The overall objectives of this thesis were threefold; (I) to gain better insight into the phenotypic heterogeneity of Alzheimer’s disease (AD) by focusing on the atypical language variant of AD; (II) to identify the effect of genetic variants involved in AD pathogenesis; and (III) to investigate the predictive value of a polygenic risk score (PGS), and its association with different AD endophenotypes in the mild cognitive impairment (MCI) stage of AD. This chapter summarizes and discusses the main findings of the studies described in this thesis. The chapter closes with clinical implications and future directions.

SUMMARY

In the first part of this thesis we focused on the logopenic variant of primary progressive aphasia (lvPPA), which is associated with underlying AD pathology. In chapter 2.1, we set out to study whether lvPPA is indeed the prototypic language profile in 22 PPA patients with biomarker evidence of underlying AD pathology. Furthermore, we aimed to correlate the language profiles with cortical atrophy patterns on MRI. Based on the evaluation of language testing results, we identified three subgroups: (I) lvPPA (n=9 (41%) patients who fulfilled lvPPA core criteria), (II) lvPPA extended (n=8 (36%) patients who fulfilled core criteria plus other language disturbances), and (III) PPA unclassifiable (n=5 (23%) patients who did not fulfill lvPPA core criteria). By visual rating and quantitative analysis of cerebral atrophy patterns we found that the patients with lvPPA showed mild global atrophy, and that the patients with lvPPA extended showed more focal cortical atrophy, predominantly at the left tempo-parietal side. These findings show that the clinical and radiological spectrum of PPA due to underlying AD pathology is broader than pure lvPPA, and support the additional use of biomarkers in PPA patients with atypical, non-classifiable language presentations.

In the second part of this thesis, we focus on the genetic complexity of AD in both familial and sporadic AD. In chapter 3.1, we aimed to detect the underlying genetic cause of AD in a clinically heterogeneous AD family with an assumed autosomal dominant inheritance pattern. Whole exome sequencing was performed on DNA from three family members affected with (preclinical) AD, and one healthy relative. We also determined Apolipoprotein E (APOE) genotype. We detected a rare missense variant in the Sortilin-related receptor (SORL1) gene, p.N674S, which in a heterozygous state segregated with disease. Sanger sequencing was used to confirm the SORL1 variant, and to detect segregation patterns in one additional affected and six unaffected family members. A combination of the SORL1 variant with APOE-ε4 homozygosity occurred in all four affected family members with (preclinical) AD, and in one younger family member who currently has no signs of cognitive decline. Three healthy relatives carried the SORL1 variant but were heterozygous for the APOE-ε4 allele. Even though all affected family members presented with a relatively late age of clinical symptom onset,
SUMMARY AND GENERAL DISCUSSION

the presence or absence of microbleeds/cerebral amyloid angiopathy (CAA) and electroencephalographic (EEG) abnormalities differed for each individual, and could not be consistently associated with \textit{APOE-ε4} homozygosity and/or carriership of the \textit{SORL1} variant. Our findings suggest that the combination of \textit{APOE-ε4} homozygosity and a rare \textit{SORL1} variant might lead to AD. Such an oligogenic disease model may also explain other genetically unexplained AD families.

Genome wide association studies (GWAS) have identified several genetic risk loci, or single nucleotide polymorphisms (SNPs), that associate with increased risk for AD. In chapter 3.2, we studied the association between 19 \textit{SORL1} SNPs and six AD endophenotypes: two cognitive tests, three cerebrospinal fluid (CSF) biomarkers (amyloid-beta (Aβ), total tau (tau), and tau phosphorylated at threonine 181 (ptau)) and hippocampal atrophy in a sample of 668 AD patients from three different data sets. We found an association between the G allele of \textit{SORL1} SNP rs2070045 and two markers of neuronal injury and neurodegeneration, i.e. high CSF levels of tau and hippocampal atrophy. Additionally, we found an association between a three SNP haplotype and higher CSF levels of tau and ptau. Our study not only confirmed the relationship between \textit{SORL1} and AD endophenotypes as in other studies, but also suggests that \textit{SORL1} variants may be implicated in the downstream pathological pathway of AD.

In chapter 4, we investigated whether a combination of 18 genetic variants associated with AD risk could predict disease progression and associated with AD endophenotypes in patients with MCI. For this we calculated a polygenic risk score (PGS), deliberately not including the variants in the \textit{APOE} gene. In chapter 4.1, we investigated the predictive value of these 18 genetic risk variants plus \textit{APOE}, both individually as well as combined on the progression from MCI to AD dementia. We selected 3216 MCI patients from four independent datasets. By single risk variant analysis, we not only confirmed \textit{APOE-ε4} as a strong risk factor for MCI to AD dementia progression, we also identified the SNP rs9331888 in the Clusterin (\textit{CLU}) locus as an independent risk factor. A PGS comprising nine established genome-wide AD risk loci, but without the \textit{APOE} loci, showed a small effect on the risk of MCI to AD dementia progression. However, a PGS comprising nine AD loci recently reported by the International Genomics of Alzheimer’s Project (IGAP) was not implicated in MCI to AD dementia progression, neither was the PGS comprising all 18 AD genetic risk variants. These findings confirm the poor predictive value of the current genome-wide AD risk loci for MCI to AD dementia progression, both individually as well as combined. One exception was the \textit{CLU} locus, which suggests a possible use of its protein product as a marker for MCI to AD dementia progression.

In chapter 4.2, we evaluated the effect of genetic variants associated with AD risk on endophenotypes closely related with AD pathology in patients with MCI. For 1730
CHAPTER 5

MCI patients we analyzed the association between non-\textit{APOE} PGS and five AD endophenotypes, including tests measuring cognitive decline over time (mini mental state examination (MMSE)) and cross-sectional CSF levels of $\text{A}\beta$, tau, and ptau. We detected a modest association between PGS and cognitive decline over time as measured by repeated MMSE, and a more consistent association with higher CSF levels of tau and ptau. Decreasing MMSE scores and increased levels of CSF tau and ptau may be considered as aspecific markers of neurodegeneration. Therefore, these findings suggest a combined effect of AD associated genetic risk variants on neuronal degeneration in general, rather than with Alzheimer-related amyloid pathology specifically.

GENERAL DISCUSSION

\textbf{Sortilin-related receptor (SORL1)}

In all four genetic studies included in this thesis, we detected an involvement of \textit{SORL1} variants in AD pathogenesis. Over the past years, genetic association studies attempting to link \textit{SORL1} with AD showed conflicting results. Despite the fact that several candidate gene studies support the association between \textit{SORL1} SNPs and increased risk on AD,\textsuperscript{10,11,15–19} other studies found no associations at all.\textsuperscript{20–23} In the early GWAS, common \textit{SORL1} SNPs showed absent or nominal association with AD risk,\textsuperscript{3,7,24,25} but in the recent large IGAP meta-GWAS risk loci in \textit{SORL1} were more strongly associated with AD risk.\textsuperscript{26} In this thesis, we show that common \textit{SORL1} risk variants associated with AD endophenotypes (\textit{chapter 3.2} and \textit{4.2}), and we found a modest association between a \textit{SORL1} SNP and progression of MCI to AD dementia (\textit{chapter 4.1}). Moreover, we found a co-occurrence of a rare \textit{SORL1} variant with \textit{APOE}-e4 in an AD family (\textit{chapter 3.1}). Three other recent studies support our findings: one study demonstrated an enrichment of rare coding \textit{SORL1} variants in patients with late-onset AD,\textsuperscript{27} a second study associated rare \textit{SORL1} variants with autosomal dominant inheritance patterns of (early onset) AD,\textsuperscript{28} and a third study showed an enrichment of \textit{SORL1} variants in both patients with sporadic and familial early onset AD.\textsuperscript{29} \textit{SORL1} is located on chromosome 11 and codes for the protein SorLA or LR11, which belongs to the vacuolar protein sorting (VPS)-10 family of sorting receptors, and to the low density lipoprotein receptor (LDLR) gene family.\textsuperscript{30} SorLA exhibits different functions, including (I) binding the amyloid precursor protein (APP) to the VPS-10 domain in the trans-Golgi network thereby preventing the secretase cleavage of APP into $\text{A}\beta$; (II) sorting APP to the different cellular compartments during its intracellular trafficking from the Golgi compartment to the late endosome\textsuperscript{15,31}; (III) lysosomal targeting and degradation of newly synthesized A$\beta$ by binding A$\beta$ to the VPS-10 domain\textsuperscript{32}; and (IV) acting as a neuronal LDL endocytic receptor for multiple ligands, resulting in uptake of ApoE rich lipoproteins and the uptake of extracellular A$\beta$ for transport to the lysosome for degradation.\textsuperscript{33,34}
Three of the variants described in this thesis (i.e. the intronic SNPs rs641120 and rs11218343, and the rare exonic variant p.N674S) are mapped in the VPS-10 domain. Pathogenic variants in this domain might cause disruption of the binding between SorLA and APP, and between SorLA and Aβ. The effect of a SORL1 variant in the VPS-10 domain, p.G511R, was functionally tested in cell culture experiments, which suggested that binding between SorLA and Aβ was disrupted and that intracellular catabolism of Aβ was decreased compared to wildtype SORL1. 32

Methodological considerations

There are several methodological considerations that have to be considered when interpreting the results presented in this thesis.

Study design and sample sizes

In the studies described in this thesis, different study designs were used. For the study investigating PPA patients with AD (chapter 2.1), we used a retrospective design. Amongst the limitations were the use of different type of MRI scanners for neuroimaging, a problem which we partially solved by measuring asymmetric atrophy patterns using FreeSurfer.35 Second, language testing was assessed by the Dutch version of the Aachen aphasia test battery (AAT), 36,37 which was originally designed for aphasia testing in patients with a cerebrovascular accident. Nevertheless, the AAT is a valid, widely used language test, and was applied by a trained speech and language pathologist. However, for future studies the use of an aphasia test specifically validated for application within PPA patients would be preferred. Currently, no such test exists. However, for monitoring of disease progression would the progressive aphasia severity scale (PASS) be usefull.38 A major strength of our study was that we tested PPA classification criteria in a relatively large, homogeneous group of PPA patients with biomarker proven AD pathology.

Three out of the five studies described in this thesis were international, multicenter studies including a substantial number of patients, suggesting a strong generalizability of the results. However, study design and study settings across different cohorts tend to be less homogeneous than in a single center cohort. We attempted to solve this problem by calculating z-scores for the association analyses, thus enabling meta-analyses. In chapter 4.2, we used linear mixed models (LMM) to analyze the effect of PGS on cognitive decline over time. LMM allows the use of all available data points, even when the number of assessments or intervals between assessments differ between individuals. When performing multicenter genetic association studies, the differences in population background of the participating patients may perturb association testing. In the studies performed with the multicenter MCI cohort (chapter 4.1 and 4.2), we aimed to minimize this problem by analyzing each study sample separately, and by computing additional heterogeneity measures ($I^2$) when performing the meta-analysis.
Genetic study in MCI

Two of our studies focus on MCI patients (chapter 4.1 and 4.2). MCI patients are at increased risk of AD dementia, but the syndrome is very heterogeneous with several different underlying pathologies. Whereas some patients may remain stable for many years, others may develop different types of dementia or may even improve to a healthy status. Therefore, carriership of genetic risk variants in patients at the predementia stage of disease may influence the disease course of each MCI patient. We envision that tools, based upon genetic profiles, that accurately predict which MCI case will develop AD dementia will be of the utmost importance for treatment options in the future. In two studies (chapter 4.1 and 4.2), we investigated the association of carriership of AD risk variants and the course of disease in MCI patients. For this we used genetic variants that were previously identified by a GWAS comparing AD patients with healthy controls. These genetic variants differed from the variants identified by a GWAS testing the association between SNPs and clinical decline in MCI patients. This may suggest that the genetic risk variants associated with the development of, or the disease progression in MCI do not overlap with the variants associated with the development of AD. This assumption is supported by a study which investigated the relation of a PGS from a set of risk variants associated with various neurodegenerative diseases (AD, Parkinson’s disease, fronto-temporal dementia-amyotrophic lateral sclerosis (FTD-ALS)) with MCI and the subsequent progression to dementia. In this study, the authors found that all three different PGS associated with presence of non-amnestic MCI at baseline, whereas only the AD-PGS associated with incident dementia, an association most pronounced for the conversion from MCI to AD dementia. Moreover, when the authors examined the risk variants separately, they found that some risk variants differentially contributed to initiating MCI than to conversion to dementia, suggesting that different genetic factors differentially drive these processes.

Endophenotypes

Genetic variants may explicitly affect specific endophenotypes underlying AD. Therefore, testing the association of genetic risk variants with specific AD endophenotypes may enhance the statistical power of detecting a heritable trait underlying AD risk. Furthermore, making an accurate clinical diagnosis can be quite a challenge, which may perturb linkage of genes to specific diseases. In this context, endophenotypes can be of great help to unravel the pathogenic effect of the AD risk variants. In our study, we found an association of a subset of AD risk variants with two non-amyloid AD endophenotypes (chapter 4.2). We hypothesize that these variants may influence non-amyloid events underlying AD, such as the accumulation of tau pathology and neuronal cell loss. However, we cannot exclude that other genetic variants may exert their effects on the amyloidogenic pathway during the earlier pre-MCI stage. Previously, novel genetic variants were associated with AD by using endophenotypes, such as
neuroimaging phenotypes and CSF levels of tau and ptau, as quantitative traits in a GWAS. The advantage of using endophenotypes over case-control studies, is that an association of an endophenotype predicts a specific biological process underlying a pathogenic effect, which might be directly tested.

**Selection of GWAS SNPs**

By design, the SNPs identified by GWAS relate to genomic regions, and are named after their closest genes. Therefore, it is not always possible to accurately determine which gene or variant is driving the association identified by GWAS. Additionally, due to the hypothesis-free design of GWAS, for many SNPs it remains unknown if they are actually involved in the pathophysiology of disease by influencing gene function or expression, or if they flag an unknown true risk factor in linkage disequilibrium (i.e. non-random association of alleles at two or more loci). One of these regions encompasses SORL1, and several SNPs in SORL1 associated with increased AD risk in a large IGAP meta-GWAS, an association replicated in different candidate gene studies. In order to learn more about the association between the variants in SORL1 and AD pathophysiology, we selected 19 SORL1 SNPs and performed a genetic association study (chapter 3.2).

In chapter 4, we describe the use of a PGS. The selection of SNPs, which were combined in one PGS, was based on the AD genetic risk loci displayed in the GWAS catalogue and/or in the large published GWAS (www.alzgene.org). In addition, for each selected loci we included a proxy SNP as backup. Unfortunately, due to technical problems, not every pre-selected SNP was adequately genotyped in the different datasets. Therefore, we cannot rule out the possibility that those missing SNPs may have affected the predictive value of the PGS on MCI to AD progression, or its usability for genotype-endophenotype association testing.

**Multiple testing**

In our three association studies (chapter 3.2, 4.1 and 4.2), we made use of several genetic risk loci identified as AD risk loci by GWAS. We used Bonferroni for multiple testing correction, which may guard against false positive findings, but is also suggested to be over-conservative. Alternatively, in chapter 3.2, we corrected for multiple testing by applying the false discovery rate (FDR). The FDR controls for the expected proportion of false positives among significant associations, and is suggested to be less stringent than the Bonferroni method.

**Strong effect of APOE-ε4**

The APOE-ε4 allele is a well-established risk factor for sporadic AD. Even though the ε4 allele exert a large effect on AD risk, a large proportion of AD patients do not carry an APOE-ε4 allele, and not all APOE-ε4 carriers develop AD. Together, this suggests that the involvement of other genes may be necessary for initiation of AD.
one AD family with many homozygous \textit{APOE-}\textepsilon4 \textit{carriers}, we detected a rare \textit{SORL1} variant, which could be considered such an additional genetic risk factor (\textit{chapter 3.1}). In the study, we mention several reasons for pathogenicity of the \textit{SORL1} variant to support our assumption that AD in this family may be caused by the combined effect of \textit{APOE-}\textepsilon4 homozygosity and the effect of the rare \textit{SORL1} variant. However, this assumption requires confirmation by a functional assay.

When performing genetic association analysis between AD risk variants and intermediate phenotypes, the strong relation between \textit{APOE-}\textepsilon4 and AD pathophysiology may mask the truly small effects of other AD genetic risk loci. Actually, evidence for this assumption has been shown by previous studies. In three studies, the associations detected between PGS and AD risk, or between PGS and AD endophenotypes, lost its significance after excluding the \textit{APOE} region from the PGS.47–49 Therefore, we deliberately constructed a PGS without the \textit{APOE} region. Additionally, we performed a univariate analysis to confirm the association between \textit{APOE-}\textepsilon4 and AD risk, and between \textit{APOE-}\textepsilon4 and endophenotypes.

**Clinical implications and future perspectives**

The link with the clinic is most easily made for the classification of PPA with evidence of underlying AD pathology (\textit{chapter 2.1}). Our study contributes to the ongoing debate about the clinical classification criteria for the PPA subtypes, and for the lvPPA subtype in specific. The study supports our clinical experience that PPA patients with underlying AD pathology may present with a heterogeneous language profile. The best strategy to define the heterogeneity of the language profile in more detail, is to assess an extensive language assessment. Additionally, the progression of disease may be monitored by using a test such as the PASS.38 Furthermore, if the clinical diagnosis remains uncertain, additional use of biomarkers like CSF analysis or PET imaging may help in adequate prediction of underlying pathology, with its importance for prognosis and trial selection.

The findings of the second part of this thesis are less easily translated to the clinic. Currently, due to the small risk estimates of the genetic variants there is no need for predictive nor for diagnostic testing of these loci in everyday clinical practice. However, multidisciplinary research with input from both clinicians and researchers is much needed, so that accurate clinical classification of the different AD phenotypes, and endophenotypes can be optimally combined with biochemical measurements and genetic tests.

In general, the use of genetic risk variants lies primarily in better understanding of the molecular pathways involved. Many years ago, identification of the increased \(\text{A}\beta\) accumulation caused by autosomal dominant mutations in \textit{APP}, \textit{PSEN1}, \textit{PSEN2} led to the development of the amyloid cascade hypothesis.50 More recently, biomarker research on pre-symptomatic autosomal dominant mutation carriers showed that
biomarker changes may occur 15-20 years prior to the expected age at onset of AD.\textsuperscript{51,52} Furthermore, the newly identified genetic risk variants by GWAS and exome sequencing studies suggest the involvement of several non-amyloidogenic pathways in AD pathogenesis; for example involvement of tau processing pathways, immune response and inflammation, lipid transport and endocytosis, and cytoskeletal function and axonal transport.\textsuperscript{6,26,53} The knowledge of such additional pathways involved in AD pathogenesis may lead to the development of novel biomarkers for diagnosing and monitoring of disease, such as specific markers of inflammation or immune response. In the future, whole genome sequencing will enable the confirmation of the associated common risk variants, and the detection of novel common and rare variants with intermediate and large effects on AD pathogenesis. Hypothetically, the accumulation of these rare risk variants combined with the common risk variants may cross the susceptibility threshold for AD. More research is warranted on this complex process of collaboration of several different genetic risk variants in the production of one phenotypic disease. In case of AD pathogenesis, it seems that not a single process is responsible for all pathology across its various stages, but that different processes might either predispose to, initiate, or promote the disease.\textsuperscript{40} Further characterization of the molecular pathways involved in AD pathogenesis by linking the variants with AD endophenotypes will potentially provide targets for effective and personalized medical treatment.
REFERENCES


SUMMARY AND GENERAL DISCUSSION


41. Shen L, Kim S, Risacher SL, et al. Whole genome association study of brain-wide...


