Genetic study of cognitive function

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Dedicated to my mother and to the memory of my father

TABLE OF CONTENTS

page

PUBLICATIONS AND MANUSCRIPTS BASED ON THE STUDIES DESCRIBED IN THIS THESIS

Chapter 1. General introduction
Pardo LM, van Duijn CM. In search of genes involved in neurodegenerative disorders. Mutat Res 2005;592(1-2):89-101.

Chapter 3.
Pardo L, MacKay I, Oostra B, van Duijn C, Aulchenko Y. The effect of genetic drift in a young genetically isolated population. Ann Human Genetic 2005;69(Pt3):288-295.

Chapter 4.
Pardo LM, Sanchez-Juan P, de Koning I, Sleegers K, van Swieten JC, Aulchenko YS, Oostra BA, van Duijn CM. The heritability of cognitive function in a young genetically isolated population (Submitted).

Chapter 5.
Pardo LM, Sanchez-Juan P, Isaacs Aaron, Sleegers K, de Koning I, Zorkoltseva IV, Axenovich TI, van Swieten JC, Aulchenko YS, Oostra BA, van Duijn CM. Age- and sex-dependent effects of the apolipoprotein E (APOE) gene on cognitive function (Submitted).

Chapter 6.
Pardo LM, Sanchez-Juan P, Yazdanpanah M, Sleegers K, de Koning I, Zorkoltseva IV, Axenovich TI, Breteler MM, Witteman JC, van Swieten JC, Aulchenko YS, Oostra BA, van Duijn CM. The effects of blood pressure, intima media thickness and four blood pressure genes on cognitive function (Submitted).

SIN TREGUA *(estrofa X)*

Llenate de ambición, ten el empeño;
ten la más loca, la más alta mira;
no temas ser espíritu, ser sueño,
ser ilusión, ser ángel, ser mentira.
La verdad es un molde, es un diseño
que rellena mejor quien más delira…
¿que la ciencia es brutal y que no sueña?
¡eso lo afirma el asno que la enseña!

Almafuerte, Argentina (1854-1917)

1

In search of genes involved in neurogenerative disorders: general introduction

Pardo LM, van Duijn CM. In search of genes involved in neurodegenerative disorders.
Mutat Res 2005;592(1-2):89-101

ABSTRACT

Dissecting the genetics of Alzheimer's disease (AD) and Parkinson's disease (PD) has contributed significantly to our understanding of the pathogenesis of neurodegeneration in these two complex disorders. For AD, three highly penetrant genes (*APP*, *PSEN1* and *PSEN2*) and one susceptibility gene (*APOE*) have been identified. For PD, seven genes (*SNCA*, *Parkin*, *UCHL1, NR4A2, DJ1, PINK1* and *LRRK2*) have been found. These genes explain only a small proportion of AD and PD patients and are mostly associated with an early onset presentation of the disease. *APOE* remains the only common gene, which increases the risk of both rare early and late onset AD. The ongoing challenge is to unravel the genetics of the most frequent forms of these complex disorders. In the present paper, we briefly review the state of the art in the genetics of AD and PD. We also discuss the prospects of finding new genes associated with common forms of these diseases in light of two hypotheses concerning the genetic variation of complex diseases: common disease/common variants and common disease/rare variants.

INTRODUCTION

Alzheimer's disease (AD) and Parkinson's disease (PD) are the most frequent neurodegenerative disorders in western societies and an important cause of disability among patients (1, 2). AD and PD are complex diseases in which multiple genes as well as environmental factors are involved (1). For both disorders, mutations associated with Mendelian forms explain only a small proportion of cases. For AD, a common polymorphism also increases the risk. In this paper we review the genes that are known to be involved in AD and PD. Further, we discuss approaches to identify new genes.

ALZHEIMER'S DISEASE

AD is the most frequent neurodegenerative disorder and the primary cause of dementia in humans. The prevalence of the disease is approximately 1% among people aged 65 to 69 years, and increases to nearly 30% by the age of 95 (3). Although different risk factors have been associated with the disease, only age and a positive history of familial dementia are the risk factors consistently identified in different studies (4, 5). In a small proportion of cases, the disease begins before age 65 years, which is referred to as presenile, or early onset, AD.

The most important clinical manifestation of AD is the progressive loss of cognitive function, short-term memory impairment being one of the first manifestations. AD patients gradually lose judgment, visuospatial skills, language, and the ability to learn new information. Clinical examination and neuropsychological testing combine to make a clinical diagnosis of probable AD, which is confirmed in 80-100% of the cases by neuropathology (6). Neuroimaging is not obligatory for the diagnosis, but does contribute to the differentiation of AD from other pathologies (7). A definite diagnosis requires histopathological examination. The pathological hallmarks of AD are extra-cellular senile plaques, neurofibrillary tangles, and neuronal loss (8). The β amyloid protein is the main constituent of senile plaques, whereas neurofibrillary tangles are composed of aggregates of hyperphosphorylated tau protein. The neuronal loss occurs in the cortex and the hippocampus, regions associated with memory and cognition (9). From a pathological perspective, there is no difference between early and late onset forms of AD.

No curative treatment is available for AD. Cholinesterase inhibitors are the standard medication to improve cognitive function, but only a small fraction of patients respond (10). Other medications alleviate the symptoms associated with late stages of AD, but do not halt the progression of the disease.

Genes and AD

Familial aggregation in AD is strong. This is true for both early and late onset forms (11, 12). The first causative gene identified for early onset AD was the amyloid precursor protein (*APP*) gene (see table 1). The *APP* gene was identified through linkage analysis in families segregating an autosomal dominant form of early onset AD (13). The *APP* gene, located at chromosome 21q21.22 (14), encodes a transmembrane protein that is normally processed into amyloid fragments. In neurons, β and γ secretases cleave the *APP* protein into smaller amyloid (Aβ) peptides. γ secretase cleaves *APP* into $A\beta_{40}$ and $A\beta_{42}$ fragments. $A\beta_{42}$ has proven to be the strongest determinant in the pathogenesis of the disease (15). So far, 18 different mutations have been reported in the *APP* gene in 50 families (http://molgen-www.uia.ac.be/ADMutations/). All 18 mutations cause amino acid changes in putative sites for the cleavage of the APP protein (16). *APP* mutations are highly penetrant. The number of carriers of the various mutations may differ across clinical populations. In the general population, not more than 0.5% of early onset AD patients carry a mutation in the *APP* gene (17). Among all AD patients, not more than 0.005% is found to carry a mutation in *APP*.

Mutations in two other genes have been found to lead to early onset autosomal dominant forms of AD (table 1). The *presenilin-1* (*PSEN1*; 14q24.3) and *presenilin-2* (*PSEN2*; 1q31-q42.3) genes were localized using linkage analysis (18, 19). These genes encode proteins that are involved in the normal cleavage of the *APP* protein, increasing the production of $A\beta_{42}$ (20, 21). Indeed, there is convincing evidence that *PSEN1* encodes a γ secretase (22). A total of 142 mutations have been found in *PSEN1* in 281 families (http://molgen-www.uia.ac.be/ADMutations/); this makes it the gene with the highest number of pathogenic mutations in AD. For *PSEN2*, 10 mutations have been found in 16 families. In the general population, mutations in the *presenilin* genes explain about 7.5% of early onset AD (6.5% for *PSEN1* and 1% for *PSEN2*). When considering all patients, both early and late onset, sporadic and familial cases, the *presenilin* genes explain less than 0.075% (17) (early onset AD represents about 1% of all AD cases).

The fourth gene related to AD is *APOE* (19q13.32), which was initially identified using linkage analysis (23). The relationship between *APOE* and AD has been confirmed in more than 100 studies conducted in diverse ethnic backgrounds (24). The gene has 3 different alleles, APOE*2, APOE*3 and APOE*4. In populations of European origin, the allele proportions are 0.08, for APOE*2, 0.77 for APOE*3 and 0.15 for APOE*4 (17). The APOE*4 allele is the variant associated with AD, and unlike the mutations found in *APP*, *PSEN1* and *PSEN2*, it is not a causal mutation for AD. Rather, it is considered to be an allele associated with an increased risk.

Table 1. Genes related to Alzheimer's disease and Parkinson's disease

Alzheimer's Disease [a]		Gene	Age at onset	Penetrance	Model of transmission	No. Mutations and other changes	PAF (%)
	Locus						
	21q21	*APP*	Early	High penetrance	Dominant	16	0.005 (17)
	14q24.3	*PSEN1*	Early	High penetrance	Dominant	140	0.065 (17)
	1q31-42	*PSEN2*	Early	High penetrance	Dominant	10	0.01 (17)
	19q13.2	*APOE*	Early-Late	Reduced penetrance		1 (risk allele)	17 (17)
Parkinson's Disease [b]							
	4q21	*SNCA*	Early	High penetrance	Dominant	3	Small
	6q25.2-27	*Parkin*	Early		Recessive	> 70	0.4-0.7 (58)
	4p14	*UCHL1*	Late	Incomplete	Dominant	1	Small
	2q22-23	*NR4A2*	Late	High penetrance	Dominant	2	Small
	1p36	*DJ1*	Early		Recessive	11	1% of early PD (108)
	1p36-35	*PINK1*	Early		Recessive	>10	Small
	12p11.2-q13	*LRRK2*	Late	Incomplete	Dominant	7	5-6 % familial cases (64)

The references of the number of mutations and the families are in http://molgen-www.uia.ac.be/ADMutations/.[a]
The references of the number of mutations and the families are in the paper. [b]
PAF= Population attributable risk fraction (fraction of patients in the population explained by mutations in the genes).

Longitudinal studies in Caucasian populations have shown that carriers of one APOE*4 allele have a two-fold increase in the risk for AD (25, 26). The increase in risk is higher in homozygotes for the APOE*4 allele; in European and American populations the risk can be as high as 8 times that of APOE*3 homozygotes (25, 26). The APOE*4 allele is also associated with an earlier onset of the disease (27). The shift in the age of onset for carriers of this variant is also dose-dependent in that homozygous subjects are affected at earlier ages than heterozygotes for APOE*4. Unexpectedly, the progression of the disease does not appear to depend on the *APOE* genotype (28). *APOE* has also been related to other neurological disorders, including cerebral amyloid angiopathy (29), Parkinson's disease (30) and Creutzfeldt-Jakob disease (31), and modulates the outcome of other cerebral insults such as trauma (29). The *APOE* gene has also been implicated in the decline of cognitive function with age, although study results have been inconsistent (32, 33).

There is also ample evidence from experimental studies supporting the role of *APOE* in AD. This gene has been implicated in different pathophysiological pathways leading to neurodegeneration, including $A\beta_{42}$ deposition and antioxidative stress (34). Neuropathological examination of brain tissue in AD patients has shown a higher density of neuritic plaques and neurofibrillary tangles in APOE*4 carriers (35, 36). Biochemical studies have demonstrated that the *APOE* gene product, apolipoprotein E, can bind both the $A\beta_{42}$ and tau protein (37); this binding appears to be isoform-specific. Transgenic knock-out mice expressing human APOE*4, have an impairment in cognitive function and also show neuropathological changes (38, 39). Finally, higher levels of *APOE* expression have been associated with AD in several studies (40, 41). However, the exact mechanisms by which the *APOE* gene increases the risk of AD remain to be determined. On the population level, the APOE*4 allele plays an important role, explaining up to 17% of all AD patients (table 1).

In summary, all of the genes identified for AD so far, appear to be involved in the $A\beta_{42}$ pathway. Most of our insights into the pathogenesis of AD come from the relatively rare dominant mutations in the *APP* and *presenilin* genes, which, essentially, elucidated this pathway. The role of the $A\beta_{42}$ protein is not limited to carriers of the *APP* and *presenilin* genes. $A\beta_{42}$ metabolism was found to be disturbed in sporadic patients without dominant mutations or the APOE*4 allele (42). The identification of rare mutations, therefore has not only led to new insights into the pathogenesis of familial forms of AD but also shed light on the pathways of sporadic forms of the disease.

The genetics of AD is far from being disentangled. It is clear that other genes must be involved in AD. The present genes explain less than 20% of all patients, a proportion that is for a large part explained by the APOE*4 allele (table 1). Linkage of AD has been shown at chromosomes 1, 4, 6, 9, 10, 12 and 19 (for a recent review

of positive linkage analysis see (43)) (table 2). A recent study suggests that the gene responsible for the positive linkage of AD at chromosome 9 is the *ubiquilin* gene (*UBQLN1*) (44). However, this finding remains to be confirmed by other studies. The genes involved in the other regions have not been identified yet. Also, a large number of candidate gene studies have been conducted with conflicting results (for a review see (45)).

Table 2. Loci identified using linkage analysis for Alzheimer's disease and Parkinson's disease

Alzheimer's disease		References for linkage analysis [a]	Candidate genes [b]
	Locus		
	1p36	(109, 110)	*MTHFR* (111, 112)
	4q35	(113, 114)	Unknown
	5p13-15	(110, 115)	Unknown
	6p21- 6p35	(110, 113, 116)	*TNF-A* (117),*HLA2* (118) *ESR-A* (119), *HFE*(120)
	9p21/q22	(109, 110, 113, 115)	*UBQLN1(44) , VLDR-R (121)*
	10q21-22/ 10q24-25	(109, 110, 113, 114)	*IDE* (122), *CDC2* (123), *GST1/2* (124),
	12p11	(109, 110, 125)	*A2M* (126),*LRP* (127)
	Xp11-21/Xq21-26	(109, 110, 113)	*MAOA* (128)
Parkinson's disease			
	2p13	(129, 130)	PARK3 *(SPR)* (131)
	1p36	(132)	PARK9 (Unknown)
	1p32	(114, 133)	PARK10 (Unknown)
	2q36-37	(134, 135)	PARK11 (Unknown)

These loci have been reported at least in two studies although overlapping samples may occur in some of them with the exception of PARK-9 [a]. Candidate genes tested within loci [b].

PARKINSON'S DISEASE

PD is the second most frequent neurodegenerative disorder in the elderly and the most common movement disorder. As with AD, age is one of the strongest risk factors associated with this disease. The prevalence of PD is about 0.5-1% among people aged 65-69 years and increases to 1-3% in people 85 years old (46, 47). The age of onset of PD has a wide distribution. The disease onset can be classified as juvenile (onset < 21 years), early (onset between 21 and 50 years) and late onset PD (onset > 50 years) (48). Besides age, several environmental factors, such as 1 methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and pesticides, can lead to Parkinsonism (49). The relationship with MPTP intoxication, as well as the weak evidence for familial clustering, have fuelled a long term dispute on the genetics of PD (50).

The main clinical feature of PD is severe motor impairment. The cardinal signs are resting tremor, rigidity, bradykinesia, and postural imbalance. Pathological changes in the brain are neuronal loss in the dopaminergic system, including the substantia nigra pars compacta, the brain stem, the autonomic nervous system, some regions in the basal ganglia, and the cortex. Histopathological findings also show Lewy body inclusions in the neurons and the dendrites (46).

Treatment for PD that prevents neuronal death progression in the dopaminergic system is not yet available. Current therapy is targeted towards improving motor and psychiatric symptoms (51). Levodopa continues to be the key medication, as it is one of the intermediate molecules in the genesis of dopamine (51). Other groups of drugs include inhibitors of monoaminooxidase enzyme, dopamine agonists, and coenzyme Q_{10}, among others (51). New treatments aimed at preventing neuronal damage are also under development.

Genes and PD

Despite weak evidence for the familial aggregation of PD, several genes have been identified. The first gene identified was *SNCA* (*PARK1*; 4q21-q23), which encodes the α-synuclein protein, one of the major constituents of Lewy body inclusions (52). Three missense mutations in *SNCA* have been identified in families segregating dominant forms of PD, including one recently reported in a Spanish family (53-55) (table 1). Furthermore, duplications and triplications of this gene leading to PD have been found (56). These mutations are rare in patients with sporadic and familial PD (57, 58).

Three other genes have been associated with dominant forms of PD. The *NR4A2* (NURR1) gene, which encodes a nuclear transcription-like protein, is involved in the generation of dopaminergic neurons (59). The *UCHL1* gene, which encodes an ubiquitin carboxy-terminal hydrolase L1, is expressed in neurons and has been found in Lewy bodies (60). Although these genes are implicated in physiological pathways of neuronal function, their actual role as causal genes for PD has not been confirmed (61). The *LRRK2* (*PARK8*; 12q12) gene has been associated with familial and sporadic late onset PD (62). The gene encodes dardarin, a 2482 amino acid protein with several domains, including a tyrosine-kinase domain. Eight different mutations have been identified in this gene (62-65). These mutations might explain up to 5-6% of familial PD and 1% of sporadic PD (64). The role of this gene in the pathogenesis of PD remains to be investigated.

Also of interest is the large number of recessive mutations found in PD. The *parkin* (*PARK2*) gene was identified in Japanese families with recessive juvenile Parkinsonism (table 1) (66). The gene, located at chromosome 6q25-q27 (67), encodes an ubiquitine E-2 protein. More than 70 mutations have been identified in

the *parkin* gene, including point mutations and genomic rearrangements (48, 68) (table1). *Parkin* mutations explain about 50% of familial cases with recessive early onset forms of the disease (69) and between 15-20% of sporadic early onset PD (70). Contributions of the gene to late onset PD are not clear, but some studies indicated that it might be important (71, 72).

Two other genes are associated with recessive forms of familial PD; *DJ1* (*PARK7*), and *PINK1* (*PARK6*). *DJ1* was identified in families derived from a Dutch isolated community (73, 74). The gene is located at chromosome 1p36, and encodes a 189 amino acid protein, which is expressed in different tissues including brain. The function of this protein in the pathogenesis of early onset PD is not known in detail. Some studies indicate that the protein might be involved in the cellular response to oxidative stress (75). A total of 11 mutations in *DJ1* have been identified (75). The alterations include single base pair changes as well as large deletions (76-78). The frequency of these alterations is estimated to explain 1% of early onset PD.

The *PINK1* gene is located at chromosome 1p35-36 and has been found to segregate in families with recessive PD (79, 80). The product of this gene is a tyrosine kinase protein that localizes in the mitochondria, suggesting a possible role in energy metabolism (81). The function of the protein has not been determined, but its location in the mitochondria suggest that the protein might play a role in protecting cells from oxidative stress (72). At least 10 different mutations have been identified in this gene (81-84) in different populations. *PINK1* mutations appear to be more frequent than *DJ1* in early onset familial PD, but their role in sporadic PD seems to be small (84-86).

Like AD, the genetics of PD are still largely unknown. Other genes must be involved in the pathogenesis of PD. Some of them have been localized to chromosomal regions (61) (table 2), but the causative genes remain to be discovered. Candidate genes have also been tested for association with PD with inconsistent results.

PERSPECTIVES FOR FINDING NEW GENES IN NEURODEGENERATIVE DISORDERS

So far, the dominant and recessive genes that have been identified for PD and AD account for a small fraction of all cases. For AD, a common genetic risk factor is known (APOE*4), which explains a substantial number of patients, but leaves more than 80% of cases undetermined. For the vast proportion of affected individuals, the cause of the disease remains unknown.

To fully understand the pathways involved in AD and PD, we need to be able to identify the other genes involved. The best approach to find these genes depends on what type of genetic background we expect: (1) a number of common variants, each of them with a small genetic relative risk (87) (i.e., APOE*4), or (2) a large number of rare mutations with a large impact on the risk of disease (i.e., *PSEN1*, *parkin*). The first hypothesis, the common variants/common disease hypothesis is, at first sight, appealing. For common forms of AD and PD that occur late in life, it is easy to envision a common allele with an associated low risk (88). One may even argue that neurodegeneration may be the result of multiple interacting genes and environmental factors, rather than a mutation with major effects on protein function. These genes may have a small effect by themselves, making it difficult to identify them (89). To explain their high frequencies, we have to assume that such variants were common in early human populations, predating the modern expansion of humans (90). These common variants are only preserved if natural selection acting upon them is weak, which is likely for late onset diseases as they affect individuals after reproductive age (88).

If the common disease/common variants hypothesis is true for AD and PD, association studies (genome wide or targeting candidate genes, which, *a priori,* are expected to be involved in the disease based on protein function) provide the most powerful approach (45, 91). In association studies, the allele proportions are basically compared between a series of patients and controls (89). So far, the results of implementing this approach have been conflicting for both AD and PD. A recent review of late onset AD (92) included 90 studies which tested for association with polymorphisms in candidate genes at 55 different loci. Genes located in only three regions (6p21, 10q24, 11q23) showed consistent association in more than two studies. Nevertheless, negative results for these regions were also reported (92).

One of the explanations for the failure of association studies relates to the fact that multiple common variants may be present in a single gene (allelic heterogeneity). When the number is large, association studies will lose power, since a very large number of patients and controls are required to detect a small increase in genetic risk for each variant (93). However, flaws in the design of association studies might also have contributed to the conflicting results reported in the literature (94, 95). Another issue is that most association studies do not consider gene-gene interactions, which are expected to be present in complex diseases (96). A single-locus design might lack the power to detect a marginal increase in risk for an allele that depends on the presence of other alleles or genes, or other environmental factors (97, 98). Such genetic interactions might be found in a single gene design, provided that very large samples are available (98).

Last, but not least, most association studies have targeted only limited polymorphisms in a gene, which account for a small proportion of all polymorphisms available in the human genome.

Contrary to the common disease/common variants hypothesis, the common disease/rare variants hypothesis predicts that large numbers of rare variants, each one with a strong effect on the risk of disease, are responsible for complex diseases (93, 99). For late onset disorders such as AD and PD, these mutations can be maintained in populations due to the fact that the diseases occur after the reproductive period of life (99). The common disease/rare variants hypothesis is consistent with the strong aggregation observed in a large number of families with multiple AD cases. Such familial aggregation does not fit the view of a large number of genes with small effects. Common variants with a small effect may explain familial aggregation in siblings, but not in extended pedigrees involving parents and grandparents, which is the pattern often observed in AD (11, 100). For PD, the weak familial aggregation is still compatible with a large number of recessive forms, which might explain patients with sporadic forms of the disease.

Linkage analysis in extended families has been successful in mapping genetic variants associated with highly penetrant Mendelian forms of AD and PD (table 1). Still, for both AD and PD, there have been examples of linkage studies that were not replicated or in which the genes have not been identified (table 2). A major problem in the identification of a new rare mutation is that, within a family, multiple genes may be involved, hampering the power of linkage analysis (95). Further, to find rare variants in a series of patients, one has to sequence a large number of patients.

Rather than being two mutually exclusive hypotheses, both the common disease/common variants and common disease/rare variants approaches may be correct for neurodegenerative disorders such as AD and PD. Particularly, for AD, there are mutations with major effects (*APP*, *PSEN1*, *PSEN2*), as well as a common variant with a small effect (APOE*4). One may argue that this is also the case for PD, although common variants with small effects have not been identified yet.

DISCUSSION

Due to the progress in dissecting the pathogenic pathways of AD and PD, new therapeutic approaches are emerging. In AD, it is becoming clear that the disease is the result of the imbalance between the production and degradation of the amyloid proteins that accumulate in the brain (101, 102). Therefore, a clear target to develop new drugs is the prevention of aggregation of amyloid protein (103). As with AD, dissecting the genetics of PD has improved knowledge regarding the pathogenesis of

the disease tremendously. The discovery of the *SNCA* gene led to the identification of the α-synuclein pathway. It has been shown that misfolding and oligomerization of this protein are associated with neuronal toxicity in the dopaminergic system (61). Further genetic research into PD revealed other disease mechanisms, including mitochondrial dysfunction, the ubiquitine-proteasome pathway, and oxidative stress. These findings open new avenues for developing treatment (61, 104).

How to further dissect AD and PD? A huge problem is the genetic and environmental heterogeneity of complex diseases. An approach to overcome this problem is to embed the studies in isolated populations, in which a reduced genetic heterogeneity and a more uniform environment are expected (105, 106).

Linkage analysis is still a powerful tool to map genes associated with neurodegenerative diseases in such populations. Since, in isolated populations, inbreeding is often increased, they also offer a powerful setting to identify new recessive mutations. Until now, most studies have focused on genome screens in isolates, but also candidate genes studies may also benefit from the reduced genetic diversity (105).

An alternative approach may be to study AD and PD not as outcomes, but, rather, to use endophenotypes, i.e., intermediate phenotypes involved in the disease pathways which are, *a priori,* expected to be strongly related to a gene (107). Typical endophenotypes for AD may be either cognitive function or $A\beta_{42}$ protein levels in blood or CSF. Indeed, using $A\beta_{42}$ protein levels as an intermediate phenotype, linkage of AD to chromosome 10 was found (70). For PD, this may involve dopamine pathology for instance, as seen on PET scan. Combining the new opportunities offered by developments in genomics with the new approaches in phenomics, may open new avenues to dissect complex traits such as AD and PD.

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2

Genetic study of cognitive function: scope of the thesis

Genetic factors play a crucial role in the risk and progression of Alzheimer's disease (AD) (1). Considerable progress has been made towards understanding the genetics of AD. As presented in **chapter 1**, four genes (*APP*, *PSEN1*, *PSEN2* and *APOE*) have been implicated in AD. These genes have been shown to contribute to less than 20% of late onset AD. Other genes must be involved in the pathogenesis of the disease, as approximately 50% of AD cases are genetic in origin (2). However, the identification of other genes underlying the pathogenesis of AD has proven to be difficult.

One of the explanations for the failure to identify genes for AD is the poor design of many linkage and association studies, that have been largely underpowered, both in terms of the number of subjects studied, as well as the number of markers used (3). One approach to follow in the near future is to study large series of cases and matched controls, which will most likely require large multicenter studies (4). Alternative approaches, which are more feasible in the short term, are to select a relatively small study group while reducing the genetic heterogeneity in AD, and to study intermediate phenotypes (endophenotypes) for AD (**chapter 1**).

Cognitive function is a potential endophenotype for AD. Cognitive impairment is an overt manifestation of the disease (5). Furthermore, it has been demonstrated that a decline in various cognitive domains occurs before the disease is diagnosed (6). There is compelling evidence that the apolipoprotein E (*APOE*) gene, which is related to AD, is also involved in cognitive function (7). Thus, dissecting the genetics of cognitive function may shed light on the genetics of AD. The aim of this thesis is to study the genetic determinants of cognitive function. To reduce the problem of genetic heterogeneity, studies are conducted in a genetically isolated Dutch population, embedded in the Genetic Research in Isolated Populations (GRIP) program. In **chapter 3** of this thesis, the influence of both genetic drift and founder effects on the genetic make-up of the GRIP population are examined. Specifically, the question of whether common traits such as AD are genetically less complex in the GRIP population is addressed.

In **chapter 4** the heritability estimates of specific cognitive domains in a sample of 2575 subjects from the GRIP population are presented. Sex- and age-specific heritability estimates and the effects of inbreeding on cognitive function are evaluated. In **chapter 5**, the role of the *APOE* gene on the variation of cognitive domains is investigated. *APOE* is one of the established determinants of AD. The aim of this study is to reveal endophenotypes for AD that are relevant for future research. In **chapter 6**, the role of blood pressure, atherosclerosis and four candidate genes for hypertension on different domains of cognitive function is examined. Finally, in **chapter 7,** the results of the thesis are summarized and discussed in a broader perspective.

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3

The effect of genetic drift in a young genetically isolated population

Pardo LM, MacKay I, Oostra B, van Duijn CM, Aulchenko YS.
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ABSTRACT

The genetic make-up of genetically isolated populations may differ from the general population as a result of genetic drift and founder effects. We assessed the extent of this deviation in a recently isolated population located in the southwest of The Netherlands and studied as part of the Genetic Research in Isolated Populations (GRIP) program. A gene-dropping experiment was performed in a large pedigree from this isolate, assuming different founder frequencies in the population founders came from. Allelic frequencies in the last generations of this pedigree were estimated. Simulation analysis showed large fluctuations, as measured by variation coefficient and sufficient loss probability, when initial frequencies were lower than or equal to 1%. For initial frequencies larger than 1%, the fluctuations were small. We also analyzed mean heterozygosity and allele diversity of 592 markers in a random sample from the GRIP population. The results were compared with a general population (CEPH sample), old large isolate (Icelandic sample) and small-sized population of Talana (Sardinia). GRIP mean heterozygosity and mean number of alleles were significantly lower as compared with CEPH and Iceland, but much higher when compared with the Talana population. We also concluded that the findings from the GRIP population for common variants (>1%) are likely to be extendable to other young isolates in Europe, as well as to outbred populations.

INTRODUCTION

Genetic drift and founder effects are major evolutionary forces that determine the fluctuation in the genetic pool of finite populations when the effects of selection and migration are neglected. The extent of such effects in a population is determined by the population size, dynamics, breeding structure and time elapsed since foundation (1). Isolated populations are by definition founded by a small sample of individuals, and geographical and or cultural barriers have restricted migration and breeding with other groups, thereby also restricting genetic influx (2). Since not all isolated populations exhibit the same genetic structure, the extent of drift and founder effects will differ according to their evolutionary history. These differential effects will be reflected in terms of the extent of linkage disequilibrium and genetic diversity.

Association studies are predicted to be a powerful tool to detect common genetic variants with small effects (3, 4). However, in outbred populations the genetic and environmental heterogeneity is high and thus, large numbers of patients and controls are required (5). Power may be increased by conducting such studies in an isolated population which exhibit less environmental variation and a more homogeneous genetic background (2, 6). Another advantage of isolated populations is that linkage disequilibrium can be found over large distances. However, the extent of linkage disequilibrium and genetic variability varies among different isolated populations, making some of them better suited for gene mapping research than others (3). Recently, the necessity to study the evolutionary history of isolated populations to optimize the use of gene mapping tools has been stressed (7, 8). The value and extent of linkage disequilibrium is an issue of active debate and has been extensively analyzed in different demographic contexts (9-12). Another crucial point is that drift and founder effects can reduce the genetic variability; in isolated populations allele variants may become unique as a joint result of drift and founder effects. That is the case in Finland, where hereditary disorders that are highly prevalent but barely found elsewhere have been reported (13, 14).

Several studies have addressed the effect of drift in isolated populations (8, 15, 16). The present analysis was intended to analyze the drift and founder effects in a young genetically isolated community in The Netherlands. In a previous study (17), the extent of linkage disequilibrium in this isolate was evaluated. Here, we assessed to what extent drift and founder effects have reduced the genetic variability in this isolated population. We simulated the stochastic process due to gamete sampling to measure the change in allelic frequencies using the complete genealogical information on a large pedigree belonging to this community. Empirical data was also used: the average heterozygosity and mean number of alleles in 592 markers from subjects derived from this isolate was compared with data available

from populations with different demographic histories (a general population, and populations of Iceland and Talana).

MATERIALS AND METHODS

Subjects

This study was performed using a pedigree derived from an isolated community, studied as part of the Genetic Research in Isolated Populations (GRIP) program. This young genetic isolate is located in the southwest of The Netherlands. From historical data we know that the population was constituted as a religious isolate in the middle of the 18th century by a limited number of founders. The population has remained in isolation, with a low rate of immigration, and experienced an exponential increase in size in the last century.

In this population, we recently started the ERF (Erasmus Rucphen Family) cohort study. This study is concentrating on unraveling genes underlying quantitative trait variation in humans. All participants are screened for many quantitative traits related to a set of diseases of interest. At present, we have collected phenotypic information on 1053 subjects who comprise the last 4-5 generations of a single large pedigree, connecting 9800 individuals. The genealogical information includes data on individuals that were living in the isolate from about the middle of the 16th century, and extends to the current inhabitants of these communities. We performed a gene-drop experiment including all people with genealogical information available (back to founders from the 16th century). The study was approved by the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam, and written consent was obtained from all subjects.

Computer simulations and summary statistics

We used the complete genealogical information of the large pedigree to simulate stochastic changes due to drift and founder effects. The program package MGA-Simulate (http://mga.bionet.nsc.ru) was used for the gene-dropping experiment. This program randomly assigns genotypes to founders given the allele frequency in a population the founders come from, and assuming Hardy-Weinberg equilibrium. The allele transmission in descendants is then simulated. In this analysis, we simulated one locus with two alleles. Eight different initial frequencies ranging from 0.01 to 50 percent were considered. For low initial frequencies (0.01, 0.1% and 1%), 10 000 simulations were used, for others (2.5, 5, 10, 25 and 50%), 5 000 simulations were performed. After each simulation, the frequency of one of the alleles was estimated in the last generations of the pedigree that at present are included in ERF study.

The distribution of allele frequencies for each one of the eight initial frequencies was obtained by repeated simulations. The means, median and quintiles of the distributions were estimated using S-plus 2000 (18)

Mean heterozygosity and mean number of alleles

We compared the heterozygosity and number of alleles between the Dutch isolate, Icelandic (deCode) and CEPH (Centre d'Etude du Polymorphisme Humain) populations using 592 Short Tandem Repeat markers typed in all three samples. These markers were genotyped in 58 random subjects from the GRIP population who participated as controls in a recent study; details are described elsewhere (17). The expected heterozygosity for each marker was calculated as: h = 1- H, where H is the homozygosity calculated as the sum of the squared frequencies of all alleles at one locus (19). The mean number of alleles was estimated by allele counting. All markers in the GRIP population were tested for deviation from Hardy-Weinberg equilibrium proportions using the library genetics for R program (http://www.r-project.org).

Information concerning the mean heterozygosity and number of alleles for the CEPH families are available at the Marshfield Clinic web page (http://research.marshfieldclinic.org/ genetics/ Map_Markers /maps/ IndexMapFrames.html), based on 8 families with 28 genotyped founders. The expected heterozygosity and mean number of alleles in the Icelandic families (deCode) were calculated (as for the GRIP population) from Kong *et al*. (2002), with 297 founder individuals. We also compared mean heterozygosity and mean number of alleles from a subset of 20 markers on chromosome 19 reported for the Talana population, based on 381 founders (20). The differences in mean heterozygosity and mean number of alleles among the GRIP population, CEPH, deCode and Talana families were tested using a non-parametric sign test. Bootstrapping was performed to study the effect of sample size on the estimation of the mean number of alleles. We derived 1000 random samples, each one of 56 chromosomes for each marker from GRIP and deCode families, and calculated the mean number of alleles per locus in all 592 markers. For a given marker, an overall mean estimate was obtained from these bootstrapped distributions. The estimates were compared with the number of alleles per marker in CEPH families using a sign test.

RESULTS

Description of the pedigree

The genealogical data set we used to do simulations comprised a total of 9800 individuals with 2296 founders. The maximal number of generations was 23 and the proportion of men in this data set was 49%. The last 4-5 generations of this large pedigree consist of 1053 individuals who are the participants of the ERF study. Out of a total of 1053 individuals, 823 (82%) had an inbreeding coefficient larger than zero, and 112 (11%) had an inbreeding coefficient higher than or equal to 0.016, which may correspond to a second cousin marriage. The average inbreeding for all individuals was 0.006. 90% of the kinship values of all possible pairs were larger than zero. The average kinship value for all the ERF pairs was 0.008, and 0.009 for the pairs with kinship values larger than zero.

Simulations

Figure 1 depicts the distribution of the simulated allele frequencies obtained from individuals of the last generations of the pedigree. The median and mean estimates of these distributions were similar to the initial values we set for founders (table 1). However, the shape of the distribution varied according to the initial frequency being skewed towards zero for low initial frequencies (figure 1 a-c), meaning that the rare alleles have disappeared. For instance, at an initial frequency (P_0) of 0.01% the allele was lost in 95.4% of the simulations (table 1). In contrast, alleles that were common in founders (P_0 >1%) were never eliminated.

Table 1 also shows that the fluctuation in allele frequencies was highest for very rare alleles, as shown by the coefficient of variation. At an initial frequency of 0.01, the coefficient of variation was 12 whereas for an initial frequency of 0.025 it was 0.5. The highest initial frequency, 0.5 in founders, corresponded to the lowest coefficient of variation, 0.09. Thus, the fluctuation in the allele frequencies in the contemporary generations is only considerable for alleles with low initial frequencies.

The results obtained in the simulations were compared with theoretical distributions expected under a simplified model. In this model, we assumed that generation time is 30 years and consequently 7 generations have passed from the foundation of the population. The pedigree data was used to compute the number of births in 30-year bins. The harmonic mean of these numbers was used to estimate the effective population size, N_e. These calculations suggest an effective size of 725.43. We assumed that the isolation of the population is complete (no immigration). Under these assumptions, the probability of loss is given by exp $\{-4N_e p_0 /t\}$= exp $\{(-414.53p_0)\}$ and the variance of the allelic frequency distribution is given by $p_0(1-p_0)1-[1-(1/(2N_e))]^t = 0.05p_0(1-p_0)$ (21). The values of the loss probability

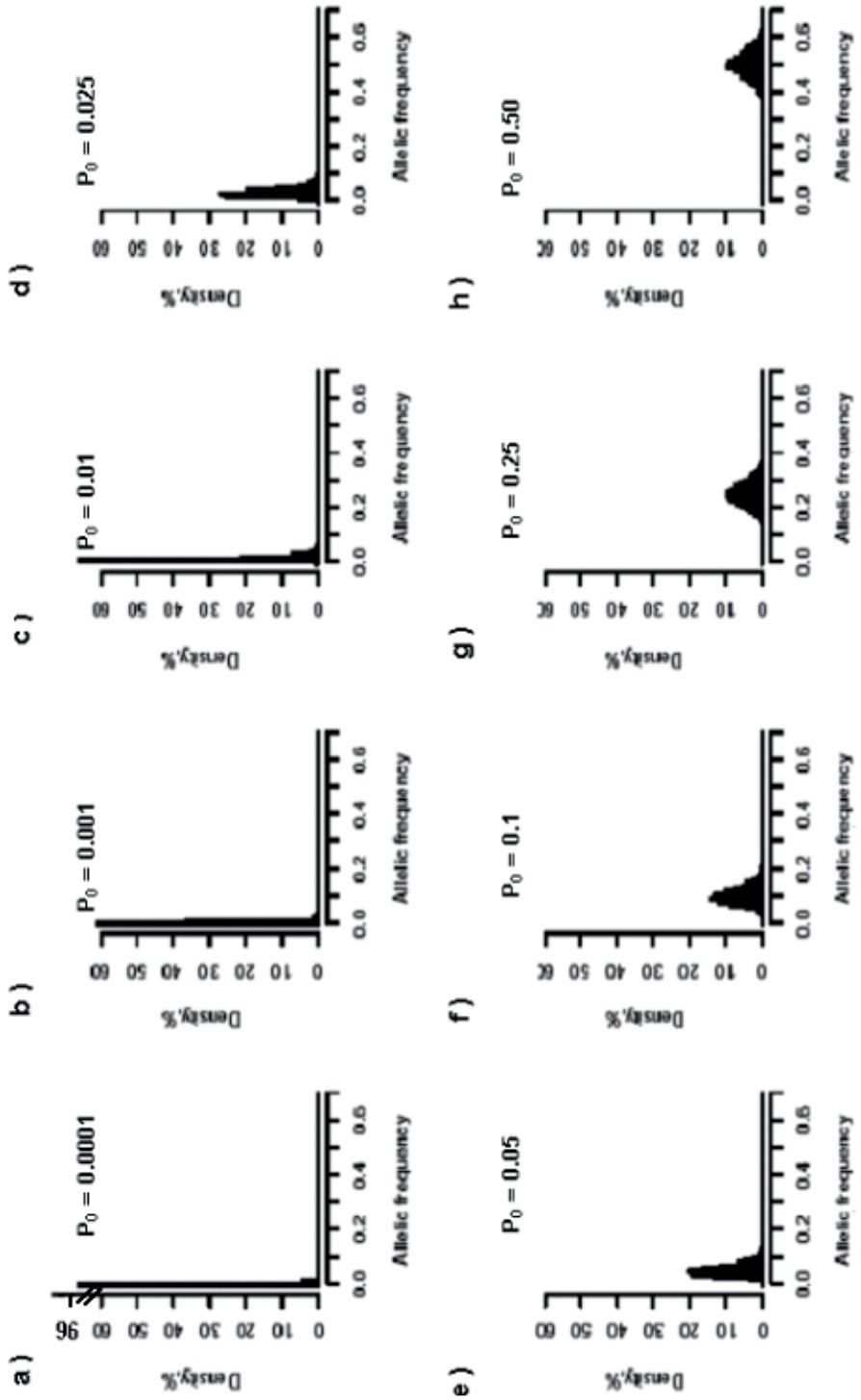

Figure 1. Distribution of simulated allele frequencies from subjects participating in ERF study given different initial frequencies, P_0.

Table 1. Distribution of allelic frequencies at last generations given different initial frequencies (P_0)

Initial freq (P_0)	Mean	Median	S.D	S.D***	Minimum	Maximum	9.5% C. I	Coefficient of Variation	Loss***	Loss/Increase in allele frequencies*			
										Loss	> than 2 times	> than 5 times	> than 10 times
0.0001	0.0001	0	0.0012	0.001	0	0.0626	0.000-0.001	12	0.973	0.954	0.046	0.046	0.026
0.001	0.001	0	0.0031	0.002	0	0.0631	0.000-0.009	3.1	0.759	0.615	0.115	0.0483	0.02
0.01	0.0099	0.0068	0.0095	0.007	0	0.0883	0.001-0.037	0.9	0.064	0.0083	0.1178	0.0068	0
0.025	0.0304	0.0268	0.0163	0.011	0.0021	0.1104	0.008-0.072	0.5	0.001	0	0.11	0	0
0.05	0.0499	0.0468	0.0207	0.015	0.0089	0.1729	0.019-0.098	0.42	0	0	0	0	0
0.1	0.1002	0.0967	0.0292	0.021	0.0278	0.2634	0.053-0.167	0.29	0	0	0	0	0
0.25	0.2496	0.2482	0.0413	0.03	0.1215	0.4306	0.175-0.335	0.16	0	0	0	0	0
0.5	0.5003	0.5	0.0468	0.035	0.3333	0.6666	0.407-0.591	0.09	0	0	0	0	0

* Estimated probability that the rare allele was lost or increased its frequency more than several times
** Coefficient of variation was calculated as standard deviation divided by the mean
*** Estimated S.D and Probability of loss as calculated from formulas (22)

and standard deviation of the distribution of allelic frequency expected according to these formulae are shown in table 1. Though it is clear that these formulas over-estimate the effects of drift, generally the agreement is good.

Mean heterozygosity and mean number of alleles

The results obtained with simulations were compared with empirical data. A sample of 592 markers from 58 random subjects derived from the GRIP population was used to calculate mean heterozygosity and mean number of alleles. There was no deviation from Hardy-Weinberg equilibrium proportions. We compared mean heterozygosity and mean number of alleles in the GRIP population with the information available for 28 founders from 8 CEPH families (http://research.marshfieldclinic.org/ genetics/ Map_Markers /maps/IndexMapFrames.html) and 297 founders from Icelandic families (22). Table 2 shows the expected heterozygosity per chromosome calculated for these three groups. Overall, the mean heterozygosity calculated over 22 chromosomes was 0.757 (standard error; 0.003), 0.759 (standard error; 0.004) and 0.768 (standard error; 0.003) for the GRIP population, deCode and CEPH families, respectively. The mean heterozygosity was slightly higher in the CEPH families, lowest in the young GRIP population with a small founder size, and intermediate for the old Icelandic isolate with a large number of founders. The mean difference was tested using the sign test and was significant when the GRIP population was compared with the deCode families ($p = 0.04$) and highly significant when the GRIP was tested against the CEPH families ($p < 0.0001$).

The mean number of alleles genome-wide and per chromosome was also calculated. As shown in table 2, the GRIP population had the smallest number of alleles (9.799; standard error; 0.116). DeCode families showed the largest allele diversity with a mean estimate of 10.059 (standard error; 0.125), whereas for the CEPH families the mean value was 9.858 (standard error; 0.139). The differences in number of alleles, tested using the sign test, was highly significant for all the comparisons among the populations (p value < 0.0001). In addition, the mean heterozygosity and mean number of alleles from a subset of 20 markers from chromosome 19 reported in the Talana population was also compared (20). The mean heterozygosity for this population was 0.685 whereas for the GRIP population this value was 0.775. Allele diversity was also lower in the Talana population. The differences in heterozygosity and mean number of alleles were statistically significant (Sign test $p < 0.001$).

The largest variability showed by the deCode families may be the result of having the largest sample size for this population. To correct for unequal sample size we performed 1000 bootstrapping replicates. In each replica we sampled from the deCode data the same number of founders as for the CEPH families (28).

Table 2. Comparison of mean heterozygosity and mean number of alleles among GRIP population, deCODE and CEPH families

		Mean Heterozygosity			Mean number of alleles		
Chromosome	**No. Markers**	GRIP	DECODE	CEPH	GRIP	DECODE	CEPH
1	55	0.751 (0.011)	0.753 (0.011)	0.762 (0.010)	9.309 (0.395)	9.399 (0.358)	9.382 (0.465)
2	49	0.765 (0.010)	0.767 (0.009)	0.776 (0.008)	10.122 (0.356)	10.204 (0.430)	10.388 (0.464)
3	33	0.763 (0.013)	0.758 (0.018)	0.780 (0.011)	10.000 (0.379)	10.152 (0.420)	9.697 (0.432)
4	42	0.752 (0.013)	0.757 (0.014)	0.769 (0.012)	9.643 (0.427)	9.833 (0.552)	9.238 (0.491)
5	33	0.768 (0.011)	0.771 (0.012)	0.782 (0.009)	10.152 (0.463)	10.424 (0.467)	10.606 (0.513)
6	32	0.773 (0.013)	0.775 (0.013)	0.772 (0.015)	10.452 (0.486)	10.903 (0.569)	10.097 (0.483)
7	27	0.773 (0.013)	0.775 (0.016)	0.781 (0.012)	10.407 (0.478)	10.481 (0.521)	11.074 (1.008)
8	28	0.746 (0.017)	0.750 (0.015)	0.774 (0.012)	9.036 (0.461)	9.357 (0.535)	9.857 (0.666)
9	33	0.757 (0.015)	0.759 (0.016)	0.767 (0.014)	9.636 (0.484)	9.848 (0.485)	9.697 (0.534)
10	29	0.756 (0.013)	0.764 (0.012)	0.762 (0.011)	9.103 (0.519)	9.310 (0.476)	9.276 (0.478)
11	30	0.745 (0.015)	0.752 (0.017)	0.766 (0.013)	9.533 (0.567)	9.667 (0.609)	9.633 (0.667)
12	27	0.741 (0.022)	0.730 (0.022)	0.757 (0.019)	9.889 (0.598)	10.519 (0.800)	9.704 (0.671)
13	18	0.740 (0.025)	0.744 (0.025)	0.755 (0.025)	10.111 (0.914)	9.944 (0.891)	8.333 (0.832)
14	21	0.767 (0.014)	0.749 (0.015)	0.770 (0.011)	8.905 (0.483)	9.381 (0.465)	9.571 (0.767)
15	19	0.774 (0.020)	0.759 (0.029)	0.772 (0.023)	11.526 (0.944)	11.368 (0.925)	11.632 (1.038)
16	22	0.754 (0.013)	0.766 (0.015)	0.764 (0.014)	9.727 (0.607)	10.227 (0.674)	10.091 (0.584)
17	21	0.744 (0.020)	0.754 (0.019)	0.741(0.021)	9.143 (0.607)	9.667 (0.670)	9.095 (0.807)
18	22	0.767 (0.017)	0.765 (0.019)	0.766 (0.015)	10.136 (0.600)	10.636 (0.653)	9.500 (0.714)
19	15	0.763 (0.021)	0.776 (0.020)	0.776 (0.018)	9.867 (0.646)	10.800 (0.698)	10.333 (0.701)
20	18	0.784 (0.015)	0.786 (0.019)	0.794 (0.016)	10.389 (0.805)	10.944 (0.887)	11.167 (0.916)
21	9	0.682 (0.047)	0.704 (0.047)	0.720 (0.041)	9.556 (0.818)	10.000 (0.667)	9.889 (0.920)
22	9	0.732 (0.027)	0.733 (0.029)	0.747 (0.028)	9.000 (0.527)	9.111 (0.539)	8.333 (0.500)
All	**592**	**0.757 (0.003)**	**0.759 (0.004)**	**0.768 (0.003)**	**9.799 (0.116)**	**10.059 (0.125)**	**9.858 (0.139)**

The mean heterozygosity and mean number of alleles per chromosome are given with standard errors.

When using bootstrap, the mean number of alleles was estimated as 7.62. The same analysis was performed for GRIP, and the estimate was of 7.24. Differences between these bootstrapped estimates and the mean number of alleles in the CEPH families were statistically significant (Sign test; p value < 0.0001)

DISCUSSION

To analyse the extent of drift and founder effects in a recent genetically isolated Dutch population, we used the complete genealogical information of a reconstructed pedigree to simulate random changes in allele frequencies. Our study showed that the fluctuation in allele frequencies over generations is small for variants attaining high frequencies in a population where the founders of the isolate come from. Alleles with an initial frequency higher than 1% are likely to be maintained in descendants since in our simulation they were never lost. This suggests that common alleles that are associated to traits in GRIP will also be found in other outbred Dutch populations. In contrast, at frequencies lower than or equal to 1%, alleles are often eliminated from our population (probability of loss was as high as 96% for initial frequencies equal to 0.01%). These results agree with the results obtained by analytical formulas under simplified assumptions. However, the probability of loss is higher for the expected distributions, because the assumption of complete isolation is not fulfilled in this isolate.

The findings obtained in the simulation analysis were supported by empirical data. Populations with a small effective number of founders are expected to show reduced genetic variability as compared to populations with larger founder size and genetic flow (19). We compared genetic variability in our isolate with populations with a different demographic history. CEPH families might be regarded as pedigrees derived from an outbred population with a heterogeneous genetic background (23). On the other hand, families from Iceland are considered to be a genetically homogeneous sample of an old isolated population with a large number of founders (24). We found lower allele diversity in our GRIP population than in both the CEPH and Icelandic populations. However the difference was not very large. The comparison with the Talana population, with a founder size of 200 and a long history of geographic isolation (25), showed that allele diversity is lower in Talana as compared to the GRIP population. Therefore, genetic variability in our isolate seems to lie between that of a population with large drift and founder effects (Talana population) and a more heterogeneous one (CEPH families).

In our study, the allele diversity in the deCode families was larger when compared with the CEPH families (table 1); theoretically, there are two explanations

for this observation. First, the difference in the number of alleles may indicate a difference in laboratory techniques applied. Second, the estimated number of alleles may be affected by the sample size. As shown by our bootstrapping experiment, the second hypothesis is the most likely explanation. By bootstrapping, we showed that when we used the same number of founders for all the populations, the GRIP population had the lowest allele diversity (7.2 alleles), and deCode families the second lowest (7.63). The decrease in the number of alleles when a smaller sample was considered was on average two, for both the GRIP population and the deCode families (from 9.8 to 7.2 in the GRIP sample and from 10.1 to 7.6 in the deCode families). Hence, this result reflects that allele diversity in a population largely depends on the sample size, as reported previously (20).

In a recent study, Aulchenko *et al* (2004) studied the amount and decay of linkage disequilibrium with genetic distance in this Dutch isolated population. The study reported a similar extent of linkage disequilibrium to other recent isolates from various parts of the world. Furthermore, linkage disequilibrium was found over large distances, highlighting the potential of this population to conduct gene mapping. In the present analysis we addressed the magnitude of the composite drift and founder effects in this isolate. We have shown that the genetic variability is reduced in the GRIP population. However, only alleles with a frequency lower than 1% are likely to be lost. The major finding of the present study is that although fluctuations in allele frequencies may occur in the GRIP population, and other recent isolates in the world due to drift and founder effects, genetic variants with a frequency higher than 1% are expected to be present in both, young genetic isolates and outbred populations. Therefore, findings of genome screens conducted in young isolates may be generalized to other demographic contexts.

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4

The heritability of cognitive function in a young genetically isolated population

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ABSTRACT

Although genetic factors influence human cognitive function, their contribution to the variation in specific cognitive domains is less clear. We estimated the heritability of different cognitive domains related to Alzheimer's disease and other dementias in 2575 subjects from a recent genetically isolated Dutch population. We also studied the effects of inbreeding, and examined sex- and age-specific genetic effects on the variation of cognitive domains. A variance components method was used to estimate the heritability of cognitive domains. We estimated sex-specific variance components in women and men. Age-specific genetic effects were estimated in two age categories (<50 years and ≥ 50 years).

The heritability of cognitive domains ranged from 10% (recognition) to 36% (visuospatial-visuoconstructive abilities). Inbreeding was associated with a small but significant decrease in general cognitive ability, visuospatial-visuoconstructive abilities and executive function. Age-specific genetic effects were found for psychomotor speed, phonological fluency and susceptibility to interference. The genetic correlations for these domains were between 0.64 and 0.73, suggesting that about 30% of the genetic expression in these domains might be age-group specific. Although we did not observe a sex-specific genetic background modulating cognitive domains, we observed differences in the magnitude of genetic and environmental effects between sexes in learning and susceptibility to interference. Our findings suggest that the genetic contribution to the variation in cognitive function differs across specific cognitive domains. We also showed that inbreeding and age-specific genetic effects modulate specific cognitive domains.

INTRODUCTION

Genetic factors have been shown to play a key role in human cognitive variation (1-3). The heritability of general cognitive ability has been consistently estimated to be approximately 50% (for a review see (2)). There is less agreement about the contribution of genetic factors to the variation of specific cognitive domains such as memory and executive function, which are commonly affected by aging, Alzheimer's disease (AD) and related disorders (4, 5). Heritability estimates range from 21% to 60% for memory (6, 7), while heritability estimates for different components of executive function vary from 35% to 79% (8, 9). The variability of these estimates might be due to the heterogeneity of the study designs (6, 7, 9, 10) and perhaps, different ethnic backgrounds (9, 10).

Age is a strong predictor of cognitive variation. Although environment is a major determinant of the age-related differences in cognitive function (11), there is also evidence that some of the genetic expression of cognitive function is age-specific (12). Thus, genes determining cognitive function at birth might differ from those that modulate cognitive decline during aging. The latter may be of relevance in light of current studies of the genetics of late onset AD and related dementias.

Along with age, sex is also related to the variation in cognitive function. Sexual dimorphism has been documented for several cognitive domains, and there is evidence that it might be under genetic control (13-16). Sex-specific genetic effects on the variation of a number of quantitative traits such as lipid levels, glucose metabolism, blood pressure and serotonin levels have been reported (17-19). To what extent sexual dimorphism in human cognitive variation is due to sex-specific genetic expression is unclear.

In certain populations inbreeding was found to contribute to the genetic variance of general cognitive ability (20). Early studies showed that inbreeding was associated with a lower cognitive performance (21). To date, studies addressing the effects of inbreeding on specific cognitive domains are scarce. The effect of inbreeding on specific cognitive domains is of interest, since recently, a role for recessive mutations in AD in an inbred population was reported (22).

Previously, we studied the heritability for various cognitive domains in 780 subjects, belonging to a single extended pedigree from a recent genetically isolated Dutch population, in relation to cardiovascular risk factors (23). In the present analysis, we extend this previous work to include a larger series of 2575 relatives to answer whether inbreeding affects specific cognitive domains (memory and executive function) and whether there are sex- and age-specific genetic effects on these domains of cognitive function.

MATERIALS AND METHODS

Study population

The present study was conducted within the setting of the Erasmus Rucphen Family (ERF) study, a family-based study of a recent genetically isolated population located in the southwest of The Netherlands (24, 25). The population was founded by a limited number of individuals in the 18th century and was characterized by minimal immigration until the last few decades. The genealogical database contains information about the current inhabitants of this area and their ancestors and includes over 80 000 records. For the ERF study, 22 families were selected that had at least 5 children baptized in the community church between 1850 and 1900. All living descendants of the families and their spouses, aged 18 and older (n = 2906), were invited to participate. The recruitment of participants started in June 2002 and finished in February 2005. The Medical Ethics Committee of the Erasmus Medical Center, Rotterdam approved the study.

Neuropsychological assessment

Trained assistants administered a battery of neuropsychological tests on the ERF participants (Sleegers *et al*, in press). Participants with a diagnosis of neurological or psychiatric disorders were excluded (n = 47) (Sleegers *et al*, in press). Five neuropsychological tests adapted to the Dutch language were used to evaluate different cognitive domains related to neurodegenerative disorders (table 1) (4, 5, 26), namely: the Dutch version of the auditory verbal learning test (AVLT) (27, 28), the trail making test (TMT) (29), the Stroop colour-word test (30, 31), the verbal fluency test (32, 33), and the block design subtest of the Wechsler adult intelligence test (WAIS) (34).

AVLT assesses different components of memory through a number of different trials (see table 1 for details), yielding several independent measures (35, 36). Four measures were derived from this test: short-term or working memory (AVLT-trial I), learning (AVLT-trials II-V), delayed recall (AVLT-trial VI) and recognition (trial-VII). The TMT consists of two tasks. The first task (TMT-A) is used to assess psychomotor speed, whereas the second one (TMT-B) evaluates mainly cognitive flexibility (5). The Stroop colour-word test is composed of three tasks. The first two tasks (word naming in card I and colour naming in card II) are used to evaluate selective attention. The third task measures susceptibility to interference, or the ability to inhibit an automatic response (interference task in card III) (35). The verbal fluency test consists of two measures, semantic fluency and phonological fluency, which are indicators of several cognitive abilities, including the ability to generate search strategies to retrieve information (executive function) (35). Here we used

Table 1. Description of the cognitive tests derived from the neuropsychological battery

Neuropsychological tests	Cognitive domains	Scores definition
Dutch Adult Reading Test (DART)	**General cognitive ability**	
DART†		No. correctly pronounced words
Auditory verbal learning test (AVLT)	**Memory**	
AVLT-trial I†	Short-term memory	No. correctly recalled words trial I
AVLT-trials II-V†	Learning	No. correctly recalled words trials` II to V
AVLT-trial VI†	Delayed recall	No. correctly recalled words trial VI
AVLT-trial VII†	Recognition	No. correctly recalled words from an interference list
Verbal fluency tests	**Verbal Fluency**	
Semantic fluency†	Speed and facility of verbal production	No. correctly named items in two categories (animals, professions)
Phonological fluency†	Speed and facility of verbal production	No. correctly named items in 3 categories (letters A, D,T)
Trail-making test (TMT)	**Executive function & psychomotor speed**	
TMT-A†	Psychomotor speed	Time in seconds to complete the task
TMT-B†	Cognitive flexibility	Time in seconds to complete the task
Stroop colour-word test	**Executive function & selective attention**	
Card I†	Selective attention (reading colours)	Time in seconds to complete the task
Card II†	Selective attention (naming colours)	Time in seconds to complete the task
Card III†	Susceptibility to interference	Time in seconds to complete the task
Block design Subtest (WAIS)	**Visuospatial and visuoconstructive abilities**	
Block Design†		Number of replicated designed blocks

† Cognitive test derived from the neuropsychological battery

the semantic and phonological fluency tests, along with TMT-B, and card III of the Stroop colour-word test, to evaluate executive function. Finally, the block design subtest (WAIS) was used to measure visuospatial and visuoconstructive abilities (35).

As part of the neuropsychological assessment we included the Dutch Adult Reading Test (DART), as a measure of general cognitive ability. This test has a high correlation with other more extensive tests that measure general cognitive function (35). The education level attained by the ERF participants in the Dutch school system was determined. We categorized the education level into three groups: elementary (8 years or less of full-time education), junior vocational training (8 to 14 years of education) and senior vocational or academic training (at least 15 years of full-time education) (36, 37).

Statistical analysis

Gender differences between the scores of the cognitive tests were tested with the Mann-Whitney U test. Age-quartile differences between the scores of the cognitive tests were tested with the Kruskal-Wallis H test. The inbreeding coefficients, the probability that 2 alleles at the same locus of a person are identical by descent (i.e. derived from the same ancestral allele), were computed for all individuals using all available genealogical information. The PEDIG package of programs was used for this purpose (38). We categorized the levels of inbreeding into quartiles.

We transformed the cognitive tests that were skewed using the natural logarithm (for AVLT-trial VII, TMT-A, TMT-B, cards I, II and III of the Stroop colour-word test and block design tests) or the square root (for DART). A full pedigree-variance components method as implemented in the SOLAR 2.1.2 software package (39) was used to estimate both the heritability as well as the effects of inbreeding on the cognitive tests. The phenotypic variance of a cognitive test, which reflects the inter-individual variation of an underlying cognitive domain, was partitioned into its additive genetic (σ^2_G) and environmental (σ^2_E) variance components (40). Heritability was estimated as the ratio of the additive genetic variance to its phenotypic variance:

$h^2 = \sigma^2_G / (\sigma^2_G + \sigma^2_E)$;

To estimate the heritability of the cognitive tests the following models were used: for general cognitive ability we adjusted for age, age^2 (to account for non-linear age effects), sex, level of education and inbreeding quartiles. For the other cognitive tests we included general cognitive ability in addition to the previous covariates to adjust for the variance that is common to all cognitive domains (41). The proportion of the phenotypic variance of cognitive tests due to covariates as well as general cognitive ability was estimated.

To evaluate sex-specific genetic effects on the variation of the cognitive tests, we used a standard approach (17, 42). In brief, we included the estimation of

genetic and environmental variances specific for men and women, as well as the correlation between genetic components of a cognitive test in men and women (ρ_G). Three different hypotheses were tested: (1) $\rho_G = 1$, which tests whether genes influencing a cognitive trait in men and women are the same; (2) $\sigma^2_{G\text{-}M} = \sigma^2_{G\text{-}F}$, which tests whether the genetic variance in men is equal to the genetic variance in women; and (3) $\sigma^2_{E\text{-}M} = \sigma^2_{E\text{-}F}$, which tests whether the residual environmental variance of a cognitive trait is equal in men and women. Rejection of hypothesis 1 implies that a different set of genes contributes to the variation in cognitive traits in men and women. Rejection of hypothesis 2 implies that the magnitude of genetic effects on the expression of a cognitive trait differs between sexes. Rejection of hypothesis 3 indicates that the magnitude of environmental effects on the expression of a cognitive trait differs between sexes. Likelihood ratio tests (one degree of freedom) were used to evaluate the above hypotheses (nested models) against a general one, in which all parameters were estimated (43).

To estimate the age-specific variance components of the cognitive tests, we split the sample into two broad age categories (< 50 years and ≥ 50 years) and followed the same approach as for sex-specific genetic effects. We selected this cut-off point because age-related differences in cognition are substantial above 50 years of age (44).

RESULTS

A total of 2575 subjects were phenotyped for specific cognitive domains. The age of the subjects ranged from 18 to 89 years (median: 48 years; 95% of the distribution: 20.38 - 75.28 years) and the proportion of men was of 49%. Of these participants, 82% were inbred (median: 0.002; 95% of the distribution: 0 - 0.02), although less than 10% had inbreeding values equal or higher than 1/64 (equivalent to a second cousin marriage). The average level of education corresponded to a junior vocational training. Six percent of the population (n = 147) had senior vocational or academic training.

Table 2 shows the median scores of the cognitive tests by gender. Men performed significantly better in the cognitive tests evaluating general cognitive ability (DART), visuospatial-visuoconstructive abilities (block design) and verbal fluency (semantic). Women performed significantly better than men in memory-related tests such as those evaluating learning, delayed recall, selective attention (card II of the Stroop colour-word test) and in susceptibility to interference. The median score of the test for working memory (AVLT-trial I) was the same for men and women, though the range of the distribution was significantly different (range

Table 2. Median score (2.5th-97.5th percentiles of the distribution) of the cognitive tests by gender and age-quartiles

	Gender			Age quartiles				
Cognitive tests	**Men**	**Women**	**P value†**	**<38.5 yr**	**38.5- 50 yr**	**50.1- 61.4 yr**	**>61.4 yr**	**P value††**
Dutch Adult Reading Test	64.0 (18.5-92.0)	60.0 (16.0-88.0)	**0.001**	72.0 (40.0- 90.0)	64.0 (26.0-90.0)	58.0 (16.0-90.0)	46.0 (10.0-86.0)	**< 0.001**
AVLT-trial I	4.0 (1.0-8.0)	4.0 (1.0-8.0)	**< 0.001**	5.0 (2.0-9.0)	5.0 (2.0-8.0)	4.0 (2.0-7.0)	3.0 (1.0-6.0)	**< 0.001**
AVLT-trials II-V	31.0 (14.0-49.0)	35.0 (16.0-51.9)	**< 0.001**	40.0 (25.0-53.0)	35.0 (19.0-51.0)	31.0 (18.0-47.0)	25.0 (11.0-41.0)	**< 0.001**
AVLT-trial VI	7.0 (2.0-13.0)	8.0 (2.0-13.0)	**< 0.001**	9.0 (4.0-14.0)	8.0 (3.0-13.0)	7.0 (2.0-12.0)	5.0 (1.0-10.0)	**< 0.001**
AVLT-trial VII	14.0 (8.15-15.0)	14.0 (9.0-15.0)	**< 0.001**	15.0 (12.0-15.0)	14.0 (10.0-15.0)	14.0 (9.0-15.0)	13.0 (7.0-15.0)	**< 0.001**
Semantic fluency	37.0 (19.0-58.0)	36.0 (17.0-55.0)	**< 0.001**	41.0 (25.0-60.0)	38.0 (22.0-57.0)	35.0 (18.0-55.0)	29.0 (14.0-48.0)	**< 0.001**
Phonological fluency	24.0 (7.0-47.0)	24.0 (7.0-47.0)	0.68	29.0 (13.0-50.0)	26.0 (10.0-47.0)	22.0 (7.0-46.0)	18.0 (4.0-40.0)	**< 0.001**
TMT-A*	33.0 (16.0-93.0)	34.0 (17.0-96.0)	0.20	25.0 (14.0-48.0)	30.0 (17.0-59.0)	38.0 (20.0-82.0)	51.0 (25.0-121.0)	**< 0.001**
TMT-B*	83.0 (35.0-450.0)	83.0 (34.0-450.0)	0.50	56.0 (29.0-133.0)	74.0 (36.0-207.0)	100.0 (46.0-450.0)	148.0 (63.0-450.0)	**< 0.001**
Card I*	48.0 (35.0-79.0)	48.0 (35.0-78.0)	0.64	44.0 (33.0-68.0)	46.0 (35.0-69.0)	50.0 (38.0-77.0)	53.0 (37.0-86.0)	**< 0.001**
Card II*	61.0 (45.0-93.0)	59.00 (42.0-89.0)	**< 0.001**	55.0 (42.0-76.0)	57.0 (43.0-86.0)	62.0 (47.0-88.0)	66.0 (46.0-106.0)	**< 0.001**
Card III*	100.0 (67.0-200.2)	97.0 (62.0-191.5)	**< 0.001**	84.0 (61.0-128.0)	92.0 (64.0-150.0)	106.0 (70.0-177.0)	124.5 (82.0-274.0)	**< 0.001**
Block Design	27.0 (10.0-64.0)	23.0 (10.0-62.0)	**< 0.001**	41.50 (12.0-67.0)	30.0 (12.0-61.0)	20.0 (10.0-52.0)	16.0 (9.0-38.0)	**< 0.001**

† p value from Mann-Whitney U test;
†† p value from Kruskal Wallis H test
Significant p values ($p \leq 0.05$) are in bold
* High scores represent low cognitive performance (for time demanding tasks)

in women: 0 - 10 recalled words; range in men: 0 - 12 recalled words). The sex differences were not statistically significant for phonological fluency, card I of the Stroop colour-word test and TMT. Table 2 also presents the median score of cognitive tests by age-quartiles. Statistically significant differences were observed for all cognitive tests (Kruskal-Wallis H test; all p values < 0.001). The highest age quartile showed the lowest performance in all cognitive tests.

Table 3 presents the estimated additive heritability of cognitive tests. The heritability of general cognitive ability was 49% (95% C.I, 40% - 53%). For memory-related components the estimates were between 10% (recognition; AVLT-trial VII) and 30% (learning; AVLT-trials II-V). For executive function (cognitive flexibility, susceptibility to interference, verbal fluency) as well as for visuospatial-visuoconstructive abilities the estimates were approximately 36%. A large proportion of the phenotypic variance of the specific cognitive domains (from 20 to 50%) was explained by age age^2, education, general cognitive ability and inbreeding quartiles (table 3). General cognitive ability accounted for between 4% and 22% of the phenotypic variance of the other cognitive domains with large effects on executive function. Table 3 also shows that inbreeding was significantly associated with lower performance on the DART, semantic and phonological fluency, TMT and block design tests.

Table 4 presents the results of the sex-specific variance components analysis. The genetic correlations of the cognitive tests between men and women were high and did not significantly differ from one (all p values > 0.10). Table 4 also shows that women displayed higher genetic variances in learning (AVLT trial II-V; p value = 0.02) and in susceptibility to interference (card III of the Stroop colour-word test; p value < 0.02), while men exhibit higher environmental variances for these domains (p value = 0.03 for learning; p value = 0.02 for susceptibility interference). The differences in the components of the variances led to substantially different heritability estimates: 40% in women versus 19% in men for learning, and 50% in women versus 15% in men for susceptibility to interference.

Age-specific variance components were estimated for two broad age groups (< 50 years and ≥ 50 years). As shown in table 5, genetic correlations were significantly smaller than one in psychomotor speed (TMT-A; p value = 0.03) and phonological fluency (p value = 0.01), and borderline significant in susceptibility to interference (card III of the Stroop colour-word test; p value = 0.06). The genetic variance was significantly higher in the old age group (50 years and above) for general cognitive ability (DART; p value < 0.001) although the genetic correlation was not significantly different from one. Likewise, we observed higher genetic variances in the advanced age group for almost all specific cognitive domains. Only in cognitive flexibility was this difference significant (TMT-B; p value = 0.026). Of

Table 3. Heritability and inbreeding effects of the cognitive tests

Cognitive tests	β inbreeding[†]	σ^2_G	σ^2_E	h^2	Proportion of variance due to all covariates	Proportion of variance due to only general cognitive ability
Dutch Adult Reading Test[‡]	**-1.329 (0.234)**	61.792 (6.581)	65.887 (5.341)	0.485 (0.041)	0.291	-
AVLT-trial I	0.001 (0.028)	0.269 (0.085)	1.820 (0.093)	0.129 (0.040)	0.279	0.047
AVLT-trials II-V	-0.161 (0.137)	13.670 (2.119)	31.909 (1.959)	0.300 (0.043)	0.431	0.088
AVLT-trial VI	-0.029 (0.048)	1.608 (0.259)	4.147 (0.245)	0.279 (0.042)	0.314	0.049
AVLT-trial VII[‡]	0.088 (0.107)	3.276 (1.156)	27.255 (1.381)	0.109 (0.037)	0.220	0.085
Semantic fluency	**-0.408 (0.159)**	21.615 (2.788)	39.133 (2.473)	0.356 (0.042)	0.365	0.148
Phonological fluency	**-0.353 (0.167)**	23.982 (3.108)	42.947 (2.746)	0.358 (0.042)	0.355	0.198
TMT-A*[‡]	**0.204 (0.062)**	2.170 (0.402)	7.430 (0.419)	0.229 (0.040)	0.447	0.051
TMT-B*[‡]	**0.434 (0.08)**	5.801 (0.766)	10.357 (0.695)	0.359 (0.043)	0.577	0.221
Card I[‡]	-0.035 (0.035)	0.766 (0.124)	2.116 (0.140)	0.265 (0.039)	0.213	0.042
Card II[‡]	-0.057 (0.031)	0.829 (0.112)	1.543 (0.110)	0.350 (0.042)	0.270	0.096
Card III[‡]	-0.038 (0.040)	1.180 (0.176)	2.593 (0.188)	0.313 (0.042)	0.482	0.156
Block Design[‡]	**-0.210 (0.083)**	5.864 (0.779)	10.485 (0.702)	0.359 (0.043)	0.460	0.086

All models were adjusted for age, age^2, sex, education level and general cognitive ability (for general cognitive ability the model was adjusted for age, age^2, sex and education level)
[†]Estimated effects of the inbreeding quartiles on the cognitive tests (1st quartile: 0; 2nd quartile: >0 - 0.00139; 3rd quartile: 0.0014 -0.00739; 4th quartile ≥ 0.0074)
Significant inbreeding effects (p value ≤ 0.05) are in bold
[‡]Transformed variables
* Positive correlation is associated with lower cognitive performance (for time demanding tasks)

Table 4. Sex- specific variance components estimates for the cognitive tests

	Women (n= 1448)			Men (n= 1127)				P values likelihood ratio tests		
Cognitive tests	$\sigma^2_{G\text{-}F}$	$\sigma^2_{E\text{-}F}$	h^2_F	$\sigma^2_{G\text{-}M}$	$\sigma^2_{E\text{-}M}$	h^2_M	ρ_G	$\rho_G = 1^*$	$\sigma^2_{G\text{-}F} = \sigma^2_{G\text{-}M}$ **	$\sigma^2_{E\text{-}F} = \sigma^2_{E\text{-}M}$ ***
Dutch Adult Reading Test‡	62.088 (8.894)	60.386 (7.586)	0.507	65.285 (11.900)	69.052 (10.631)	0.486	0.948 (0.124)	0.339	0.803	0.486
AVLT-trial I	0.274 (0.130)	1.805 (0.140)	0.132	0.290 (0.161)	1.814 (0.171)	0.138	0.913 (0.424)	0.422	0.939	0.969
AVLT-trials II-V	18.522 (3.269)	27.386 (2.895)	0.403	8.361 (2.709)	36.746 (2.849)	0.185	1.000	1.000	**0.024**	**0.031**
AVLT-trial VI	1.581 (0.361)	4.243 (0.353)	0.271	1.647 (0.438)	4.019 (0.418)	0.291	1.000	1.000	0.919	0.710
AVLT-trial VII‡	3.172 (1.572)	26.691 (1.831)	0.106	3.409 (1.925)	27.983 (2.217)	0.109	1.000	0.190	0.988	0.097
Semantic fluency	23.352 (4.157)	32.820 (3.701)	0.416	23.381 (5.533)	43.373 (5.195)	0.350	0.869 (0.144)	0.160	0.437	0.392
Phonological fluency	23.011 (4.708)	43.970 (4.356)	0.344	29.114 (5.656)	37.766 (5.056)	0.435	0.877 (0.126)	1.000	0.913	0.683
TMT-A‡	2.143 (0.585)	7.546 (0.608)	0.221	2.203 (0.667)	7.282 (0.686)	0.232	1.000	1.000	0.979	0.839
TMT-B‡	5.651 (1.064)	10.163 (0.993)	0.357	7.169 (1.502)	9.482 (1.350)	0.431	0.844 (0.134)	0.125	0.395	0.685
Card I‡	0.773 (0.205)	2.074 (0.210)	0.272	0.811 (0.219)	2.121 (0.232)	0.277	0.933 (0.209)	0.378	0.998	0.792
Card II‡	0.859 (0.163)	1.391 (0.155)	0.382	0.857 (0.234)	9.683 (1.307)	0.337	0.934 (0.185)	0.947	0.941	0.363
Card III‡	8.590 (1.345)	9.346 (1.181)	0.479	2.488 (0.915)	13.761 (1.106)	0.153	1.000 (0.000)	1.000	**0.002**	**0.018**
Block Design‡	68.057(12.675)	105.176(11.581)	0.393	62.138 (17.500)	131.624 (16.933)	0.321	0.989 (0.199)	0.472	0.766	0.184

All models were adjusted for age, age^2, sex, education level, inbreeding and general cognitive ability (for general cognitive ability the model was adjusted for age, age^2, sex, inbreeding and education level). ‡Transformed variables

σ^2_G: Genetic variance; σ^2_E: environmental variance; ρ_G: genetic correlation of the expression of cognitive variables between sexes

*p value testing $\rho_G = 1$; ** p value testing whether σ^2_G is different between women and men; *** p value testing whether σ^2_E is different between women and men. Significant p values (p value ≤ 0.05) are in bold

Table 5. Age- specific variance components of the cognitive tests

	Younger than 50 years (n= 1387)			50 years and older (n= 1188)				P values likelihood ratio tests		
Cognitive tests	$\sigma^2_{G\text{-}Y}$	$\sigma^2_{E\text{-}Y}$	h^2_Y	$\sigma^2_{G\text{-}O}$	$\sigma^2_{E\text{-}O}$	h^2_O	ρ_G	$\rho_G = 1^*$	$\sigma^2_{G\text{-}Y} = \sigma^2_{G\text{-}O}$ **	$\sigma^2_{E\text{-}Y} = \sigma^2_{E\text{-}O}$ ***
Dutch Adult Reading Test‡	105.872 (15.100)	78.665 (12.207)	0.574	192.709 (43.029)	124.531 (33.545)	0.607	1.000	1.000	**<0.001**	**0.016**
AVLT-trial I	0.174 (0.126)	1.617 (0.139)	0.097	0.049 (0.145)	1.308 (0.284)	0.036	0.948 (0.475)	0.458	0.314	0.078
AVLT-trials II-V	14.402 (3.852)	29.991 (3.691)	0.324	14.751 (8.613)	27.598 (7.831)	0.348	0.932 (0.187)	0.361	0.967	0.639
AVLT-trial VI	1.684 (0.513)	4.149 (0.500)	0.289	1.616 (1.079)	4.362 (1.117)	0.270	0.899 (0.206)	0.323	0.915	0.747
AVLT-trial VII‡	6.247 (3.003)	30.608 (3.140)	0.170	12.713 (9.774	38.820 (7.504)	0.247	0.834 (0.550)	0.410	0.243	**0.045**
Semantic fluency	26.531 (5.283)	28.181 (4.725)	0.485	30.280 (12.738)	16.731 (7.938)	0.644	0.827 (0.143)	0.131	0.694	**0.042**
Phonological fluency	34.141 (6.553)	32.983 (5.741)	0.509	42.166 (15.816)	26.947 (11.093)	0.610	0.683 (0.118)	**0.007**	0.399	0.39
TMT-A‡	3.829 (1.022)	6.841 (0.960)	0.356	6.019 (2.768)	6.019 (2.768)	0.459	0.642 (0.180)	**0.035**	0.152	0.788
TMT-B‡	8.414 (1.651)	12.274 (1.491)	0.407	13.794 (4.648)	17.314 (3.917)	0.443	1.000	1.000	**0.026**	**0.022**
Card I‡	0.924 (0.267)	2.113S (0.267)	0.304	1.235 (0.664)	2.124 (0.559)	0.368	0.995 (0.210)	0.483	0.532	0.807
Card II‡	0.980 (0.292)	1.644 (0.276)	0.374	1.142 (0.653)	1.974 (0.623)	0.366	0.879 (0.183)	0.895	0.827	0.5
Card III‡	1.416 (0.414)	3.199 (0.415)	0.307	1.343 (0.840)	5.288 (1.129)	0.203	0.734 (0.155)	0.064	0.547	**0.007**
Block Design‡	3.374 (0.873)	11.276 (0.906)	0.230	0.555 (0.810)	13.738 (2.602)	0.039	1.000	1.000	**0.002**	0.162

All models were adjusted for age, age^2, sex, education level, inbreeding and general cognitive ability (for general cognitive ability the model was adjusted for age, age^2, sex, inbreeding and education level). ‡Transformed variables
σ^2_G Genetic variance; σ^2_E environmental variance; ρ_G genetic correlation of the expression of cognitive variables between age groups
*p value testing $\rho_G = 1$; ** p value testing whether σ^2_G is different between < 50 years group and ≥50 years group; *** p value testing whether σ^2_E is different between < 50 years group and ≥50 years group. Significant p values (p value ≤ 0.05) are in bold

interest, the genetic variance of the block design test was not significant for the advanced age group, which suggests that the significant heritability estimate we observed when considering the whole sample was mainly driven by the young age group. The environmental variances were significantly higher in the advanced age group in general cognitive ability as well as in verbal fluency (semantic), cognitive flexibility (TMT-B), recognition (AVLT-trial VII) and susceptibility interference (card III of Stroop colour-word test).

DISCUSSION

In this study we found a heritability estimate of 49% for general cognitive ability. For specific cognitive domains, we observed a wide variation in the heritability estimates (from 11% to 36%) after adjusting for the common variability attributed to general cognitive ability. In addition, we found that inbreeding was negatively associated with general cognitive ability as well as with visuospatial-visuoconstructive abilities (block design), and with various components of executive function, namely: cognitive flexibility (TMT-B) and with lower ability to generate searching strategies (semantic and phonological). Furthermore, we did not find evidence for a sex-specific genetic background modulating cognitive domains. However, we observed differences in the genetic and environmental variances between men and women for learning and susceptibility interference. This indicates that although sharing the same set of genes, the magnitude of genetic and non-genetic (environmental) effects differs between sexes. In contrast to sex-specific genetic effects, our results indicated that different genes might be involved in cognitive domains at different ages.

The heritability estimates of general cognitive ability as well as of specific cognitive domains in our analysis were consistent with previous studies (9, 45). The highest estimates of heritability were 30% (95% C.I, 21% - 39%) for learning and 36% (95% C.I, 27% - 45%) for cognitive flexibility. Both these estimates are in the lower range of those reported by others (21% - 60% for memory and 35% - 79% for executive function) (46). However, some of these studies used twin-based design, which may be prone to overestimate heritability when no other sources of familial correlations are considered (47). The fact that we used extended families to estimate the heritability of cognitive domains and adjusted for general cognitive ability, which accounted for up to 22% of the phenotypic variance for some cognitive tests, may explain partly why our findings were in the lower range.

In our study, inbreeding was significantly associated with general cognitive ability and with specific cognitive domains. Although the effects of inbreeding were significant, it only accounted for a relatively small proportion of the total variance

of the cognitive tests (1% on average, data not shown). Inbreeding results in an increased probability of homozygosity for any allele at any loci, which may have adverse or beneficial effects. However, there is evidence that supports an association of inbreeding with decline in cognitive function (21). Our results are in line with the findings of Morton (1978) who showed that the decrease in cognitive performance as a result of inbreeding was due to the effect of recessive alleles (20). Further, our findings suggest that the genetic background underlying specific cognitive domains is constituted, at least partly, by recessive alleles.

In spite of the recent indications for genetic influences on the sexual dimorphism in cognitive function from both human and functional animal studies (13-16), we could not demonstrate sex-specific genetic effects. Although in the crude analysis we observed significant gender differences in general cognitive ability and in specific cognitive domains, these differences were not explained by sex-specific genetic effects on the variation of both general cognitive ability and specific cognitive domains between men and women. Thus, other factors may account for the gender-related differences in cognitive function documented in other studies. However, in our study we estimated the net effect of all genes (40), which does not preclude that one or several loci are expressed differently between sexes.

We found evidence for age-specific genetic effects on the variation of psychomotor speed, phonological fluency and susceptibility to interference, with genetic correlations between 0.64 and 0.73 that were significantly different from one. This means that about 30% of the genetic expression in these cognitive domains might be age-group specific. In addition, significantly higher genetic variances and higher heritability estimates were observed in the advanced age group (≥ 50 years) for general cognitive ability and as well as for cognitive flexibility. This is in line with the observation of higher heritability estimates in both general cognitive ability and other cognitive domains with increasing age (45). These findings suggest that age-specific genetic expression modulates cognitive function at old age. A possible point of concern is that we only considered two broad age groups and therefore we might have missed higher differences in cognitive domains between more narrow age groups. Given the preponderant role of age as a determinant of variation in cognition, these results need confirmation in other populations.

Genetically isolated populations may exhibit both increased genetic drift and inbreeding and thus may deviate in their genetic composition from the general population. Moreover, in genetic isolated populations, there is an increased chance of loss or fixation of some genetic variants potentially leading to lower genetic diversity and consequently lower heritability. However, in a recent study (24) we have shown that the effects of drift are relatively small in our young genetically isolated population and that there is a very small chance of losing an allele unless

it had a very low frequency in the original population. Thus, our population should not deviate much from the general population in its genetic composition and we do not expect that our heritability estimates would substantially differ from those in the general Dutch population.

In summary, we found significant heritability in cognitive domains after adjustment for general cognitive ability. We also provide evidence that inbreeding influences the variation in specific cognitive domains. The results of sex- and age-specific heritability estimation highlight the potential interaction between genes and age- and sex-related factors in the expression of cognitive domains.

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5

Age- and sex-dependent effects of the apolipoprotein E (APOE) gene on cognitive function

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ABSTRACT

The *APOE* gene that encodes Apolipoprotein E is a well-established risk factor for Alzheimer's disease (AD). The APOE*4 allele has been shown to have a strong correlation with AD-like alterations in the brains of subjects with mild cognitive impairment (MCI) and of cognitively intact subjects. Thus far there is no consensus about which specific early changes in cognitive function are related to APOE*4. In the present study, we investigated the association between the APOE*4 allele and cognitive function in non-demented subjects to identify the cognitive profile associated with APOE*4. Furthermore, we evaluated whether age and gender modulate the effects of the APOE*4 allele on cognitive function.

Carriers of the APOE*4 allele had lower short-term memory ($p = 0.05$) and cognitive flexibility ($p < 0.001$) than non-carriers. These effects were driven by subjects of 50 years and older. In this age group we also found a significant relationship between APOE*4 and susceptibility to interference ($p = 0.04$). Gender differences in the effects of the APOE*4 allele were also found. APOE*4 carrier women had poorer short-term memory and executive function than non-carriers. No differences were observed in men. We concluded that APOE*4 contributes to the variation of short-term memory and executive function, which might represent early changes in cognitive function in AD. The contribution of APOE*4 to these cognitive domains is age- and sex-dependent, with effects being pronounced at older age and in women.

INTRODUCTION

The apolipoprotein E gene, *APOE*, is a pleiotropic gene, with a large range of effects on human traits including lipid metabolism (1), longevity (2) and cognitive function (3). Three common alleles of *APOE* (APOE*2, APOE*3 and APOE*4) that encode three different isoforms of the protein (ε-2, ε-3 and ε-4) have been identified. The APOE*4 allele is a well-established genetic risk factor for late onset Alzheimer's disease (AD) (4). Although APOE*4 is neither necessary nor sufficient to cause AD, it has been associated with lower cognitive function and with the progression from mild cognitive impairment (MCI) to AD (5-7). Neuroimaging studies in subjects with MCI (8), as well as in cognitively intact individuals at high risk for AD (9), have shown that APOE*4 is correlated with alterations in the brain which are similar to those seen in the brains of AD patients (10).

Therefore, although there is considerable evidence to support a role for APOE*4 in cognitive decline in non-demented individuals, some important questions remain unanswered. Firstly, it is still not clear whether the effect of APOE*4 on cognitive function is global or correlates predominantly with specific cognitive domains (3). Secondly, there is still debate about whether *APOE* affects cognitive function early in life. Most of the studies that found an association between *APOE* and cognition focused on aged individuals (6). There is evidence that cognitive decline during ageing is faster in APOE*4 carriers (7), suggesting that this variant modulates processes in the brain that lead to neurodegeneration. Animal studies have shown that the *APOE* protein has significant effects on early brain development (11), indicating that *APOE* may also have an impact on early cognitive reserve. Nevertheless, it has not yet been shown whether *APOE* has early effects on cognitive function in humans. Thirdly, it has not yet been established whether there are gender-specific effects of the APOE*4 allele on cognition. There is some evidence for gender-specific effects of APOE*4 on cognition in relation to AD (12). The influence of gender on the neuropsychological profile associated with APOE*4 has only been addressed in a few studies, with conflicting results (6, 13).

In the present study we evaluated the effects of the APOE*4 allele on specific cognitive domains over a wide age-range in participants of a family-based study. The main aim was to study the cognitive profile associated with the APOE*4 allele in non-demented subjects. Further, we addressed whether the effect of this variant is homogenous across different ages and whether it is modulated by gender.

MATERIALS AND METHODS

Study population

Subjects were participants of the Erasmus Rucphen family (ERF) study, a family-based cohort study conducted in a recent genetically isolated population in The Netherlands (14, 15). The population was founded in the middle of 18th century by approximately 150 people and remained isolated until the last few decades. An extensive genealogical database including over 80 000 records is available for this population. For the ERF study, 22 families that had at least 5 children baptized in the community church between 1850 and 1900 were selected. All living descendants of the families and their spouses, aged 18 and older (n = 2906), were invited to participate in the ERF study. Thus, participants from the ERF study were selected according to genealogical, and not disease, status. The Medical Ethics Committee of the Erasmus Medical Center, Rotterdam approved the study. Participants followed a standardized protocol that included a medical questionnaire and an interview. Subjects were extensively phenotyped for different clinical measures organized into cardiovascular, ophthalmologic, cognitive and anthropometrical blocks. The recruitment started in June 2002 and finished in February 2005. Genotypes for the *APOE* gene for 1024 participants were available at the time of the current study. Using the genealogical database we connected all genotyped participants in a single large pedigree consisting of 9800 subjects.

Data collection

A battery of neuropsychological tests was administrated to the participants of the ERF study. We used the following tests: the Dutch version of the auditory verbal learning test (AVLT) (16), the trail making test (TMT) (17), the Stroop colour-word test (18), the verbal fluency test (17) and the block design subtest of the Weschler adult intelligence test (WAIS) (19). These tests are widely used to screen for cognitive deficits in AD and other dementias (20) and were chosen to assess cognitive domains over a wide age range.

AVLT assesses different aspects of memory through a series of trials (trial I to VII) (21). Different scores were derived from AVLT: short-term memory: number of correctly recalled words in the first trial (AVLT trial I); learning: sum of correctly recalled words from trial II to V (AVLT trials II-V); delayed recall: number of correctly recalled words after a 20 minute delay (AVLT trial VI) and recognition: number of correctly recognized words from a 30-word list (AVLT trial VII) (21, 22). The TMT evaluates psychomotor speed (part A) and cognitive flexibility (part B) (23). The scores were defined as time in seconds to complete the task in parts A and B separately. The Stroop colour-word test assesses selective attention (reading

task in card I; naming colours in card II) and susceptibility to interference (Stroop interference in card III) (21). The scores were defined as time in seconds to complete the task in cards I, II and III separately. The verbal fluency test consists of semantic and phonological fluency tests that evaluate searching strategies to retrieve stored information (21). The scores for verbal fluency were defined as the sum of the named words starting with letters D, A, and T (phonological fluency), and the sum of all named animals and professions (semantic fluency). Cognitive flexibility (TMT-B), susceptibility to interference (Stroop card III) and verbal fluency are measures of executive function (21). The block design subtest assesses visuospatial and visuoconstructive abilities (21). This test was scored as the maximum number of replicated blocks per time. Furthermore, we assessed the general cognitive ability of the participants with the Dutch Adult Reading Test (DART) (21)

The education level attained by the subjects within the Dutch schooling system was determined (22). We categorized the highest level of education attained by the participants into three groups: elementary education (8 years or less of full-time education), junior vocational training (8 to 14 years of education) and senior vocational or academic training (at least 15 years of full-time education) (22).

Genotyping

Genomic DNA was extracted from whole blood samples obtained at the baseline examination, utilizing the salting out method (24). Samples were genotyped for the *APOE* C112R (APOE*4 allele) and *APOE* R158C (APOE*2 allele) polymorphisms with a Taqman allelic discrimination Assay-By-Design (Applied Biosystems, Foster City, CA). Primer sequences are available on request. The assays utilized 5 nanograms of genomic DNA and 2 microliter reaction volumes. The amplification and extension protocol was as follows: an initial activation step of 10 min at 95°C preceded 40 cycles of denaturation at 95 °C for 15 s. and annealing and extension at 50 °C for 60 s. Allele-specific fluorescence was then analysed on an ABI Prism 7900HT Sequence Detection System with SDS v 2.1 (Applied Biosystems, Foster City, CA).

Statistical analysis

The *APOE* genotypes were grouped according to the number of APOE*4 alleles, namely: zero (2/2, 2/3 and 3/3 genotypes), one (2/4 and 3/4 genotypes) and two copies (4/4 genotypes); or as carriers and non-carriers of the APOE*4 allele. General characteristics of the study population among the genotypic groups were compared using the one-way ANOVA test (normally distributed) or Kruskal-Wallis test (not normally distributed) for continuous variables. For dichotomous variables we used the chi-square test. These analyses were performed with SPSS V.11.0 (SPSS

Inc. Chicago IL). The observed frequencies of the *APOE* genotypes were tested for deviations from Hardy-Weinberg equilibrium proportions using the chi-square test.

Inbreeding coefficients were computed based on all available genealogical information using PEDIG software (25). As the inbreeding values were not normally distributed, we categorized the levels of inbreeding into quartiles, namely: 25th percentile: 1.55 x10^{-3}, 50th percentile: 0.004, and 75th percentile: 0.009.

We evaluated the effects of the APOE*4 allele on cognitive function in subjects belonging to a single large pedigree using linear mixed models to allow adjustment for family structure. The genotypes were pooled into APOE*4 allele carriers and non-carriers assuming a dominant model, which is consistent with the role of this variant in cognitive function (6). The models were adjusted for age, age^2 (to account for non-linear age effects), sex, inbreeding and general cognitive ability (26). Other confounders considered were hypertension, atherosclerosis, body mass index (BMI), alcohol consumption and smoking. Because of the skewed distributions of the AVLT trial VII, TMT-A, TMT-B, cards I, II and III of the Stroop colour-word and block design tests, the data were log-transformed to improve normality ($\log_{20}$ (100 * cognitive test)).

To evaluate whether age and sex modified the effect of the APOE*4 allele on cognitive function, we included interaction terms between *APOE* and two age categories (< 50 years and ≥ 50 years). We selected this cut-off point because age-dependent influences on cognitive function are substantial after the age of 50 years (27). We also performed a stratified analysis by gender. All analyses were performed using the SOLAR software package (28)

RESULTS

The frequencies of the *APOE* alleles were 0.05 for the APOE*2 allele, 0.75 for the APOE*3 allele and 0.20 for the APOE*4 allele. The allele and genotype distributions were in Hardy-Weinberg equilibrium (p = 1.00). Table 1 presents the general characteristics of the population by number of APOE*4 alleles. A significant increase in serum levels of total cholesterol and triglycerides was observed with increasing number of *APOE*4* alleles (p ≤ 0.02).

Table 2 presents the median and the inter-quartile range of the distribution of the cognitive tests by *APOE* genotype, as well as the p values of the adjusted analysis. Short-term memory (AVLT trial I) and cognitive flexibility (TMT-B) were significantly associated with APOE*4 in both the crude analysis (AVLT trial I; p = 0.02; TMT-B; p = 0.003) and in the analysis adjusted for family structure (AVLT trial I; p = 0.05; TMT-B; p < 0.001). Findings for card II of the Stroop colour-word test were not

Table 1. Demographic characteristics and risk factors per APOE genotypes

	APOE			
Variable	2/2, 3/2, 3/3 (n =646)	4/2, 4/3 (n =307)	4/4 (n =49)	P value†
Age (years)	54.1 (15.91)	55.2 (14.21)	51.9 (12.73)	0.31
Inbreeding	0.006 (0.007)	0.007 (0.008)	0.006 (0.006)	0.06
Men (%)	0.40	0.39	0.51	0.50
Body Mass Index (kg/cm^2)	0.3 (0.05)	0.3 (0.05)	0.3 (0.04)	0.34
IMT (mm)	0.86 (0.20)	0.88 (0.22)	0.84 (0.20)	0.14
Fasting glucose (mmol/l)	4.79 (1.11)	4.73 (1.07)	4.56 (0.63)	0.36
Cholesterol (mmol/l)	5.50 (1.10)	5.73 (1.19)	5.68 (1.22)	0.02
Triglycerides (mmol/l)	1.35 (0.80)	1.46 (0.77)	1.65 (0.95)	0.02
HDL (mmol/l)	1.29 (0.38)	1.24 (0.32)	1.13 (0.27)	<0.001

Values presented are means (standard deviations) or proportions
IMT (common carotid Intima Media Thickness)
HDL (High density lipoproteins)
† p value of 1-way ANOVA test (continuous variables) or chi-square test (proportions)

consistent, as this test did not show any significant association with APOE*4 in the analysis adjusted for family structure (card II of the Stroop colour-word test; p = 0.23). The inclusion of other cardiovascular variables did not substantially change the association of the APOE*4 allele with the cognitive tests.

APOE was not associated with DART, the test that assesses general cognitive ability. Table 3 presents the effects of the APOE*4 allele on the cognitive tests in two age categories (< 50 years; ≥ 50 years). Significant interactions were observed for cognitive flexibility (TMT-B; p = 0.001), recognition (AVLT trial VII; p = 0.03) and susceptibility to interference (card III of the Stroop colour-word test; p = 0.04). In the group of 50 years and older, a significant association between the APOE*4 allele and short-term memory (AVLT trial I; p = 0.05), cognitive flexibility (TMT-B; p < 0.001) and susceptibility to interference (card III of the Stroop colour-word test; p = 0.03) was found. The effect of the APOE*4 was also stronger for the AVLT trial VII test (recognition) in the group of 50 years and older, although the relation to APOE*4 was not statistically significant in the stratified analysis. None of the cognitive tests were significantly associated with the APOE*4 allele in the younger age group.

Table 4 presents the gender-specific effects of the *APOE* genotype on the cognitive tests. Significant interactions were observed for learning (AVLT trials II-V; p = 0.03), recognition (AVLT trial VII; p = 0.05), semantic fluency (p = 0.04) and cognitive flexibility (TMT-B; p = 0.04). In the stratified analysis, the APOE*4 effects were only significant in the female group. As shown in table 4, the APOE*4 allele was significantly associated with lower performance in tests measuring short-term memory (AVLT trial I; p = 0.03), cognitive flexibility (TMT-B; p < 0.001) and susceptibility to interference (card III of the Stroop colour-word test; p = 0.04). The

Table 2. Median score (25th-75th quartiles) of the cognitive tests by APOE genotypes

	*APOE*4*				
Cognitive test	0 (n= 646)	1 (n= 307)	2 (n = 49)	P value†	P value††
AVLT-trial I *(short-term memory)*	4.0 (3.0-5.0)	4.0 (3.0-5.0)	4.0 (3.0-4.0)	**0.018****	**0.049**
AVLT-trials II-V *(learning)*	31.0 (24.0-38.0)	29.0 (24.0-37.0)	29.5 (21.5-34.5)	0.062	0.521
AVLT-trial VI *(delayed recall)*	7.0 (5.0-9.0)	7.0 (5.0-9.0)	7.0 (4.0-9.0)	0.391	0.926
AVLT-trial VII *(recognition)*	14.0 (13.0-15.0)	14.0 (12.0-15.0)	14.0 (12.8-15.0)	0.725	0.818
Semantic fluency *(verbal fluency)*	34.0 (28.0-41.0)	32.0 (26.5-39.0)	32.0 (26.0-44.0)	0.207	0.932
Phonological fluency *(verbal fluency)*	23.0 (16.0-30.0)	23.0 (14.5-32.0)	20.0 (14.8-27.3)	0.269	0.089
TMT-A *(psychomotor speed)**	37.0 (28.0-53.0)	40.0 (29.0-58.0)	40.0 (31.5-61.5)	0.107	0.186
TMT-B *(cognitive flexibility)**	97.0 (63.0-149.5)	106.0 (69.0-210.0)	112.5 (83.5-258.3)	**0.003**	**<0.001**
Card I *(selective attention)**	50.0 (44.0-59.0)	52.0 (46.0-60.5)	48.0 (44.0-55.0)	0.161	0.844
Card II *(selective attention)**	61.0 (54.0-71.0)	64.0 (57.0-72.5)	61.0 (55.0-72.0)	**0.022**	0.225
Card III (*susceptibility to interference)**	103.0 (87.8-123.0)	108.0 (90.0-133.0)	105.0 (85.5-122.5)	0.109	0.260
Block Design *(visuospatial abilities)*	21.0 (12.0-34.0)	18.0 (12.0-33.0)	17.5.0 (11.8-30.5)	0.231	0.511

AVLT (Auditory verbal learning test)
TMT (Trail Making Test)
Card I, II and III of the Stroop colour-word test
† P value of Kruskal-Wallis test (significant p values are in bold)
†† P value of the adjusted analysis (age, age^2, sex inbreeding, education, DART and familial structure)
* Higher scores cognitive performance (for time demanding tasks)
** For this test the median score were the same, but the differences in the distribution were significant

Table 3. Effects (standard error) of the APOE*4 allele on cognitive tests in two age categories

	< 50 years	>= 50 years
Cognitive tests	**APOE*4 ††**	**APOE*4 ††**
AVLT-trial I *(short-term memory)*	-0.181 (0.162)	**-0.253 (0.125)**
AVLT-trials II-V *(learning)*	0.232 (0.785)	-0.788 (0.683)
AVLT-trial VI *(delayed recall)*	0.091 (0.247)	-0.189 (0.248)
AVLT-trial VII† *(recognition)*	0.079 (0.045)	-0.105 (0.071)
Semantic fluency *(verbal fluency)*	-0.307 (0.986)	0.148 (0.729)
Phonological fluency *(verbal fluency)*	1.490 (0.951)	0.493 (0.840)
TMT-A† *(psychomotor speed)**	1.276 (1.114)	0.723 (1.166)
TMT-B† *(cognitive flexibility)**	1.549 (1.331)	**7.105 (1.541**)
Card I† *(selective attention)**	-0.442 (0.678)	0.376 (0.629)
Card II† *(selective attention)**	-0.132 (0.569)	0.960 (0.611)
Card III† *(susceptibility to interference)**	-0.635 (0.636)	**1.769 (0.790)**
Block Design† *(visuospatial abilities)*	0.004 (1.656)	0.520 (1.242)

The values are the regression coefficients of the APOE*4 carriers onto the cognitive tests.
The models were adjusted for age, age^2, sex, inbreeding, education, DART and familial structure
† Log-transformed cognitive tests
†† The genotypes were pooled into APOE*4 non carriers versus carriers of at least one APOE*4 allele
* Positive effects correlate with lower performance (time demanding tasks)
Significant effects (p-value< 0.05) of the APOE*4 allele are in bold

Table 4. Effects (standard error) of the APOE*4 allele on cognitive tests by gender

	Men	Women
Cognitive tests	**APOE*4 ††**	**APOE*4 ††**
AVLT-trial I *(short-term memory)*	-0.121 (0.148)	**-0.276 (0.127)**
AVLT-trials II-V *(learning)*	0.583 (0.786)	-1.224 (0.670)
AVLT-trial VI *(delayed recall)*	0.355 (0.275)	-0.301 (0.233)
AVLT-trial VII† *(recognition)*	0.034 (0.078)	-0.062 (0.056)
Semantic fluency *(verbal fluency)*	1.500 (0.937)	-1.253 (0.779)
Phonological fluency *(verbal fluency)*	1.705 (0.951)	0.390 (0.867)
TMT-A† *(psychomotor speed)**	0.549 (1.299)	1.508 (1.091)
TMT-B† *(cognitive flexibility)**	1.038 (1.713)	**6.544 (1.401)**
Card I† *(selective attention)**	-0.649 (0.796)	0.556 (0.572)
Card II† *(selective attention)**	0.546 (0.747)	0.513 (0.518)
Card III† *(susceptibility to interference)**	0.124 (0.812)	**1.396 (0.686)**
Block Design† *(visuospatial abilities)*	1.612 (1.693)	-0.284 (1.290)

The values are the regression coefficients of the APOE*4 carriers onto the cognitive tests.
The models were adjusted for age, age^2, sex, inbreeding, education, DART and familial structure
† Log-transformed cognitive tests
†† The genotypes were pooled into APOE*4 non carriers versus carriers of at least one APOE*4 allele
* Positive effects correlate with lower performance (time demanding tasks)
Significant effects (p-value< 0.05) of the APOE*4 allele are in bold

effects of the variant on the test that assesses learning were borderline significant (AVLT trials II-V; p = 0.07). No significant evidence for associations between the APOE*4 allele and the cognitive tests were found in men.

DISCUSSION

In this family-based study, we found significant associations between the APOE*4 allele and reduced short-term memory and lower cognitive flexibility. These effects were driven by the group of 50 years and older, and were not significant in the younger age group. In addition, we found significant gender differences in the effects of the APOE*4 allele on these cognitive domains. Female APOE*4 carriers performed less well in memory tests than non-carriers, with stronger effects in short-term memory and borderline differences in learning.

In our study the association between the APOE*4 allele and cognitive function was specific to short-term memory (and learning in women), and executive function, with larger effects on cognitive flexibility. This is in line with previous studies that demonstrated specific effects of APOE*4 on memory and executive function (3, 6). The lower cognitive performance in both short-term memory and executive function in carriers of at least one APOE*4 allele also fits with the domains predominantly affected in MCI (29). Our findings suggest that both cognitive flexibility and short-term memory, as measured by AVLT and TMT-B, might be early markers of the cognitive deficits related to AD-like pathology.

Most of the studies that have found an association between *APOE* and cognition have focused on aged individuals, and have provided evidence for a role for *APOE* in cognitive decline in APOE*4 carriers (6). Animal studies, however, have shown that apolipoprotein E has a role in early brain development (11). We found that the effects of the APOE*4 allele were modified by age, with significant effects only in the advanced age group (≥ 50 years). We did not find any significant effect of the APOE*4 allele in those aged 50 years and younger. The fact that the APOE*4 allele has little or no effect on cognitive function early in life, whereas it influences cognitive flexibility and short-term memory in late life, suggests that APOE*4 is related to cognitive decline, rather than cognitive reserve (7, 30).

It has been shown that the ε-4 isoform of apolipoprotein E is less effective in the maintenance of neuronal repair than the other two apolipoprotein E isoforms (ε-3 and ε-2) (31). The effect of APOE*4 late in life may be explained by the fact that the deficiency in repair only becomes manifest at advanced age as the cumulative damage to the brain that occurs during ageing increases. However, there are alternative pathways that may help explain the effects of APOE*4 in late life.

Apolipoprotein E is also involved in the oxidative stress response (31), and APOE*4 carriers might be more susceptible to oxidative damage. The effects of APOE*4 on specific cognitive domains may be due to the higher susceptibility of neurons to oxidative stress in specific brain areas such as hippocampus (32, 33).

There is evidence for gender-specific effects of *APOE* on cognitive function (6, 13). In our analysis, women showed differences in cognitive performance by APOE*4 status whereas, in men, the effect was less pronounced and non-significant. This is line with a previous study that showed higher cognitive decline in female APOE*4 carriers, especially in executive function (13). A higher cognitive decline in male APOE*4 carriers in memory and executive function was found in another study (6). However, the small number of APOE*4 women carriers might account for the lack of APOE*4 effects in women in this study (6). Our findings are in line with the strong effects of APOE*4 on AD found in a previous meta-analysis (12). Moreover, more frequent neurodegenerative lesions in the brain of women with AD have been reported (34). The fact that the differences between men and women were observed in both short-term memory and executive function, both of which are predominantly affected in AD, supports the hypothesis that women carriers of APOE*4 may be an important high risk group from a clinical and public health perspective.

There is recent interest in finding endophenotypes for AD. These are defined as intermediate phenotypes that are in the pathways leading to a disease, but with a simpler genetic architecture than the disease itself (35). Endophenotypes should be correlated with the disease, should be heritable and should be associated with the disease risk factors in unaffected subjects (10). In previous work, we showed that AVLT, TMT and the Stroop colour-word test are highly heritable (26). Here we show that APOE*4 contributes to the variance in these tests only in subjects aged 50 years and above. These results suggest that these tests may be valuable endophenotypes for AD.

In summary, in this cross-sectional study, we showed that the cognitive profile associated with the APOE*4 allele consists of changes in short-term memory and executive function. The lower cognitive performance in these domains was significant in women and in individuals 50 years and older.

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6

The effects of blood pressure, intima media thickness and four blood pressure genes on cognitive function

Pardo LM, Sanchez-Juan P, Yazdanpanah M, Sleegers K, de Koning I, Zorkoltseva IV, Axenovich TI, Breteler MM, Witteman JC, van Swieten JC, Aulchenko YS, Oostra BA, van Duijn CM.
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ABSTRACT

The genetic variation underlying vascular pathology may contribute to lower cognitive function. In this study the effects of systolic blood pressure (SBP), diastolic blood pressure (DBP), intima media thickness (IMT) and polymorphisms of the angiotensinogen (*AGT*), angiotensin II type 1 receptor (*AT1R*), α-adducin-1 (*ADD1*) and G-protein β-3 (*GNβ3*) genes, which have all been associated with vascular pathology, were evaluated in relation to cognition. Participants of a family-based study were genotyped for the *AGT* M235T, *AT1R* C575T, *ADD1* G460W and the *GNβ3* rs2301339 G/A polymorphisms. Cognitive function was assessed with a battery of neuropsychological tests. The effects of SBP, DBP, IMT and the four genetic variants on the cognitive tests were evaluated using linear mixed models. The effect of the four genetic variants was evaluated independently as well as by combining the risk alleles.

DBP was associated with worse performance in the trail-making test A (TMT-A). IMT was associated with lower performance in the Stroop-colour word test and semantic fluency. In contrast, SBP was associated with a better performance in the TMT-A and in card II of the Stroop colour-word test. The effects of SBP on TMT-A were driven by the group of 55 years and older. Significant effects of the *AGT* 235T allele on recognition were found. In contrast, worse performance in phonological fluency was associated with the *AAD1* 460W polymorphism, while a poorer performance in TMT-B and card III of the Stroop colour-word test were associated with the *GNβ3* rs2301339 G allele. Moreover, lower performance in tests evaluating learning and in susceptibility to interference with an increasing number of risk alleles was found. We concluded that vascular factors and genes involved in blood pressure regulation contribute to the variation in cognitive function.

INTRODUCTION

Vascular pathology plays a key role in the development and progression of cognitive decline and dementia including Alzheimer's disease (AD) and vascular dementia (1, 2). Vascular damage may lead to hypoperfusion and ischaemia, resulting in neuronal death (3-5). Hypertension and atherosclerosis are the main causes of vessel damage and there is compelling evidence that each contributes to the risk for AD, vascular dementia and reduced cognitive function (6-10).

The Renin-Angiotensin Aldosterone System (RAAS) modulates vascular tone and water and salt homeostasis (11, 12), and seems to play a role in hypertension and atherosclerosis (3). Four genes involved in RAAS are known to be involved in blood pressure regulation: the angiotensinogen (*AGT*) gene (13); the angiotensin II type 1 receptor (*AT1R*) gene (14); the G-protein β3 subunit (*GNβ3*) gene (15, 18) and the α-adducin 1 (*AAD1*) gene. *AGT* and *AT1R* are part of the Renin-Angiotensin System. *GNβ3* and *AAD1* are salt-sensitivity genes in the aldosterone pathway (15-17).

In the present analysis we examined to what extent blood pressure, atherosclerosis and polymorphisms of the *AGT, AT1R, ADD1* and *GNβ3* genes contribute to cognition. We studied four polymorphisms in the RAAS genes, which have been related to blood pressure regulation: G460W of *ADD1* (19), the M235T of *AGT* (13), the C573T of *AT1R* (14) and the rs2301339 G/A of *GNβ3*.

MATERIALS AND METHODS

Study population

This study was conducted within the Erasmus Rucphen Family (ERF) study, which is embedded in the Genetic Research in Isolated Populations (GRIP) program. The ERF study was designed to find genetic determinants of quantitative traits in a recent genetically isolated population in The Netherlands (20, 21). An extensive genealogical database, including over 80 000 records is available for the GRIP population. For the ERF study, 22 families that had at least 5 children baptized in the community church between 1850 and 1900 were selected. All living descendants of the families and their spouses, aged 18 and older (n = 2906), were invited to participate and the study was approved by the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam. Participants of the ERF study followed a standardized protocol that included a medical questionnaire and an interview. Subjects were phenotyped for different clinical measures including cardiovascular, ophthalmologic, cognitive and anthropometrical outcomes. The recruitment of subjects started in June 2002 and finished in February 2005. The present analysis is

based on a sample of 1041 participants, from whom genotypic information of the four genetic polymorphisms was available.

Data collection

A battery of neuropsychological tests was administrated to the participants of the ERF study. The following neuropsychological tests were chosen to assess different cognitive domains over a wide age-range: the Dutch versions of the auditory verbal learning test (AVLT) (22), the trail-making test (TMT) (23), the Stroop colour-word test (24), the verbal fluency test (23) and the block design subtest of the Weschler Adult Intelligence Test (WAIS) (25).

AVLT assesses different aspects of memory through a series of trials (trial I to VII) (26). Different scores were derived from AVLT: short-term memory: number of correctly recalled words in the first trial (AVLT trial I); learning: sum of correctly recalled words from trial II to V (AVLT trials II-V); delayed recall: number of correctly recalled words after a 20 min-delay (AVLT trial VI) and recognition: number of correctly recognized words from a 30-words list (AVLT trial VII) (26, 27). TMT evaluates psychomotor speed (part A) and cognitive flexibility (part B) (28). The scores were defined as time in seconds to complete the task in part A and B separately. The Stroop colour-word test assesses selective attention (reading task in card I; naming colours in card II) and susceptibility to interference (colour interference in card III) (26). The scores were defined as time in seconds to complete the task in cards I, II and III separately. The verbal fluency test consists of semantic and phonological fluency tests that evaluate searching strategies to retrieve stored information (26). The scores for verbal fluency were defined as the sum of the named words starting with letters D, A, and T (phonological fluency), and sum of all named animals and professions (semantic fluency). Cognitive flexibility (TMT-B), susceptibility to interference (card III of the Stroop colour-word test) and verbal fluency are measures of executive function (26). The block design subtest assess visuospatial and visuoconstructive abilities (26). This test was scored as the maximum number of replicated blocks per time. We used the Dutch Adult Reading Test (DART) as a measure of general cognitive ability (26).

We determined the education level attained by the subjects within the Dutch schooling system (27). We categorized the highest level of education attained by the participants into three groups: elementary education (8 years or less of full-time education), junior vocational training (8 to 14 years of education) and senior vocational or academic training (at least 15 years of full-time education) (27).

Blood pressure was measured twice in the sitting position from the right upper arm with an automated device (OMRON 711, automatic IS; Vernon Hills Illinois, USA). The average of the two measurements was used in the analyses. Hypertension

was defined as mean systolic blood pressure (SBP) ≥ 160 mmHg or diastolic blood pressure (DBP) ≥ 100 mmHg or use of antihypertensive medication (29). Common carotid intima media thickness (IMT) was measured with a 7.5-MHz linear array transducer (ATL Ultra-Mark IV; Advanced Technological Laboratories, Bethell, Washington, USA). The conditions and procedures for the IMT assessment have been described previously (30).

Genotyping

The genotyping of the *ADD1* G460W, the *AGT* M235T, the *AT1R* C573T and the *GNβ3* rs2301339 G/A polymorphisms was performed using TaqMan allelic discrimination Assays-By-Design (Applied Biosystems, Foster City, CA) . The primer sequences used to genotype these polymorphisms are available on request. The assays utilized 5 nanograms of genomic DNA and 5 microliter reaction volumes. The amplification and extension protocol was as follows: an initial activation step of 10 min at 95°C preceded 40 cycles of denaturation at 95°C for 15 s. and annealing and extension at 50°C for 60 s. Allele-specific fluorescence was then analysed on an ABI Prism 7900HT Sequence Detection System with SDS v 2.1 (Applied Biosystems, Foster City, CA).

Covariate adjustment and data analysis

The general characteristics of the study population were compared for the total sample as well as in two age categories (< 55 years; ≥ 55 years) using either the t-test (continuous variables) or chi squared test (categorical variables). Inbreeding coefficients were computed based on all available genealogical information using PEDIG software (31). As the inbreeding values were not distributed normally, we categorized these into quartiles.

The observed frequencies of the genotypes were tested for deviations from Hardy-Weinberg equilibrium proportions using the chi-square test (one degree of freedom). As the participants of the study were connected to a single extended pedigree, we used linear mixed models to evaluate the effect of SBP, DBP, IMT and genetic variants on the cognitive tests. Linear mixed models allow adjustments for family structure to be made. The models were adjusted for age, age^2 (to account for non-linear age effects), sex, inbreeding, general cognitive ability (DART) and anti-hypertensive medication (32). Because of the skewed distribution, the AVLT trial VII, TMT-A, TMT-B, block design and cards I, II and III of the Stroop colour-word test were log-transformed to improve normality.

Next we evaluated the effect of the polymorphisms in the four blood pressure genes on the cognitive tests, based on an earlier analysis (manuscript in preparation). Genotypes of the four genes were pooled as follows: GG = 0, GW/WW = 1 for

ADD1 (17); MM/MT = 0, TT = 1 for *AGT* (33); CT/TT = 0 CC = 1 for *AT1R* and AA = 0, AG/GG = 1 for *GNβ3*. The latter two polymorphisms were pooled based on the blood pressure levels in our population (van Rijn MJ *et al.* 2006, manuscript in preparation). To evaluate the effects of the combined genetic polymorphisms, a variable was created for additive effects of the risk alleles (0, 1, 2, 3 and 4 risk alleles). The models were adjusted for the aforementioned covariates, except for anti-hypertensive mediation. All analyses were performed using the SOLAR package (34).

RESULTS

All 1041 subjects were connected in a single pedigree consisting of 9800 individuals. The general characteristics of the whole study population, as well as of the two age categories, are presented in table 1. The mean age of the total population was 55 years (SD ±15.10). The mean level of inbreeding was significantly higher in the group of 55 years and above. Hypertension and atherosclerosis were significantly more prevalent in the old age group (p value < 0.001) (table 2). The mean level of education was significantly lower in the group of 55 years and above.

Table 2 shows the effects of the vascular risk factors on cognitive function in the total sample and in two age categories (< 55 years and ≥ 55years). Overall, DBP was significantly associated with a worse performance on the TMT-A test and card II of the Stroop colour-word test. In contrast, SBP was significantly associated with a better performance in the TMT-A. IMT was significantly related to a worse performance in all of the tasks in the Stroop colour-word test (cards I-III), as well as to a worse performance on semantic fluency. In the age-stratified analysis, SBP was

Table 1. Characteristics of the study population by two age categories

Demographic variables	All sample	<55 years (n=520)	≥ 55 years (n=521)
Age (years)	54.27 (15.10)	41.77 (9.02)	66.68 (9.01)†
Men (%)	40.8	38.7	41.3
Inbreeding	0.006 (0.007)	0.005 (0.006)	0.007 (0.008)†
University degree or higher (%)	3.5	6.3	1.0†
Systolic blood pressure (mm Hg)	141.44 (21.86)	132.32 (16.72)	152.08 (22.36)†
Diastolic blood pressure (mm Hg)	80.22 (10.09)	78.97 (10.27)	81.64 (9.73)†
Hypertension (%)	49.0	28.5	73.1†
Anti-hypertensive medication use (%)	21.71	10.5	33.0†
Common carotid IMT	0.86 (0.21)	0.74 (0.12)	1.02 (0.19)†

Continuous values are presented as means (SD)
IMT (Carotid Intima Media Thickness)
†p value < 0.05 compared with people older than 55 years

Table 2. Effects of blood pressure and IMT on the cognitive tests in the whole sample and in two age categories

	All sample			*< 55 years (n=520)*			*≥ 55 years (n=521)*		
Cognitive tests	**SBP**	**DBP**	**IMT**	**SBP**	**DBP**	**IMT**	**SBP**	**DBP**	**IMT**
AVLT-trial I	-0.003 (0.003)	0.002 (0.006)	-0.276 (0.339)	-0.003 (0.006)	-0.003 (0.010)	-0.204 (0.690)	-0.002 (0.004)	0.008 (0.009)	-0.313 (0.369)
AVLT-trials II-VI	-0.005 (0.017)	-0.047 (0.033)	-2.000 (1.750)	0.019 (0.030)	-0.085 (0.047)	-0.836 (3.264)	-0.013 (0.022)	-0.028 (0.048)	-2.830 (2.058)
AVLT-trial VI	0.001 (0.006)	-0.006 (0.011)	-0.643 (0.611)	0.003 (0.010)	-0.008 (0.016)	-0.661 (1.126)	-0.001 (0.008)	-0.007 (0.017)	-0.632 (0.759)
AVLT-trial VII	0.002 (0.002)	-0.003 (0.003)	-0.156 (0.158)	0.002 (0.002)	-0.003 (0.003)	0.241 (0.202)	0.002 (0.003)	-0.001 (0.006)	-0.236 (0.247)
Semantic fluency	0.025 (0.020)	-0.055 (0.037)	**-5.288 (1.994)**	0.066 (0.036)	-0.103 (0.057)	**-9.002 (4.097)**	0.012 (0.022)	-0.016 (0.048)	-3.321 (2.113)
Phonological fluency	0.037 (0.021)	-0.058 (0.039)	-3.178 (2.103)	0.061 (0.035)	-0.094 (0.056)	0.273 (3.915)	0.034 (0.026)	-0.044 (0.057)	-3.428 (2.503)
TMT-A†*	**-0.079 (0.027)**	**0.159 (0.052)**	4.681 (2.798)	-0.062 (0.045)	**0.164 (0.071)**	2.911 (5.128)	**-0.094 (0.036)**	0.134 (0.080)	4.456 (3.501)
TMT-B†*	-0.047 (0.036)	0.127 (0.068)	3.582 (3.675)	0.035 (0.055)	0.008 (0.087)	4.380 (6.290)	**-0.101 (0.051)**	0.192 (0.111)	0.432 (4.755)
Card I†*	-0.026 (0.015)	0.055 (0.029)	**5.182 (1.548)**	-0.048 (0.026)	0.059 (0.041)	**5.846 (2.937)**	-0.026 (0.020)	0.062 (0.043)	**5.268 (1.901)**
Card II†*	**-0.031 (0.014)**	**0.059 (0.026)**	**6.384 (1.392)**	-0.037 (0.021)	0.058 (0.033)	4.379 (2.409)	-0.033 (0.020)	0.061 (0.043)	**7.329 (1.874)**
Card III†*	-0.001 (0.017)	0.029 (0.032)	**4.941 (1.761)**	-0.017 (0.025)	0.044 (0.039)	3.664 (2.831)	0.005 (0.026)	0.014 (0.056)	**5.824 (2.486)**
Block Design†*	-0.016 (0.033)	0.008 (0.063)	-2.007 (3.422)	-0.071 (0.060)	0.061 (0.096)	1.545 (6.556)	-0.018 (0.035)	0.033 (0.078)	-2.130 (3.406)

The values are the regression coefficients (standard errors) of the systolic blood pressure (SBP), diastolic blood pressure (DBP) and IMT onto the cognitive tests
All models were adjusted for age, age^2, sex, inbreeding, education, DART, antihypertensive medication and family structure
† Log-transformed cognitive tests
Significant regression coefficients (p values <0.05) are in bold
* Positive correlation is associated with lower cognitive performance (for time demanding tasks)

significantly associated with better performance in both tasks of TMT in the group or 55 years and older. IMT was significantly associated with lower performance in the Stroop colour-word test (cards I-III) in this age group. In those younger than 55 years, DBP showed an association with a poorer performance in TMT-A, whereas IMT was significantly associated with worse performance in semantic fluency and the Stroop colour-word test.

The genotype frequencies of the *AGT* M235T, the *AT1R* C573T, the *ADD1* G460W, and the *GNβ3* rs2301339 *G/A* polymorphisms were in Hardy-Weinberg Equilibrium proportions (p value = 0.72 for *AGT,* p value = 0.30 for *AT1R*, p value = 0.58 for *ADD1* and p value = 0.18 for *GNβ3)*. Table 3 presents the effects of the polymorphisms of the *AGT, AT1R, ADD1* and *GNβ3* genes on the cognitive tests. The *AGT* M235T polymorphism was associated with better performance in the AVLT trial VII test (recognition task; p value = 0.01). Carriers of at least one *AAD1* 460W allele had a lower performance in phonological fluency than non-carriers (p value = 0.04), whereas carriers of the *GNβ3* G allele had lower performance in both TMT-B (cognitive flexibility; p value = 0.02) and in card III of the Stroop colour-word test (susceptibility to interference; p value = 0.05).

Table 3. Effects of four blood pressure genetic polymorphisms of blood pressure genes on cognitive tests

Cognitive tests	*AGT*	*AT1R*	*AAD1*	*GNβ3*
AVLT-trial I (short-term memory)	-0.123 (0.105)	0.014 (0.093)	-0.029 (0.092)	0.152 (0.160)
AVLT-trials II- V (learning)	-0.635 (0.559)	-0.758 (0.504)	-0.779 (0.493)	-0.740 (0.842)
AVLT- trial VI (delayed recall)	-0.022 (0.192)	-0.013 (0.173)	-0.221 (0.168)	-0.150 (0.293)
AVLT- trial VII† (recognition)	**0.129 (0.048)**	0.060 (0.044)	0.007 (0.042)	-0.085 (0.075)
Semantic fluency (verbal fluency)	0.041 (0.641)	-0.712 (0.584)	-0.643 (0.572)	0.756 (0.961)
Phonetic fluency (verbal fluency)	-0.236 (0.688)	0.188 (0.619)	**-1.256 (0.608)**	0.976 (1.035)
TMT-A† (psychomotor speed)*	0.038 (0.893)	-1.134 (0.808)	1.449 (0.791)	-0.661 (1.327)
TMT-B† (cognitive flexibility)*	0.703 (1.160)	-0.400 (1.054)	1.749 (1.034)	**4.071 (1.746)**
Card I† (reading abilities)*	0.475 (0.508)	0.599 (0.460)	-0.073 (0.449)	-0.298 (0.766)
Card II†(reading abilities)*	0.432 (0.453)	-0.064 (0.410)	0.374 (0.403)	-0.044 (0.676)
Card III† (susceptibility to interference)*	0.920 (0.561)	0.566 (0.510)	0.420 (0.501)	**1.676 (0.841)**
Block Design† (visuospatial abilities)	1.089 (1.112)	-0.290 (1.000)	-0.451 (0.985)	1.165 (1.643)

Values presented are the regression coefficient of the genetic polymorphism (s.e. are in brackets) onto the cognitive tests
AVLT (Auditory verbal learning test)
TMT (Trail Making Test)
†Log-transformed cognitive tests
All models were adjusted for age, age^2, sex, inbreeding, education, DART, antihypertensive medication and family structure
The genotypes were pooled as: *AAD1*: GG=0, GW/WW =1; *AGT*: MM/MT =0, TT=1; *GNβ3*: AA=0, AG/ GG=1; *AT1R*: TT/TC=0 CC=1
Significant regression coefficients (p value < 0.05) are in bold
* Positive correlation is associated with lower cognitive performance (for time demanding tasks)

Table 4. Median score (25th- 75th percentiles) of the cognitive tests per number of combined risk alleles of four blood pressured genes

	Number of risk alleles†					
Cognitive tests	**0 (n= 26)**	**1 (n=304)**	**2 (n = 443)**	**3 (n =204)**	**4 (n=20)**	**P value‡**
AVLT- trial I (short-term memory)	4.00 (2.00-5.00)	4.00 (3.00-5.00)	4.00 (3.00-5.00)	4.00 (3.00-5.00)	3.00 (3.00-4.00)	0.645
AVLT-trials II- V (learning)	35.00 (23.50-41.50)	31.00 (25.00-38.00)	31.00 (24.00-37.00)	29.00 (23.00-36.00)	25.00 (21.00-32.00)	**0.007**
AVLT- trial VI (delayed recall)	8.00 (5.00-11.00)	7.00 (5.00-9.00)	7.00 (5.00-9.00)	7.00 (5.00-9.00)	6.00 (4.00-8.00)	0.299
AVLT- trial VII (recognition)	15.00 (13.00-15.00)	14.00 (13.00-15.00)	14.00 (12.00-15.00)	14.00 (12.00-15.00)	14.50 (13.00-15.00)	0.071
Semantic fluency (verbal fluency)	38.00 (30.00-45.00)	34.00 (27.00-41.00)	34.00 (28.00-40.00)	33.00 (26.00-39.00)	32.00 (27.00-40.00)	0.337
Phonological fluency (verbal fluency)	25.00 (15.00-29.00)	23.00 (15.25-32.00)	23.00 (16.00-30.00)	20.00 (14.00-29.00)	19.00 (12.00-27.00)	0.411
TMT-A (psychomotor speed)*	39.00 (27.50-52.50)	37.00 (30.00-56.00)	38.00 (29.00-54.00)	38.00 (29.00-58.00)	47.00 (32.00-74.00)	0.954
TMT-B (cognitive flexibility)*	84.00 (60.50-129.00)	99.50 (63.75-151.25)	103.00 (67.00-167.00)	102.00 (68.00-199.00)	124.50 (89.00-199.50)	0.060
Card I (reading abilities)*	49.00 (44.00-60.25)	50.00 (46.00-58.00)	50.00 (44.00-60.00)	51.00 (45.00-61.00)	53.00 (48.00-85.00)	0.469
Card II (reading abilities)*	62.00 (54.75-67.75)	62.00 (55.00-70.00)	63.00 (55.00-72.00)	63.00 (56.00-72.00)	66.00 (58.00-77.00)	0.461
Card III (susceptibility to interference)*	96.50 (76.25-109.50)	104.00 (89.00-122.75)	105.00 (90.00-128.00)	106.00 (87.75-131.00)	109.00 (95.75-148.00)	**0.015**
Block design (visuospatial abilities)	19.00 (12.00-33.00)	19.00 (12.00-33.00)	18.50 (12.00-32.00)	18.00 (12.00-34.50)	22.00 (11.50-31.00)	0.750

Values presented are the median and 50% of the score distribution of the cognitive tests per number of risk alleles

† Combined risk alleles: 0,1,2,3 and 4 risk alleles: *AAD1*: GW/WW =1; *AGT*: TT=1; *GNβ3*: GG=1; *AT1R*: CC=1

†† P value of the adjusted analysis (age, age^2, inbreeding, education, DART and familial structure)

* Higher score represent worse cognitive performance (for time demanding tasks)

The effects of the combined risk alleles of the four blood pressure genes are presented in table 4. There was a trend towards lower performance in all cognitive tests with increasing number of risk alleles. There was a significant difference of 10 words in AVLT trials II-V (learning) when comparing carriers of the four risk alleles against non-carriers (learning; p value = 0.007). The difference between these groups for the card III of the Stroop colour-word test was also statistically significant (susceptibility to interference; p value = 0.02). The effect of the combined risk alleles on the TMT-B was borderline significant (cognitive flexibility; p value = 0.06). Further stratified analysis showed that the effect of the combined alleles was significant for those younger than 55 years old for AVLT trials II-V (learning; p value = 0.007), whereas in the card III of the Stroop colour-word test, the effect of the combined alleles was driven mainly by the group of 55 years and older (susceptibility to interference; p value = 0.003).

DISCUSSION

In this family-based study, we found that DBP was associated with lower cognitive performance in both TMT-A and card II of the Stroop colour-word test. IMT was significantly associated with a worse performance in all tasks of the Stroop test as well as with semantic fluency. In contrast, SBP was associated with a better performance in TMT-A, and in card II of the Stroop colour-word test. Furthermore, the *AGT* 235T allele was associated with better performance in the recognition task. Lower phonological fluency in carriers of at least one *ADD1* 460W allele was found whereas the *GNβ3* A allele was related to a poorer cognitive performance in tests evaluating cognitive flexibility and susceptibility to interference. The combined risk alleles of the four genes were significantly associated with lower performance in tasks evaluating learning and susceptibility interference. This association was borderline significant for cognitive flexibility.

In this study, most of the cognitive tests were adversely associated with blood pressure and atherosclerosis, which is in line with previous studies (8, 35). However, SBP was associated with improved performance in TMT-A and TMT-B in those aged 55 years and older. The better cognitive performance in those with high SBP is counter-intuitive, but in line with a number of epidemiological studies (for a review see (36)), suggesting that with aging the brain may become more susceptible to hypoperfusion leading to lower cognitive function (5). High SBP may prevent hypoperfusion in the brain, thereby improving cognitive function. On the other hand, high blood pressure at a young age may lead to cerebral vessel damage, as shown by findings in the Framingham study (35).

This is the first study in which the four RAAS genes involved in blood pressure regulation have been evaluated in relation to cognitive function. In this analysis, polymorphisms of two genes involved in salt-sensitivity variation, the *GNβ3* and *AAD1* genes, were associated with a poorer performance in tasks evaluating executive function (cognitive flexibility, susceptibility to interference and verbal fluency). When combining all alleles associated with increased blood pressure, there was a decrease in the performance in all cognitive tests, but this was statistically significant only in the tests evaluating learning and susceptibility to interference. The association of the *AGT* 235T allele with better recognition is unexpected as this allele has been associated with higher blood pressure through the effects of increased angiotensinogen levels (13). However, angiotensin II has been associated with improved memory in several studies (for a review see (12)). A major issue of this analysis is that the assumption of the additive effects of the risk alleles might not be true, as most of the gene-gene interactions in previous studies are modelled assuming multiplicative effects. However, it has been recently suggested that modelling gene-gene and gene-environment interactions using an additive scale may indeed be of biological relevance (37).

In our study, multiple tests were conducted with candidate genes. However, the polymorphisms we studied were chosen based on previous research, which suggested that these polymorphisms are associated with blood pressure variation among the participants of our study. Here, we tested whether these specific polymorphisms were also associated with cognitive function. Our findings of association between blood pressure genes and cognitive function seem to contradict our earlier study, in which no significant genetic correlations between vascular factors and cognitive function were found (32). However, the lack of genetic correlation may be explained partly by the small number of subjects studied.

In conclusion, we have shown significant associations between vascular factors and the polymorphisms of four blood pressure regulating genes with cognitive functions. Polymorphisms of genes involved in salt-sensitivity pathways had significant effects on lower executive function. These findings suggest that genes involved in blood pressure explain a small proportion of the variation in cognitive function.

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7

General discussion

INTRODUCTION

Alzheimer's disease (AD) is a late-onset neurodegenerative disorder that afflicts more than 24 million individuals worldwide (1). It is expected that this number will double in the next 20 years (1). Dissecting the genetics of AD is one of the most challenging endeavours for researchers in the field of human genetics and genetic epidemiology. A major difficulty in the identification of genes involved in AD pathogenesis is that it is a complex disorder. Multiple genes, each with a small effect, interacting with each other and with environmental factors, can confer an increased risk for the disease, while rare genetic variants with large phenotypic effects can also cause AD. *APOE* is an example of a gene that can confer an increased risk of AD while *APP*, *PSEN1* and *PSEN2* are examples of genes with rare variants that cause AD.

As a consequence of the complexity of AD, large sample sizes are required in order to estimate the multiple parameters describing a complex system. Alternatively, one may attempt to study the system in a setting in which the complexity is expected to be reduced.

Studies in genetically isolated populations are an example of a method for reducing the genetic complexity of AD. In such populations, the genetic and environmental heterogeneity of complex diseases is expected to be reduced. Genetically isolated populations have been proven to be a powerful setting for finding genes for both Mendelian and complex disorders (2, 3).

Further, it may also be possible to identify a less complex subsystem of a complex disease that can be measured as continuous trait, and concentrate on elucidating the genetics of this trait. Endophenotypes provide an example of subsystems that can reduce the complexity of diseases, which are caused by alterations in different biological pathways. Endophenotypes are traits that are in a strong causal relationship with the disease of interest, but are more closely associated with the genes underlying the disease. Thus, these traits have a less complex genetic architecture (4).

In this thesis the genetic determinants of cognitive function, which is a potential endophenotype for AD, were studied in a young genetically isolated population. In this chapter the results of the thesis are discussed. In the first section, the advantages and disadvantages of using genetically isolated populations to study complex diseases are considered. In the second section, the characteristics of potential endophenotypes for AD are discussed. In the third and fourth sections, the genetics of cognitive function are discussed. In the last section, possible directions for future research in this and other populations are discussed.

GENETIC RESEARCH IN ISOLATED POPULATIONS

A genetically isolated population usually implies a relatively small group of people who have a common population history. This history is described by the time since foundation or most recent bottleneck, the number of founders, the population growth history (whether the population has undergone expansion or not), the rate of immigration, and the pattern of marital choice. The interactions between these parameters dictate how large founder and drift effects will be and how large the extent of linkage disequilibrium (LD) in the population is.

In any study of a genetically isolated population it is very important to determine the magnitude of founder/drift effects and LD in the study population (5). Both effects are double-sided. Strong drift and founder effects tend to deviate the genetic pool of isolated populations from that of the general population (3). This makes isolated populations suited to the identification of rare genetic variants since these variants might reach higher frequencies in particular isolates. However, large deviations from the allele frequencies found in the general population reduce the chance that any findings can be extended to other populations, as these variants can become population-specific.

If a very strong LD between markers and a putative disease locus can be expected, the marker coverage of the genome required for association studies may be sparse compared to that required in the general population. On the other hand, high LD may limit the precision of fine mapping of a locus region (3).

In this context, young genetically isolated populations can present a very attractive compromise between reduced heterogeneity and extendibility. Such populations, like any relatively small genetically isolated population, are expected to have a more homogenous environmental background, and should exhibit moderate amounts of LD. Yet, these populations may be very similar to the general population, except for the frequencies of rare variant alleles.

GRIP population: a young genetically isolated population

The studies described in this thesis have been conducted within the framework of the Genetic Research in Isolated Populations (GRIP) program. The study population is located in the Southwest of The Netherlands and it is known from historical records that the population was founded in the 18th century by a limited number of individuals (<400). In this population new genes and mutations were identified for disorders such as early-onset Parkinson's disease (PD) (*DJ-1* gene) (6) and hereditary hemochromatosis (*SLC11A3* gene) (7). In the past three years, research has been conducted to clarify the extent of LD in the GRIP population. Aulchenko *et al,* studied the pattern of LD across the whole genome in a sample

of 58 unrelated subjects using micro-satellite markers (8). Moderate LD was found. The extent of LD was higher than that observed in the general population (UK) but lower than in an old, large genetically isolated population (Finland). The degree of LD in GRIP was similar to that of other young, genetically isolated populations such as in Palau and the Central Valley of Costa Rica. These findings have been confirmed in a more recent study in which a sample of 200 unrelated individuals from the GRIP population was analysed. Service *et al*, studied LD in 12 genetically isolated populations, using a dense map of 2486 SNPs covering chromosome 22 (9). They have shown that LD in the GRIP population is similar to that in other young, genetically isolated populations. For these populations, it was estimated that an approximately 30% reduction in the number of SNP markers would still achieve the same efficiency of coverage in a whole genome screen compared to the general population. However, the overall magnitude of LD was different across the different young isolated populations (9), which highlights the fact that other factors such as founder population size, inbreeding and admixture are also major determinants of the LD in a given population.

In chapter 3 of this thesis, genetic drift and founder effects in the GRIP population were studied (9). It was shown that drift and founder effects are strong only for variants with frequencies lower that 1%. At the same time, common genetic variants identified in the GRIP population are likely to be present at similar frequencies in the general population. Table 1 presents empirical support for these theoretical findings by comparing the frequencies of common variants of three genes (*APOE, AGT* and *CETP*) in the GRIP population with their frequencies in other Caucasian and non-Caucasian populations. The frequencies of these common variants in the GRIP population are similar to the frequencies in the general Dutch population and other populations of Caucasian origin. In contrast, the frequency

Table 1. Allele frequencies of the variants genotyped in GRIP compared with Caucasian and non-Caucasian populations

Population	**APOE*4**	***AGT* T235**	***CETP* V405**
Caucasian			
GRIP	0.20*	0.48*	0.30**
Finland	0.19 (54)	0.46 (55)	0.37 (56)
General Dutch population	0.15 (57)	0.40**	0.32 (58)
Other Caucasian populations	0.14 (54)	0.45 (59)	0.30 (60)
Non-Caucasian			
African descendants	0.31 (54)	0.83 (61)	0.695†
Asia (Japanese-Chinese)	0.117 (54)	0.76 (61)	0.568†

* This thesis (chapter 5,6)
** Unpublished data
† dbSNP (rs5882; http//www.ncbi.nlm.nih.gov/SNP)

of the *DJ-1* deletion that causes recessive PD was approximately 0.9% in the GRIP population. Although mutations in the *DJ-1* gene were found in other populations, the specific deletion was absent (10). These results confirm that genetic variants identified in young isolated populations can be extended to more general contexts, provided that they are relatively common (frequency > 1%).

In conclusion, the GRIP population represents a typical example of a young genetically isolated population. Work presented in this thesis shows that the genetic composition of the GRIP population does not deviate much from that of the general population.

Gene-mapping in genetically isolated populations

Genetically isolated populations represent a methodological and computational challenge to genetic epidemiologists. One of the major issues when conducting research in isolated populations is that the probability that 2 alleles at the same locus are identical by descent (IBD) is higher than in outbred populations (11). This holds both for 2 alleles at the same locus in one individual (inbreeding) and for 2 alleles randomly sampled from 2 different individuals (kinship). Considerable caution must therefore be taken in the interpretation of any findings in isolated populations using current statistical methods (linkage and association studies) (11).

In standard affected sib-pair methods, which are commonly used to search for loci in complex diseases, sibs are expected to have 0, 1 or 2 alleles identical by descent (IBD), with probabilities of 0.25, (0 alleles), 0.50 (1 allele) and 0.25 (2 alleles) (the null hypothesis). In affected sib-pairs analysis, any excess of sharing between sibs is considered a signal of linkage (12). When parents are either related or inbred, the expected shared IBD probabilities between sibs might increase under the null hypothesis, as the expected shared IBD probabilities were derived assuming unrelated parents. The probability of false positive linkage findings increases if this is not taken into account (12). Likewise, in genetic mapping using dominant models and in homozygosity mapping, higher rates of false positive linkage results are obtained when not all kinship and/or inbreeding information is used (Fan *et al*, 2006 in press).

Higher kinship and inbreeding also affect the type-1 error in association studies, if patients are substantially more closely related than controls (13). The association studies of quantitative traits described in chapters 5 and 6 were performed using a linear mixed modelling approach that accounted for the complete pedigree information, including thousands of individuals and multiple loops. Therefore, it is unlikely that the findings in these chapters are due to cryptic relationships between individuals in the study population.

Another problem is the computational burden caused by the complex pedigrees derived from isolated populations. The extensive genealogical records that are available for some isolated populations reveal that individuals are inter-related through multiple connections. These extended pedigrees become too complex for linkage analysis using standard computer programs (11). An alternative is to decompose these complex pedigrees into smaller pedigrees, although this might reduce the power of the study (11) and increase the risk of type-1 errors (Fan *et al*, 2006 in press). For association studies there are methods that account for complete genealogical information (14), but they require extensive computational time. High performance parallel computing provides one option to speed-up the analysis. Faster methods, which account for pedigree structure, such as the restricted maximum likelihood method are also an alternative.

ENDOPHENOTYPES FOR ALZHEIMER'S DISEASE (AD)

Endophenotypes are the intermediate traits that lie in the pathways relating genes to diagnostic entities, but have a simpler genetic architecture than the disease itself (4, 15). Criteria to describe an endophenotype have been developed in the field of psychiatric genetics (4, 15, 16): (*i*) the endophenotype has to be associated with the disease (*ii*) the endophenotype should be heritable; (*iii*) the endophenotype should manifest in both affected and unaffected individuals and (*iv*) the endophenotype should occur with a higher frequency in relatives of probands than in the general population (15, 16). Current endophenotypes for the genetic analysis of AD can be grouped into cognitive tests, brain imaging-derived measures and biochemical markers (17).

Cognitive tests

AD is characterized by insidious cognitive decline (18). Although patients with AD may also exhibit behavioural and motor symptoms, the main feature of AD is global cognitive impairment (18). Cognitive deficits can be assessed using neuropsychological tests (18), which have high sensitivity and specificity to differentiate between different dementia subtypes (18, 19).

Cognitive traits are suitable for genetic analysis. The heritability of general cognitive ability has been estimated to be as high as 50% (20), although for other specific cognitive domains the estimates are lower and vary according to the study design and the population studied (chapter 4). Furthermore, lower cognitive function is observed in the relatives of AD cases, especially early onset AD (21).

An important caveat in using cognitive domains as endophenotypes for genetic analysis is that other variables, such as educational achievement, or depression, may affect the assessment of cognitive function (22). For this reason, neuropsychological assessment might have lower sensitivity and specificity than neuroimaging to identify pre-clinical AD (23). A major advantage of neuropsychological assessment is that it is easy to implement by trained personnel and allows evaluation of large numbers of individuals.

Neuroimaging

There is evidence that brain volume is an inheritable trait. A high heritability of both grey matter (90%) (24) and white matter (87%) (25) was demonstrated by using magnetic resonance imaging (MRI) in twins. Significant heritabilities for regional brain volumes have also been demonstrated (26). Measures derived from neuroimaging studies have been used as endophenotypes for AD (22), as they correlated with AD (27). In addition, first-degree relatives of AD cases show a higher degree of structural and functional changes in the brain, without any clinical manifestation (28).

A complication when using these quantitative imaging measures is the high implementation cost and time as specialized personnel are required to analyse the imaging data. Thus far, such studies were based on relatively small sample sizes. Developments in neuroimaging may make large-scale studies feasible in the future.

Biochemical markers

The β-amyloid protein (Aβ) is a key element in the cascade of events that leads to AD (29). Increased concentrations of the $A\beta_{42}$ and $A\beta_{40}$ fragments of the Aβ protein in blood have been associated with AD in some studies (30, 31). Recently, it has been shown that low levels of the $A\beta_{42}$ fragment, together with the levels of tau protein, in cerebral-spinal fluid (CSF) have high sensitivity and specificity to predict AD (31). Aβ levels are heritable, with estimates of 56% for $A\beta_{42}$ levels and 41% for $A\beta_{40}$ levels in AD families (32). In addition, several studies have shown that first-degree relatives of late onset AD cases are at risk of exhibiting higher levels of Aβ (32).

One major issue is that the assessment of $A\beta_{40}$ and $A\beta_{42}$ levels in CSF is an invasive procedure. $A\beta_{40}$ and $A\beta_{42}$ levels can also be determined in plasma. However, the levels of these proteins vary with age and with the levels of serum creatinine, and correlate poorly with the levels in CSF (33). In addition, in cross-sectional studies a large overlap between the levels on these proteins in AD patients and in controls has been found (33). In summary, these endophenotypes await further technical development.

GENETIC STUDIES OF COGNITIVE FUNCTION

Heritability studies

The classical approach to model the genetic variation of a continuous trait is to partition the phenotypic variance (V_P) of a trait onto its genetic and environmental variance components (V_G and V_E respectively) (34): $V_P = V_G + V_E$

The proportion of phenotypic variance that is explained by genetic factors is denominated heritability (34).

Twin studies are often used to estimate heritability (35). In this design, the heritability is estimated as twice the difference of the intra-class correlation of monozygotic twins minus the intra-class correlation of dizygotic twins (26). The main limitation of twin studies is that shared genetic and environmental effects cannot be discriminated using only sib-correlations (35). Therefore twin studies tend to give inflated heritability estimates. It has been argued that for cognitive function this does not represent a problem, as early shared environmental effects may not influence cognitive abilities in late life. Yet, it has been shown that non-genetic influences may have an important contribution to specific cognitive domains such as verbal fluency (36). In addition, the assumption of homogenous environmental effects for both monozygotic and dizygotic twins might not apply (37).

The above caveats for the twin studies may be overcome by using adoption studies that compare correlations between twins reared apart and adopted children with shared environments (35). These studies are powerful in discriminating between shared environmental and genetic effects, but they are limited by the scarcity of suitable subjects.

Alternatively, heritability can be also estimated by including other types of relatives in the analysis. Distant relatives are not expected to share a common environment and therefore studies based on extended pedigrees may provide more reliable estimates of heritability. These studies are less discriminative than adoption studies, but have the advantage of the availability of large numbers of subjects.

Heritability estimates range across different populations as well. Differences in the heritability estimates for cognitive function have been attributed to differences in the genetic background, but even more to differences in the environment. Despite these shortcomings, heritability analysis is a powerful method to determine whether genes are accounting for a large proportion of the variance of a cognitive trait.

Definitions of phenotypes for genetic analysis

Cognition is a composite of multiple, highly correlated cognitive abilities (18). Early studies of the variation of psychometric intelligence test results among individuals showed that a general factor (general intelligence or "*g*" factor) explained a large

proportion of the variation of cognitive tests (38). Furthermore, multivariate genetic studies of intelligence have also shown that "*g*" explains a large proportion of the genetic variance of psychometric tests (38). From a genetic perspective, this might imply that the genetic make-up of cognitive function is constituted by "general" genes that are common to all cognitive domains rather than specific genes that affect only a single domain (39).

However, cognition is a complex trait. As for other complex traits, the genetic architecture of cognition will most likely consist of multiple genes each with a mild effect on the expression of human cognitive variation (40). With this assumption in mind, it might be more powerful to study the genetics of cognitive function in discrete entities, rather than considering cognition a single unit (41), especially if the goal is to find genetic and environmental determinants for AD.

Functional studies have revealed that the *APOE* variant APOE*4 is associated with alterations in the brains of non-demented subjects at high risk for AD (such as first-degree relatives or carriers of early-onset AD mutations) that are similar to those observed in AD patients (28). Therefore this genetic variant may also be valuable to underscore changes in domains of cognition that are stronger correlated with AD and its prodromal phases. For example, in chapter 5 of this thesis, the relation between *APOE* and cognitive function was evaluated. The APOE*4 allele was associated with a worse performance in cognitive tests measuring short-term memory and executive function. There was no effect on other cognitive domains. These results suggest that reduced short-term memory and executive function might be early markers for AD and therefore potential endophenotypes for genetic studies.

Other risk factors may have effects on specific cognitive domains. For example hypertension has been shown to affect executive function (43), whereas visuospatial abilities appear not to be affected (44). In chapter 6, the relationships between blood pressure and atherosclerosis with cognitive function were studied. Increased blood pressure and IMT were associated mainly with lower executive function whereas memory-related tests did not show any association. The findings presented in chapter 5 and 6 demonstrate that cognitive domains are differentially affected when comparing the APOE*4-related spectrum (typical from AD) and hypertension-related spectrum (typical of vascular dementia).

Inbreeding, AD and cognitive function

Although genetic factors have been shown to play an important role in the pathogenesis of late onset diseases such as AD, the genetic background of such disorders is poorly understood. There is some evidence that recessive or partially recessive variants might be implicated in late onset disorders (45). Such variants might escape strong selective constraints because they only have detrimental effects

during post-reproductive life (45). The mutation-accumulation hypothesis predicts that these alleles accumulate in the genome, contributing to aging and related disorders (46). This is in line with the increase in the genetic variance observed for several late onset disorders (45).

There is evidence that inbreeding has an impact in late onset diseases such as hypertension (47, 48). Inbreeding increases the genetic homozygosity and thus enriches the genome of individuals with recessive variants (49). For AD, the evidence is scarce. Only one study has reported a higher prevalence of AD associated with inbreeding (50). Furthermore, mutations in 3 genes that cause early onset AD are clearly dominant (29). However, these mutations have a minor contribution to the total burden of the disease in the general population (51). Thus, it is likely that other genetic variants with less strong effects on the fitness of individuals may be part of the genetic background for late onset AD.

Studies of inbreeding and cognitive function date back to as early as 1978 (52). Morton showed that inbreeding had detrimental effects on cognitive performance. In addition he showed that these effects were entirely due to recessive variants. In chapter 4 of this thesis, the effects of inbreeding on cognitive function in a sample of 2575 subjects from the GRIP population were investigated. Although the percentage of inbred participants in the GRIP population is high (75%), the inbreeding coefficients were on average low (mean value 0.006; lower than the value corresponding to a second-cousin marriage: 0.02). In the GRIP population, inbreeding was associated with lower visuospatial abilities and executive function. The effect was very small, but the findings suggest that inbreeding contributes to the variation in general cognitive function in adults. Furthermore, the results demonstrate that the GRIP population may be a useful setting in which to find rare recessive variants associated with late onset AD.

POWER OF ASSOCIATION STUDIES IN ERF

A family-based study was conducted in the GRIP population. The Erasmus Rucphen Family (ERF) study was designed to find susceptibility genes associated with quantitative traits related to complex diseases. The ERF study includes 2996 participants belonging to 22 5-generation families, selected on the basis of the number of sibs (at least 5) in the second generation (5). This is the setting for the genetic epidemiological studies to search for genes implicated in cognitive function described in this thesis. One of the important questions to be addressed is: How powerful is the ERF study to detect genetic variants with small phenotypic effects?

Several genes have been genotyped in approximately 1000 participants of the ERF study. Preliminary power studies have shown that this sample has about 80% of the power required to detect a candidate polymorphism explaining as little as 1% of a trait's variation at the nominal significance level of 0.05. In this thesis, the associations between cognitive functioning and the *APOE* gene were investigated. In the study described in chapter 5, the APOE*4 variant was associated with specific cognitive domains, contributing to less than 2% of the variance of those domains, which was in line with previous studies (42, 53). This provides an empirical proof of the high power of our study sample to detect gene variants with modest effects on phenotype.

FUTURE RESEARCH

In the ERF study, extensive phenotypic information has been gathered for 2996 subjects belonging to 22 extended pedigrees. In addition, genotyping of approximately 6000 SNPs (1 SNP every 0.5 cM on average) is on-going. This tremendous amount of information represents a methodological and computational challenge. It is also not yet quite clear what are the most powerful analytical methods in the setting of the ERF study.

The most interesting and powerful approach is perhaps Genome Wide Association (GWA) analysis. As demonstrated in this thesis, the GRIP population is a young genetically isolated population that is representative of both young isolates, and the general Caucasian population. We expect that common variants found in relation to cognitive function in the GRIP population will also have a role in determining cognitive ability in other populations. We have also shown that the ERF study is sufficiently powerful to detect small genetic effects such as those of the *APOE* gene on specific cognitive domains. It is likely that other loci are implicated in cognitive function, as this gene only explained a small proportion of the variance of the cognitive domains. In addition, in the GRIP population there is moderate LD between genetic markers. This improves the marker coverage of the genome in ERF participants.

It may be expected, however, that there are also rare variants affecting cognitive function in the GRIP population. Under this assumption, linkage analysis should be a powerful method in the ERF study. The ERF families were selected based on large sibships that are critical for genome-wide linkage analysis. For cognitive function, inbreeding was associated with reduced executive function and visuospatial abilities (chapter 4). Therefore, homozygosity mapping may be an interesting approach to identify genetic variants associated with specific cognitive functions.

However, the same strict criteria employed for both association and linkage studies need to be applied to the ERF study to reduce both the type-1 and -2 errors associated with linkage and association studies. There are major methodological challenges, including the definition of phenotypes, to be overcome ahead of performing genome-wide analyses in the ERF study. The findings presented in this thesis indicate that specific cognitive domains will be suited to genetic analysis, especially in the context of AD. Memory and executive function, for example, might be valuable endophenotypes for AD in the ERF study, as shown by the significant effects of APOE*4, the well-known risk factor for AD, on these 2 cognitive domains. In addition, for both memory and executive function the heritability estimates were relatively high. Phenotypic definition with other tools such as neuroimaging will also help to identify better endophenotypes, as well as select subjects at risk for late AD.

The work presented in this thesis has provided the foundation upon which to construct further studies aimed at elucidating the genetics of cognitive function as well as that of onset AD.

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Summary - Samenvatting - Resumen

Alzheimer's disease (AD) is the primary cause of dementia in Western societies, affecting approximately 30% of the population aged 90 years or over. Despite the huge effort in the scientific community to identify the genes involved in AD, the genetics of late onset AD are largely unknown. Intermediate phenotypes of AD (endophenotypes) may help to identify genetic and non-genetic modifiers of the disease. In this thesis we aimed to identify genetic determinants of cognitive traits that are used as intermediate phenotypes for AD in a young genetically isolated population in the Southwest of The Netherlands. The research was performed as part of the Genetic Research in Isolated Populations (GRIP) program.

In **chapter 1,** the literature of the genetics of AD and Parkinson's disease (PD) was reviewed in light of the current hypotheses available to explain the genetic background of complex disorders: common diseases/common variants and common diseases/rare variants. Several strategies to increase the power of genetic studies of neurodegenerative disorders were also delineated.

In **chapter 2** the aims and general outline of the research described in this thesis are presented.

In **chapter 3,** founder and drift effects on the GRIP population were examined. A large extended pedigree, including 9800 individuals derived from the GRIP population, was used to simulate the drift and founder effects. We found that only variants attaining initial frequencies equal or lower than 1% will be subjected to drift and founder effects, with a high probability of loss and a reduced chance of fixation. To provide empirical confirmation of the simulated data, the expected heterozygosity and mean number of alleles of 592 microsatellite markers from 58 subjects from this population were estimated. These parameters were compared with those from samples with a different demographic history, namely the CEPH families (general population), Icelandic pedigrees (old and large isolated population) and the Talana (a sub-isolate with strong founder effects). We observed a lower mean heterozygosity and a lower number of alleles in the GRIP population than in the CEPH families (general population) and the Icelandic population, but higher than in the Talana population. We concluded that the fluctuations in the genetic pool of the GRIP population have occurred due to genetic drift and founder effects, but that these effects have only had an impact for rare genetic variants.

In this thesis, the genetics of cognitive traits that are potential endophenotypes for AD was examined. In **chapter 4,** the heritability of specific cognitive domains, as assessed by standard neuropsychological tests, was estimated in participants from a family-based study (The Erasmus Rucphen Family (ERF) study) in the GRIP population. The effects of inbreeding on cognitive variation were also examined, along with sex- and age-specific genetic effects on the phenotypic expression of cognitive function. The heritability estimates varied from 10% to 30% for memory-

related traits, while for executive function the estimate was 36%. Inbreeding was associated with worse executive function and reduced visuospatial abilities. This analysis also showed that age-specific effects might account for age-related differences in psychomotor speed and susceptibility to interference. In contrast, there was no evidence for sex-specific genetic effects on cognitive function. These findings suggest that the contribution of genetic factors to the variation in cognitive function is domain-specific. Furthermore, the significant effects of inbreeding indicate that recessive variants may, at least partially, determine the genetic make-up of cognitive function.

In **chapter 5**, we studied the relationship between the *APOE* gene and cognitive function to examine the cognitive profile associated with the APOE*4 allele in non-demented subjects. In addition, we evaluated whether age and sex modulated the effect of this genetic variant on the cognitive domains. Carriers of at least one APOE*4 allele had worse cognitive performance than non-carriers in short-term memory, cognitive flexibility and susceptibility to interference. These effects were modulated by both age and gender, as we observed that the effect was stronger in those 50 years and older and also in women. The significant associations between the APOE*4 allele and memory and executive function suggests that these cognitive tests may be markers of early changes in cognition.

In **chapter 6**, the association between blood pressure, atherosclerosis and polymorphisms in the angiotensinogen (*AGT*), angiotensin II type 1 receptor (*AT1R*), the α-adducin 1 (*AAD1*) and the G-protein β-3 subunit (*GNβ3*) genes were evaluated in relation to cognitive function. We found adverse effects of blood pressure and atherosclerosis on cognitive tests, mainly with those tests that evaluate executive function. The effect of the angiotensinogen *AGT* M235T, the *AT1R* C573T, the *AAD1* G460W and the *GNβ3* rs2301339 polymorphisms on the cognitive tests were also evaluated. There was a significant association between the *AGT* 235T allele with a better performance in the recognition task. In contrast, lower cognitive performance in phonological fluency in carriers of the *AAD1* 460W allele was found. In addition, a worse performance in the tests that evaluate susceptibility to interference, with carriers of the *GNβ3* G allele was observed. The joint effect of the risk alleles of the four genes was also evaluated. Interestingly, worse performance in all cognitive tests with increasing number of risk alleles was observed, although it was significant only for learning and susceptibility to interference. There were no major differences in the stratified age-analysis. Our results confirm the substantial role of vascular factors in reduced performance in cognitive function. In addition, genes underlying the variation in blood pressure also contribute to the variation in cognition. The association of the *AGT* 235T allele with better performance in recognition suggests that angiotensinogen might have pleiotropic effects in brain.

The results of this thesis underscore the value of the GRIP population as a powerful setting to do genetic research. Future genetic studies of cognitive function in the setting of the ERF study are also promising.

De ziekte van Alzheimer (Alzheimer's Disease, AD) is de meest voorkomende oorzaak van dementie in de Westerse wereld. Circa 30% van de bevolking van 90 jaar en ouder lijdt hieraan. Ondanks grote inspanningen binnen de wetenschap om genen te identificeren die betrokken zijn bij AD, blijven de genetische aspecten van de zgn. "late onset AD" grotendeels onbekend. Intermediaire phenotypes van AD (endophenotypes) kunnen mogelijk eraan bijdragen om genetische en non-genetische factoren te identificeren, die de ziekte kunnen beïnvloeden. Het doel van dit proefschrift is het identificeren van genetische determinanten van cognitie, die gebruikt worden als intermediaire phenotypes van AD in een jonge, genetisch geïsoleerde populatie in het Zuid-Westen van Nederland. Het onderzoek maakt deel uit van het GRIP programma (Genetic Research in Isolated Populations).

In **hoofdstuk 1**, wordt literatuur over de genetica van AD en de ziekte van Parkinson (Parkinson's Disease, PD) beschreven in het kader van de huidige hypotheses over de genetische achtergrond van complexe ziektes: veel voorkomende/zeldzame varianten van algemeen voorkomende ziektes. Verscheidene methoden om de "power" van genetische studies over neurodegeneratieve afwijkingen te vergroten worden ook besproken.

In **hoofdstuk 2** worden het doel en de algemene opzet van het in dit proefschrift beschreven onderzoek uiteengezet.

In **hoofdstuk 3** worden "founder" en "drift" effecten binnen de GRIP populatie bestudeerd. Een grote en uitgebreide stamboom bestaande uit 9800 personen, afkomstig uit de GRIP populatie, is gebruikt om deze "founder" en "drift"effecten te simuleren. Wij vonden dat alleen varianten met een initiële frequentie van 1% of minder onderhevig zijn aan deze effecten, met een grote kans op verlies en een verminderde kans op fixatie. Om empirische bevestiging te verkrijgen van de bevindingen van de gesimuleerde data, zijn de verwachte graad van heterozygotie en het gemiddelde aantal allelen van 592 "microsatellite" markers in 58 personen uit deze populatie geschat. Deze data is vergeleken met data van personen met een andere demografische achtergrond, afkomstig van de CEPH families (algemene bevolking), uit IJslandse stambomen (oude en grote genetisch geïsoleerde populatie) en van de Talana populatie (een sub-isolaat met sterke "founder" effecten). Wij vonden een lagere gemiddelde heterozygotie en een kleiner aantal allelen in de GRIP populatie dan in de CEPH families (algemene bevolking) en de IJslandse populatie, maar daarentegen een hoger aantal dan in de Talana populatie. Wij concluderen dat fluctuaties in de genetische "pool" van de GRIP populatie hebben plaatsgevonden door genetische "drift" en "founder" effecten, maar dat deze effecten alleen de zeldzame genetische varianten hebben beïnvloed.

In dit proefschrift zijn genetische determinanten van cognitie bestudeerd die potentiële endophenotypes vormen van AD. In **hoofdstuk 4** is de

erfelijkheid van bepaalde cognitieve domeinen, beoordeeld middels standaard neuropsychologische testen, geschat bij deelnemers aan een familie-onderzoek (ERF Studie, Erasmus Rucphen Familie Studie) binnen de GRIP populatie. De effecten van bloedverwantschap tussen ouders op variatie in cognitie zijn ook bestudeerd, tezamen met geslachts- en leeftijdsspecifieke genetische effecten op phenotypische expressie van cognitieve functie. Schattingen van de erfelijkheid variëren van 10 tot 30% voor geheugen gerelateerde eigenschappen, terwijl voor uitvoerende functies de schatting 36% was. Bloedverwantschap tussen ouders was geassocieerd met verslechterde uitvoerende functies en verminderde ruimtelijke en gezichtsfuncties. Deze analyse toonde ook dat leeftijdsspecifieke effecten mogelijk leeftijdsgerelateerde verschillen in psychomotorische snelheid en vatbaarheid voor afleiding ("interference") kunnen verklaren. Daarentegen was er geen bewijs voor geslachtsspecifieke genetische effecten op cognitie. Deze bevindingen suggereren dat de bijdrage van genetische factoren aan de variatie in cognitieve functie domein-specifiek is. Voorts geven de significante effecten van bloedverwantschap tussen ouders aan dat recessieve varianten, ten minste voor een deel, mogelijk de genetische basis van cognitieve functie bepalen.

In **hoofdstuk 5** hebben wij de relatie tussen het *APOE* gen en cognitieve functie bestudeerd teneinde het cognitieve profiel, geassocieerd met het APOE*4 allel, in niet-dementerende personen te onderzoeken. Voorts hebben wij onderzocht of leeftijd en geslacht het effect van deze genetische variant op cognitieve domeinen beïnvloedt. Dragers van ten minste één APOE*4 allel hadden een verminderde cognitieve prestatie t.o.v. niet-dragers wat betreft korte termijn geheugen, mentale flexibiliteit en vatbaarheid voor afleiding. Deze effecten werden beïnvloed door zowel leeftijd als geslacht, daar wij zagen dat het effect sterker was bij hen die 50 jaar of ouder waren, alsmede bij vrouwen. De significante associaties tussen het APOE*4 allel en geheugen en uitvoerende functies kunnen erop wijzen dat deze cognitieve testen mogelijk markers zijn van vroege veranderingen in cognitie.

In **hoofdstuk 6** wordt de associatie tussen zowel bloeddruk, atherosclerose en polymorphismen in het angiotensinogeen (*AGT*) gen, het angiotensine II type 1 receptor (*AT1R*) gen, het α-adducin 1 (*AAD1*) gen en het G-protein β-3 subunit (*GNβ3*) gen in relatie tot cognitieve functie bestudeerd. Wij vonden een negatief effect van bloeddruk en atherosclerose op cognitieve tests, vnl. op die tests die uitvoerende functies evalueren. Het effect van de *AGT* M235T, *AT1R* C573T, *AAD1* G460W en *GNβ-3* rs2301339 polymorphismen op cognitieve tests werd ook bestudeerd. Er was een significante associatie tussen het *AGT* 235T allel en een betere prestatie bij herkenning. In tegenstelling werd een verminderde cognitieve prestatie in phonemische vloeiendheid bij dragers van het *AAD1* 460W allel gevonden. Daarnaast observeerden wij bij dragers van het *GNβ3* G allel een

slechtere prestatie in tests die de vatbaarheid voor afleiding beoordelen. Ook werd het effect van een combinatie van de risico-allelen van deze vier genen geëvalueerd. Het was daarbij interessant dat een verslechterde prestatie bij alle cognitieve tests werd waargenomen bij een toenemend aantal risico allelen, alhoewel deze bevinding alleen voor leervermogen en vatbaarheid voor afleiding significant was. Er waren geen grote verschillen in analyses in verschillende leeftijdsgroepen. Onze resultaten bevestigen de grote rol van vasculaire factoren bij verminderde prestatie op cognitief gebied. Voorts dragen genen die variatie in bloeddruk beïnvloeden ook bij aan de variatie in cognitie. De associatie van het *AGT* 235T allel en verbeterde herkenning duidt mogelijk op de pleiotrope effecten op de hersenen die angiotensinogeen zou kunnen hebben.

De resultaten van dit proefschrift benadrukken het belang van de GRIP populatie als een krachtige opzet om genetisch onderzoek te verrichten. Toekomstige studies van cognitieve functie binnen de ERF studie zijn ook veelbelovend.

La enfermedad de Alzheimer es la primera causa de demencia en sociedades occidentales afectando aproximadamente un 30% de la poblacion mayor de 90 años de edad. A pesar del enorme esfuerzo de la comunidad científica para identificar el component genético de de la enfermedad de Alzheimer, los genes responsables de las formas más comunes esta patología no han sido completamente determinados. Fenotipos intermedios de la enfermedad de Alzheimer (endofenotipos) podrían ayudar en la identificación de determinantes genéticos y no genéticos de este desorden neurodegenerativo. La función cognitiva, entre otros, es un potencial endofenotipo de la enfermedad de Alzheimer. Esta tesis presenta estudios realizados para identificar determinantes genéticos de la función cognitiva en una poblacion genéticamente aislada en el suroeste de Holanda. Este trabajo hace parte del programa llamado "Genetic Research in Isolated Populations (GRIP)".

En el **capítulo 1,** una revisión de la literatura acerca de la genética de la enfermedad de Alzheimer y la enfermedad de Parkinson es presentada a la luz de las hipótesis mas aceptadas acerca de la variación genética de las enfermedades complejas. Además, algunas estrategias para aumentar el poder de los estudios genéticos de enfermedades neurodegenerativas es delineada.

En el **capítulo 2,** el objetivo general de esta tesis es presentado.

En el **capítulo 3,** los efectos de deriva genética (genetic drift/ founder effects) en la poblacion de GRIP fueron investigados. Para ello, una familia extendida que incluyó 9800 individuos fue usada para simular los efectos de deriva genética. En este estudio se observó que las frecuencias alélicas iguales a 1% o menores, están sujetas a deriva genética, con una alta probabilidad de ser eliminadas de la población. Para confirmar los resultados del estudio de simulación, la Heterozigosidad Esperada (EH) y la diversidad alélica fueron estimados usando 592 microsatelites (short-tanden repeats) en una muestra de 58 personas de la población de GRIP. Estos parametros (EH y numero promedio de alelos) fueron comparados con los obtenidos de poblaciones con una historia demográfica diferente: familias CEPH (muestra de una población general), familias de Islandia (representativas de una población genéticamente aislada y antigua) y familias de la población de Talana (representativas de una población aislada sometida a gran deriva genética). En este estudio se observó que la EH y numero promedio de alelos en la población de GRIP es menor respecto de las familias de CEPH y Islandia, pero mayor que en la población de Talana. Nosotros concluimos que ha habido fluctuaciones en el acervo genético de la población de GRIP debido a deriva genética, pero que estas fluctuaciones solo tienen impacto en variantes alélicas que son raras en la población (frequencias ≤ 1%).

En esta tesis, la genética de pruebas cognitivas (potentiales endofenotipos para la enfermedad de Alzheimer) fue investidada. **En el capítulo 4**, la heredabilidad

(heritability) de dominios cognitivos específicos fue estimada en participantes del estudio de ERF (Erasmus Rucphen family-ERF) en la población de GRIP. Además, los effectos de consaguidad (inbreeding) y la heredabilidad específica de sexo y edad en la variación de la función cognitiva de los participantes de ERF fueron estimados. La heredabilidad estimada fluctuó entre 10 a 30% en pruebas cognitivas relacionadas con memoria, mientras que los estimados de heredabilidad para las pruebas relacionadas con función ejecutiva fue en promedio 36%. Además, se observo que el grado de consaguinidad entre los padres de un individuo esta asociado a un menor rendimiento en pruebas cognitivas que evaluan función ejecutiva y habilidades visoespaciales. Este análisis también mostró que la expresión de genes específica por edad, podría explicar differencias en "rapidez psicomotora" y susceptibilidad a la interferencia". Por otro lado, no encontramos evidencia de que las diferencias en la variación en función cognitiva entre mujeres y hombres sea explicada por differencias en la expression de genes. Los resultados sugieren que la magnitud de la expresión de genes que modulan cognición varía de acuerdo a dominios especificos. Además, las asociación de consaguinidad con ciertos dominios cognitivos parece indicar que parte del material genético que modula cognición esta constituido por variantes que actuan de manera recesiva.

En el **capítulo 5**, la relación entre el gen que codifica para la apolipoproteina E (APOE) y función cognitiva fue investigada, para examinar el perfil cognitivo asociado con el alelo APOE*4. También investigamos si la edad y el género modulan los efectos de este variante genético en determinados dominios cognitivos. Se observó que personas con una o dos copias del alelo APOE*4 presentaron un menor rendimiento en las pruebas cognitivas que evaluan "memoria de corto plazo", "flexibilidad cognitiva" y "susceptibilidad a la interferencia" respecto de las personas que no son portadoras del alelo APOE*4. Estos efectos son modulados por edad y sexo, dado que el efecto fue significativo únicamente en el grupo de sujetos mayores de 50 años o en mujeres. Estos efectos estadísticamente significativos entre el variante APOE*4 y función cognitiva sugieren que estas pruebas cognitivas podrían ser macadores tempranos de deterioro cognitivo asociado con la enfermedad de Alzheimer.

En el **capítulo 6**, la asociación entre presion arterial ateroesclerosis y polimorfismos en los genes angiotensinogeno (*AGT*), receptor tipo 1 de la angiotensina II (*AT1R*), alfa aducina 1(*AAD1)* y subunidad 3 de la proteína G (*GNβ3*) fueron evaluados en relación con la función cognitiva. Se observaron effectos adversos de la presión arterial (sistólica y diastólica) sobre función cognitiva particularmente en las pruebas que evaluan función ejecutiva. Además, se evaluó el efecto sobre la función cognitiva de los siguientes polimorfismos: *AGT* M235T, *AT1R* C535T *AAD1* G460W y *GNβ3* rs2301339. Encontramos una asociación significativa

entre el alelo T del gen *AGT* con un mejor rendimiento en la prueba cognitiva que evalua "reconocimiento". Por otro lado, observamos un menor rendimiento en la prueba que evalua "fluencia fonologica" en portadores del alelo 460W del gen *AAD1,* al igual que menor rendimiento la prueba que evalua "susceptibilidad a la interferencia" en portadores del alelo G del gen *GNβ3*. También estudiamos el effecto combinado de los alelos de riesgo de los cuatro genes asociados con presion arterial sobre la función cognitiva. Un menor rendimiento fue observado en todas las pruebas cognitivas por aumento en el numero de alelos de riesgo, aunque solo se alcanzó significancia estadística en las pruebas que evaluan "aprendizaje" y "susceptibilidad a la interferencia". No se observó ninguna diferencia en el análisis estratificado por edad. Nuestros resultados confirman el efecto substancial de factores vaculares en la disminución de la función cognitiva. Además, los genes que influencian presión arterial contribuyen también a la variación en la función cognitiva. La asociación del gen de *AGT* con un mejor rendimiento en la prueba que evalua "reconocimiento" sugiere que este gen podría tener efectos pleiotrópicos en el cerebro.

Los resultados de esta tesis subrayan el valor de la poblacion de GRIP para realizar estudios genéticos. Futuros estudios en el contexto del estudio de ERF son igualmente prometedores en la busqueda de genes involucrados en la función cognitiva y la enfermedad de Alzheimer.

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CURRICULUM VITAE

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