gen. Dit gen codeert voor een eiwit wat betrokken is bij mitochondriale fusie. Disregulatie van mitochondriale fusie kan gerelateerd zijn aan neurodegeneratieve ziektes in mensen. Verder zijn er aanwijzingen dat het FAM73B gen gerelateerd is aan botfenotypes in muizen. Samenvattend, omdat het effect van de variant op het gen onbekend is, hebben we replicatie en functionele studies nodig om onze bevinding te bevestigen.

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Het gebruik van bètablokkers was niet gerelateerd aan valrisico (hoofdstuk 2.3). Maar het gebruik van de subgroep niet-selectieve bètablokkers was gerelateerd aan een verhoogd valrisico, terwijl selectieve en lipofiele bètablokkers niet gerelateerd waren aan valrisico. Verder vonden we geen relatie tussen bètablokkergebruik en valrisico binnen CYP2D6 genotypen. Het CYP2D6 enzym is belangrijk voor het metabolisme van een aantal bètablokkers, zoals metoprolol. Een mogelijke verklaring voor de tegenstrijdige bevindingen tussen selectieve- en niet-selectieve bètablokkers kan komen door het verschil in bindingscapaciteit voor β1-, β2- en α-receptoren, wat resulteert in verschillende klinische effecten en mogelijke bijwerkingen. In vergelijking met selectieve bètablokkers, verminderen niet-selectieve bètablokkers niet alleen de hartslag en het samenknijpen van het hart, maar induceren ze ook perifere vasoconstrictie, inclusief van de bloedvaten in en naar de skeletspieren. Desondanks is er vanuit de literatuur bekend dat er geen substantiële verschillen in bijwerkingen zijn tussen de verschillende bètablokkers. Daarom is er meer onderzoek nodig om een mogelijk negatief effect van niet-selectieve bètablokkers op valrisico te bevestigen.

Naast medicatie-gerelateerd vallen onderzochten we de relatie tussen medicatiegebruik en homocysteïne waardes (hoofdstuk 3.2). De resultaten laten zien dat gebruikers van diuretica, medicatie welke aangrijpt op het renine-angiotensine systeem, en metformine licht verhoogde homocysteïne waardes hadden. Voor diuretica was dit specifiek te zien bij sulfonamiden loop-diuretica ('high-ceiling' sulfonamiden diuretica). De verhoging was echter zeer beperkt, wat suggereert dat het onwaarschijnlijk is dat medicatiegebruik bijdraagt aan klinische relevante veranderingen in plasma homocysteïne-waardes in de bestudeerde populatie.

Vanuit de literatuur is een relatie tussen selectieve serotonine-heropnameremmer (SSRI) gebruik en fractuurrisico bekend. Deze relatie is ook gevonden in de populatie van de Rotterdam Studie. De relatie zou kunnen komen door een verhoogd valrisico en/of door een direct negatief effect op de botten. Echter, in ons onderzoek vonden we geen relatie tussen SSRI gebruik en BMD of verandering in BMD, ook niet wanneer er rekening gehouden werd met de duur van het SSRI gebruik (hoofdstuk 3.3). Ons onderzoek liet zien dat er geen relatie is tussen SSRI gebruik en BMD. Verder is het onduidelijk welke rol serotonine precies speelt in botten. Daarentegen is er vanuit de literatuur duidelijk bewijs voor een relatie tussen SSRI gebruik en valrisico. Daarom denken we dat de relatie tussen SSRI en valrisico het meest logische onderliggende mechanisme is voor de relatie tussen SSRI gebruik en fractuurrisico. Hoewel SSRI's ook op een andere manier effect zouden kunnen hebben op bot dan wordt aangeduid met BMD. Zo zouden ze effect kunnen hebben op andere determinanten van botsterkte, botmodellering of botgeometrie.

In de discussie (hoofdstuk 4) concluderen we dat, gebaseerd op onze eigen resultaten en de literatuur, benzodiazepines en antidepressiva de twee belangrijkste medicatiegroepen zijn die gerelateerd zijn met een verhoogd valrisico. Wanneer clinici het valrisico van een ouder individu evalueren is het belangrijk dat er op deze twee medicatiegroepen gelet wordt. Aangezien genetische variatie een rol bleek te spelen in benzodiazepine-gerelateerd valrisico, maar de exacte rol onduidelijk is, is het belangrijk dat dit verder onderzocht wordt.

5.3 PhD portfolio

Name: Annelies Christine Ham
Erasmus MC Department: Internal Medicine
Research School: MolMed
PhD period: 2011-2017
Promotors: Prof.dr. A.G.Uiterlinden, Prof.dr. B.H.C. Stricker
Supervisor: Dr. N. van der Velde

General courses
2013   Biomedical English Writing and Communication
2011   BROK (Basiscursus Regelgeving Klinisch Onderzoek)

Specific course
2014   Supervision in Science
2012   Basic course on R
2011   Principals of genetic epidemiology (NIHES)
2011   Genomics in Molecular Medicine (NIHES)
2011   Genome Wide Association Analysis (NIHES)
2011   Pharmaco-epidemiology (NIHES)
2011   Genetics for Dummies
2011   SNP course VIII
2011   Research management for PhD students

Seminars and workshops
2015   Symposium 'Personalised Medicine'
2015   GGG-congress ZonMw
2014   Science days
2014   PCDI Postdoc Retreat
2013   Science days
2013   NCHA meeting
2012   Molmed day
2012   NCHA meeting
2012   Phd day
2012   Science days
2011   Phd day

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(Inter)national conferences and presentations

2015	Oral presentation at the EUGMS
2015	Oral poster presentation at the ECTS
2014	Oral presentation at the Dutch 'Val-symposium'
2014	Oral presentation at the EUGMS
2014	Oral presentation at the Dutch 'Geriatrie dag'
2014	Poster presentation at the Erasmus MC Internal Medicine 'Science days'
2013	Poster presentation at the Dutch NCHA meeting
2012	Attending the Dutch 'Geriatrie dag'

Supervising Master's theses

2012	Pharmacy student Maarten de Pater
2012	Medical student Riekske van Zwienen

5.4   List of publications

1. Swart KM, Enneman AW, van Wijngaarden JP, et al. Homocysteine and the methylenetetrahydrofolate reductase 677C-->T polymorphism in relation to muscle mass and strength, physical performance and postural sway. *Eur J Clin Nutr.* 2013;67(7):743-748.

2. van Dijk SC, Smulders YM, Enneman AW, et al. Homocysteine level is associated with aortic stiffness in elderly: cross-sectional results from the B-PROOF study. *J Hypertens.* 2013;31(5):952-959.

3. Ham AC, Enneman AW, van Dijk SC, et al. Associations between medication use and homocysteine levels in an older population, and potential mediation by vitamin B_{12} and folate: data from the B-PROOF Study. *Drugs Aging.* 2014;31(8):611-621.

4. Ham AC, Swart KM, Enneman AW, et al. Medication-related fall incidents in an older, ambulant population: the B-PROOF study. *Drugs Aging.* 2014;31(12):917-927.

5. van der Zwaluw NL, Dhonukshe-Rutten RA, van Wijngaarden JP, et al. Results of 2-year vitamin B treatment on cognitive performance: secondary data from an RCT. *Neurology.* 2014;83(23):2158-2166.

6. van Wijngaarden JP, Swart KM, Enneman AW, et al. Effect of daily vitamin B-12 and folic acid supplementation on fracture incidence in elderly individuals with an elevated plasma homocysteine concentration: B-PROOF, a randomized controlled trial. *Am J Clin Nutr.* 2014;100(6):1578-1586.

7. Brouwer-Brolsma EM, Dhonukshe-Rutten RA, van Wijngaarden JP, et al. Cognitive Performance: A Cross-Sectional Study on Serum Vitamin D and Its Interplay With Glucose Homeostasis in Dutch Older Adults. *J Am Med Dir Assoc.* 2015;16(7):621-627.

8. Enneman AW, Swart KM, van Wijngaarden JP, et al. Effect of Vitamin B_{12} and Folic Acid Supplementation on Bone Mineral Density and Quantitative Ultrasound Parameters in Older People with an Elevated Plasma Homocysteine Level: B-PROOF, a Randomized Controlled Trial. *Calcif Tissue Int.* 2015;96(5):401-409.

9. Oliai Araghi S, van Dijk SC, Ham AC, et al. BMI and Body Fat Mass Is Inversely Associated with Vitamin D Levels in Older Individuals. *J Nutr Health Aging.* 2015;19(10):980-985.

10. Sohl E, de Jongh RT, Swart KM, et al. The association between vitamin D status and parameters for bone density and quality is modified by body mass index. *Calcif Tissue Int.* 2015;96(2):113-122.

11. van Dijk SC, Enneman AW, Swart KM, et al. Effects of 2-year vitamin B_{12} and folic acid supplementation in hyperhomocysteinemic elderly on arterial stiffness and cardiovascular outcomes within the B-PROOF trial. *J Hypertens.* 2015;33(9):1897-1906; discussion 1906.

12. van Dijk SC, Sohl E, Oudshoorn C, et al. Non-linear associations between serum 25-OH vitamin D and indices of arterial stiffness and arteriosclerosis in an older population. *Age Ageing.* 2015;44(1):136-142.

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13. van Dijk SC, Swart KM, Ham AC, et al. Physical Fitness, Activity and Hand-Grip Strength Are Not Associated with Arterial Stiffness in Older Individuals. *J Nutr Health Aging.* 2015;19(7):779-784.

14. Winkler TW, Justice AE, Graff M, et al. The Influence of Age and Sex on Genetic Associations with Adult Body Size and Shape: A Large-Scale Genome-Wide Interaction Study. *PLoS Genet.* 2015;11(10):e1005378.

15. Brouwer-Brolsma EM, Dhonukshe-Rutten RA, van Wijngaarden JP, et al. Low vitamin D status is associated with more depressive symptoms in Dutch older adults. *Eur J Nutr.* 2016;55(4):1525-1534.

16. Brouwer-Brolsma EM, Vaes AM, van der Zwaluw NL, et al. Relative importance of summer sun exposure, vitamin D intake, and genes to vitamin D status in Dutch older adults: The B-PROOF study. *J Steroid Biochem Mol Biol.* 2016;164:168-176.

17. de Koning EJ, van der Zwaluw NL, van Wijngaarden JP, et al. Effects of Two-Year Vitamin B_{12} and Folic Acid Supplementation on Depressive Symptoms and Quality of Life in Older Adults with Elevated Homocysteine Concentrations: Additional Results from the B-PROOF Study, an RCT. *Nutrients.* 2016;8(11).

18. Swart KM, Ham AC, van Wijngaarden JP, et al. A Randomized Controlled Trial to Examine the Effect of 2-Year Vitamin B_{12} and Folic Acid Supplementation on Physical Performance, Strength, and Falling: Additional Findings from the B-PROOF Study. *Calcif Tissue Int.* 2016;98(1):18-27.

19. van Dijk SC, de Jongh RT, Enneman AW, et al. Arterial stiffness is not associated with bone parameters in an elderly hyperhomocysteinemic population. *J Bone Miner Metab.* 2016;34(1):99-108.

20. van Dijk SC, Enneman AW, Swart KM, et al. Effect of vitamin B_{12} and folic acid supplementation on biomarkers of endothelial function and inflammation among elderly individuals with hyperhomocysteinemia. *Vasc Med.* 2016;21(2):91-98.

21. Ham AC, Ziere G, Broer L, et al. CYP2C9 Genotypes Modify Benzodiazepine-Related Fall Risk: Original Results From Three Studies With Meta-Analysis. *J Am Med Dir Assoc.* 2017;18(1):88 e81-88 e15.

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5.6   About the author

Annelies Christine Ham was born on July 3rd 1987 in Rotterdam, the Netherlands. After finishing secondary school at Commenius College in Capelle aan den IJssel in 2005, she started her bachelor study 'Nutrition and Health'. She obtained her degree in 2008 and continued with the master program 'Nutrition in Health and Disease', which she finished in 2010. During her master she did an internship at the university of Bergen, Norway. In 2011 she started a PhD project at the Erasmus Medical Centre, department of internal medicine. The project was a collaboration between the geriatric en genetic section. She mainly worked on the B-PROOF study, a multi-centre study including Wageningen University and Research centre and VU University Medical Centre, but she also worked on the Rotterdam Study, a large prospective cohort study. In October 2015 she started as project manager of the Rotterdam Periconception Cohort (Predict study), a prospective cohort study investigating the relation between gene-environment interactions, and embryonic growth and (adverse) pregnancy outcomes.

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