Chapter 1

INTRODUCTION

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Mr. J., 56 years of age, presents at the VUmc Alzheimer center with progressive word-finding difficulties. The problems had started three years ago during a stressful period at his work, and had initially been attributed to a burn-out. Nevertheless, after toning down his responsibilities at work, the word-finding difficulties persisted and slowly progressed. Over time, he endured more problems at work due to his disturbed speech with frequent word pauses. The last few months, his wife also noticed that he forgets appointments and he once mixed up two important rental payments. The mother of the patient had Alzheimer's disease with symptom onset at 82 years of age. The patient and his wife are anxious that he might become demented, and that also their children might be at risk. After additional investigations at the Alzheimer center,[1] Mr. J. is diagnosed with Alzheimer's disease with predominant language disturbances.

Alzheimer's disease (AD) is the most common cause of dementia, and typically affects patients at an older age with a gradual decline in cognitive function. In general, patients present with deterioration in memory, and as the disease progresses, disturbances in other cognitive domains such as executive function, praxis, language and visuoperception occur.[2] Eventually, the cognitive dysfunctions are severe enough to interfere with daily living. The neuropathological hallmarks of AD are extraneuronal plaques consisting of deposition of the amyloid-beta (Aβ) protein, and intraneuronal neurofibrillary tangles consisting of hyperphosphorylated tau (ptau) protein.[3] The main theory underlying AD pathophysiology is the amyloid cascade hypothesis, which posits that changes in Aβ homeostasis lead to the formation of amyloid plaques and tau tangles, which causes neuronal cell and synapse loss.[4] Eventually, this process of neurodegeneration results in cognitive dysfunction.

AD dementia may be preceded by a prodromal phase called mild cognitive impairment (MCI). MCI is a syndrome characterized by the impairment of one or more cognitive domains, but without interference with normal daily activities.[5] Although MCI is a heterogeneous disorder, within ten years about half of the patients progress to AD dementia.[6]

Phenotypic heterogeneity

The case of Mr. J. illustrates the clinical heterogeneity of AD. Patients with an atypical variant of AD may present a heterogeneous cognitive profile with an early age of symptom onset, defined by an age below the arbitrary age of 65 years. In the initial phase of disease, patients experience predominant disturbances in language, praxis or visuoperception, while memory function is relatively spared.[7] Since 2011, the clinical criteria for probable AD dementia acknowledge three so-called non-memory presentations[8]: (I) the logopenic variant of primary progressive aphasia (lvPPA); (II)

the posterior cortical atrophy (PCA); and (III) the dysexecutive variant of AD.[9–12] The clinical profile of Mr. J. fits such an atypical variant, particularly the language variant of AD; lvPPA. The umbrella term primary progressive aphasia (PPA) refers to a slowly progressive, and initially isolated language disorder of a neurodegenerative nature, and includes three subtypes; (I) semantic variant; (II) non-fluent or agrammatic variant, and (III) logopenic variant. Whereas the first two variants are usually associated with underlying fronto-temporal lobe degeneration (FTLD), the logopenic variant is mainly associated with underlying AD pathology. However, non-classifiable PPA patients with underlying AD pathology have been described, as well as lvPPA cases without biomarker evidence of underlying AD pathology.[13,14] These inconsistent findings support our clinical observation that the language profiles of PPA in AD may be quite heterogeneous, which makes the diagnosis of this PPA variant in AD often difficult to establish.

Genetic background of AD

Many patients like Mr. J. are concerned that they have inherited AD and that they may in turn pass it on to their children. However, AD is a complex and heterogeneous disease, not only from the clinical point of view, but also from the genetic point of view. Genetic variants play an important role in the development of AD, but the effect on disease progression differs for each variant, and its interplay with an unique genetic background may differ for each individual. Approximately 10% of patients with early onset AD have familiar AD.[15] These families possess a monogenic, fully penetrant gene mutation that underlies AD development. These mutations, and thus the AD phenotype, are inherited from parent to child, in a Mendelian, autosomal dominant inheritance pattern. By linkage analysis, autosomal dominant mutations that cause familiar AD have been identified in three genes: the amyloid precursor protein (*APP*) gene, the presenilin 1 (*PSEN1*) gene, or the presenilin 2 (*PSEN2*) gene.[16–18] All these mutations alter Aβ processing, affirming the involvement of Aβ processing in AD, and these genetic findings were at the basis of the formulation of the amyloid hypothesis. Nothwithstanding, in a French longitudinal cohort following a large group of families with early onset AD, 23% of the families with an autosomal dominant inheritance pattern did not harbor such a causative mutation, suggesting that other, yet to be discovered, genes are also involved in AD.[19]

The majority of patients presents with the sporadic form of AD, with a late onset of disease. Often, the pattern of inheritance is not straightforward and is most likely caused by the combination of several genetic and environmental factors. Twin studies predicted the heritability (i.e. the estimated variation due to genetic variation among individuals) of sporadic late-onset forms to be approximately 80%, explaining the high prevalence of familial late onset AD.[20] For many years, just one genetic risk factor for sporadic AD was firmly implicated; the ε4 allele of the Apolipoprotein gene (*APOE*-ε4).[21] The

exact mechanism behind the detrimental effect of *APOE*-ε4 on dementia risk remains unknown. Patients with one *APOE*-ε4 allele have a threefold increased risk of AD, carriers of two ε4 alleles even a 11 till 15 fold increased risk.[22] Even though *APOE*-ε4 homozygosity is a major risk factor for late onset AD, only 50% of individuals with AD carry an *APOE*-ε4 allele, and not all *APOE*-ε4 homozgygotes will develop the disease.[23] Therefore, over the last few years several different types of studies have been performed in search of other genetic risk variants involved in the pathogenesis of late onset AD.

Genome wide association studies (GWAS) have been performed with a case-control design, investigating the association between single nucleotide polymorphisms (SNPs) and AD. In GWAS, SNP arrays are used to read millions of genetic variants covering the whole genome from the DNA of ten thousands of patients and controls. If an allele, i.e. one type of a genetic risk variant, was detected far more frequently in the patients than in the controls, the allele was said to be associated with AD. By design, the SNP arrays contain pre-selected and common genetic variants, and the identified risk variants mark a genomic region, not a specific gene.
Up till now, by using GWAS and meta-GWAS more than 20 common genetic risk variants of AD have been identified.[24–29] However, most of these associated common genetic risk loci only have a small effect on AD risk, leaving a large proportion of the genetic heritability unexplained.[30] By design, low-frequency and rare variants associated with AD risk remain undetected by GWAS, even though they could exert a large effect on AD pathogenesis. Novel techniques like next generation sequencing, enabled the identification of such variants. By whole genome sequencing the entire genome is captured and by whole exome sequencing the focus lies on the protein coding part of the DNA, the exome. By sequencing and subsequent comparison of the sequences, we may identify novel causal mutations in Mendelian disorders, but also the involvement of rare variants with intermediate to large effect in complex non-Mendelian disorders. Important discoveries made by whole genome and exome sequencing of large patient and control groups were the identification of rare coding variants in *TREM2*, and loss-of-function variants in *ABCA7*, both associated with increased risk for AD,[31–33] and identification of a rare coding variant in *APP* with a protective effect against AD and cognitive decline in elderly without AD.[34]
The schematic overview in figure one shows the relation between risk allele frequency and strength of genetic effect for some of the studies described in this thesis.

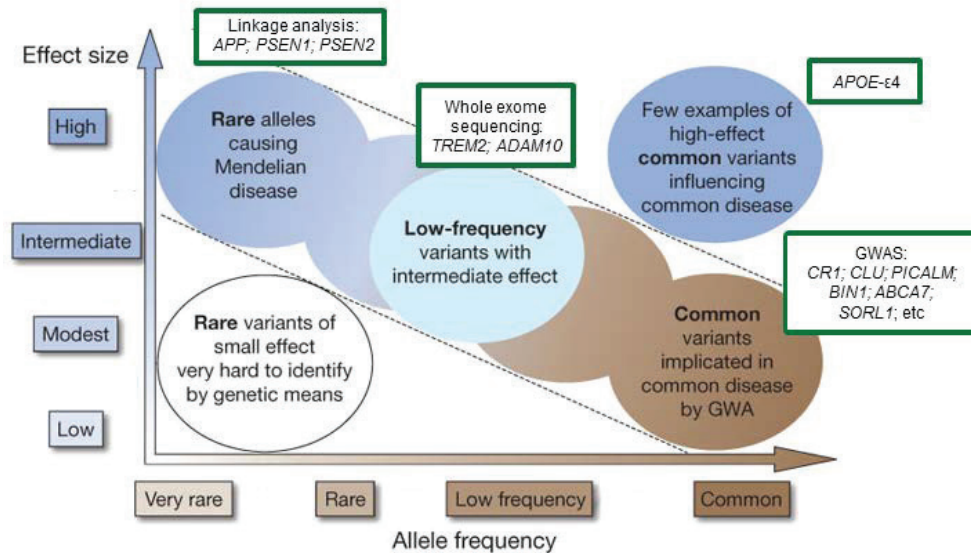**Figure 1.** Overview of different types of genetic risk variants associated with Alzheimer's disease.

Figure adapted after the original figure by Manolio et al, Nature 2009.[30]

Endophenotypes of Alzheimer's disease

The identification of novel genetic risk loci has increased our understanding of the molecular pathways involved in AD pathogenesis, like the involvement of the tau processing pathway or lipid transport and endocytosis.[35] One way to learn more about the involvement of both amyloidgenic and non-amyloidgenic pathways is to investigate the association between the risk variants and the different endophenotypes of AD. Endophenotypes are intermediate phenotypes, which lie more closely to the effects of genetic variation than dichotomized affection status alone. Because the endophenotypes more directly interact with the genetic risk variance, they provide more statistical power to detect small genetic effects on AD pathogenesis. Usable endophenotypes of AD are cerebrospinal fluid (CSF) biomarkers (Aβ, tau, ptau), measurements of hippocampal atrophy on MRI, and neuropsychological test results. By testing the association between these AD endophenotypes and the genetic risk loci we may learn when, and where in the pathological cascade of events the genetic risk variants exert their influence.

Polygenic risk score

Even though in the past years more and more genetic risk variants have been identified, each of these variants individually confers only a small effect on disease risk.[30] Therefore, the clinical usability of these risk variants remains low. In general, the common SNPs identified by GWAS alter AD risk by a modest 10-15%. By combining these risk variants with their individual small effect sizes into one cumulative polygenic

risk score (PGS), we may improve identification of their effect on AD risk.[36] Previous studies have investigated the usability of PGS in the prediction of progression from the predementia MCI stage to AD dementia. The studies generated conflicting results because the predictive capability of the PGS resulted to be almost completely driven by *APOE*-ɛ4 alone, or because the association behaved differently in amnestic-MCI compared to non-amnestic MCI.[37,38]

A complementary approach assesses the relationship between PGS and AD endophenotypes, which will further identify the genetic risk variants underlying the perturbation of the molecular mechanisms that lead to specific measurable alterations in the AD biomarkers. In AD patients, previous studies showed that CSF biomarkers and memory associated with AD risk variants joined in one PGS (without the *APOE* region, i.e. non-*APOE* PGS).[39–41] In MCI patients, little research has been performed on this subject. More understanding of the genetic factors influencing the pathological pathways at the MCI stage may eventually improve the prognostic accuracy and the development of effective treatment in the predementia stage of disease.

AIMS OF THIS THESIS

This thesis focuses on the association of genetic risk variants with several AD endophenotypes. The main aims of this thesis are threefold:

(I) To gain better insight into the heterogeneous nature of clinical AD phenotype, by focusing on the applicability of clinical consensus criteria for the language variant of AD, i.e. the logopenic variant of primary progressive aphasia.
(II) To identify specific genetic risk variants and their association with the clinical presentation of AD in both familial and sporadic AD.
(III) To investigate the predictive value of the polygenic risk score in progression from MCI to AD dementia, and the association of the polygenic risk score with AD endophenotypes in patients with MCI.

THESIS OUTLINE

The first aim is addressed in **chapter 2**, where we evaluated the different language profiles and cerebral atrophy patterns present in a group of PPA patients with proven underlying AD pathology. **Chapter 3** covers the second aim of this thesis. In **chapter 3.1**, we describe a clinically heterogeneous AD family with an assumed autosomal dominant inheritance pattern of AD, in which we detected a rare genetic risk variant in the Sortilin-related receptor (*SORL1)* gene co-occurring with *APOE*-ɛ4 homozygosity. In **chapter 3.2**, we investigated the effect of *SORL1* risk variants on different AD endophenotypes in a group of AD patients. The **fourth chapter** is dedicated to the investigation of the usability of a PGS created out of 18 genetic risk variants for AD. In **chapter 4.1**, we test the predictive value of individual AD genetic risk variants and of

the combined PGS on progression from MCI to AD dementia. In **chapter 4.2**, we used different AD endophenotypes in MCI patients to learn more about the combined effect of AD genetic risk variants on AD pathophysiology in the predementia state of disease. In **chapter 5**, the main findings of this thesis are summarized and discussed, with recommendations for future research.

Chapter 1

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