Unraveling the biological mechanisms in Alzheimer's disease — Lessons from genomics

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ABSTRACT

Alzheimer's disease (AD) is the most common form of dementia and the most common neurodegenerative disease, with a complex genetic background. Genome-wide association studies (GWAS) have yielded important new insights into genetic mechanisms of AD pathology. Current results unequivocally confirm apolipoprotein E (APOE) as a major genetic risk factor for development of late onset AD. Additional associations of more than twenty genes have also been identified and replicated in subsequent genetic studies. Despite the exciting new GWAS data which have emerged in the last few years, it has become clear that common variants within the genome cannot fully explain the underlying genetic risk for AD. Novel approaches such as genome-wide analysis of copy number variations (CNV) or low-frequency rare functional gene variants may provide additional insight into genetic basis of AD. In this review we summarize the findings of eighteen GWAS studies in AD performed to date, with an emphasis on potential future developments in the quest for genetic risk factors of AD.

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1. Introduction

Alzheimer's disease (AD) is the most common form of dementia and the most common neurodegenerative disease. The estimated prevalence of the disease in 2006 was approximately 27 million people, with the highest number of patients present in the regions of Asia and Europe (Brookmeyer et al., 2007). The number of patients with dementia is increasing by roughly 4.5 million people annually (Ferri et al., 2005), and is projected to reach more than 115 million by the year 2050 (Prince and Jackson, 2009).

The disease is characterized histopathologically by the accumulation of β-amyloid plaques and neurofibrillary tangles, leading to progressive neuronal and synaptic loss in the limbic and association areas of cortex and in subcortical nuclei (Selkoe, 1991; Chertkow et al., 2001; Tiraboschi et al., 2004; Takashima, 2009). The AD patients exhibit a plethora of symptoms related chiefly to progressive cognitive and functional decline (Waldemar et al., 2007). The pathogenesis of AD still remains not fully elucidated, but deposition of β-amyloid (Aβ) 1–42 derived from amyloid precursor protein (APP), appears to be a key element contributing to oxidative stress, tau pathology, mitochondrial insufficiency and synaptic failure. Aβ, which is the major component of senile plaques, is cleaved from APP first by β-secretase, and then by the γ-secretase.

Genetic background of the disease is heterogeneous and complex, without a straightforward mode of inheritance. The patients can be divided in two major forms of the disease, namely those with an early age of onset, usually below 65 years of age, and patients with the late onset AD (LOAD), typically well beyond 65 years. The patients with

early onset of the disease show Mendelian transmission and are affected by mutations in three genes, which are all involved in the production of Aβ (Tanzi and Bertram, 2005). The mutations affect the APP (Goate et al., 1991), presenilin 1 (PSEN1; Sherrington et al., 1995) and presenlin 2 (PSEN 2; Levy-Lahad et al., 1995; Rogaev et al., 1995) genes, interfering with the normal cleavage of APP by the γ-secretase complex.

Overwhelming majority of AD patients exhibit the late onset of the disease and show less-obvious familial aggregation. Despite strong evidence of heritability (Bergem et al., 1997; Gatz et al., 2006), genetic mechanisms involved in late onset AD have been much more difficult to elucidate. The disease pathophysiology in these patients is most likely linked to a whole set of susceptibility genes affecting various pathways, including those involved in Aβ production, such as SORL1, GAB2 or CH25H (Andersen et al., 2005; Zerbinatti et al., 2008), aggregation, such as CST3 or PRNP (Kaeser et al., 2007; Schwarze-Eicker et al., 2005), and clearance, such as ACE (Bertram and Tanzi, 2009; Sleegers et al., 2010). The role of several other susceptibility genes has also been implicated in other pathophysiological pathways, such as TF, MAPT and GAB2 in oxidative stress (Yamamoto et al., 2002; Ballatore et al., 2007; Nizzari et al., 2007), CHRNB2 in Ach transmission (Oddo and LaFerla, 2006), CR1 and CLU in inflammation damage (Khera and Das, 2009; Zanjani et al., 2005) or PICALM in intracellular trafficking of synaptic vesicle proteins (Harel et al., 2008). Over past several decades, more than 500 genes have been associated with increased risk of AD, mainly by utilizing the candidate gene approach (Bertram and Tanzi, 2008).

Despite the large number of candidate genes, only a few have been reproducibly shown to influence disease risk or onset age (Bertram et al., 2007). Among those genes, the ε4-allele of the apolipoprotein E gene (APOE) has shown the strongest risk effect for the development of AD (Strittmatter et al., 1993; Saunders et al., 1993). APOE is found within senile plaques (Namba et al., 1991), binds Aβ (Strittmatter et al., 1993), may influence neuritic formation of plaques in mouse models of the disease (Holtzman et al., 2000) and is involved in Aβ deposition and clearance in the brain (Holtzman, 2004). Despite the strong evidence for its role in disease pathophysiology, APOE as a genetic risk factor is not fully penetrant, and is neither necessary nor sufficient for the development of AD (Ertekin-Taner, 2010). Hetero-zygous carriers of the ε4 genotype exhibit a two to four times greater AD odds ratio when compared to homozygous ε3 carriers. In homozygous ε4 carriers the odds ratio increases to 6 to 30, as shown in population-based studies in subjects of European origins (Ertekin-Taner, 2007). However, the effect of APOE ε4 seems to be age dependent, and its use as a diagnostic and predictive factor in a clinical setting is not feasible (Knopman et al., 2001).

Deciphering of the human genome and development of high-throughput genomic technologies (Manolio and Collins, 2009) have accelerated the efforts aimed at unraveling of underlying pathophys-iological mechanisms involved in AD. These achievements opened up novel major avenues of scientific effort in research of AD. A new impetus was given to the search for candidate genes associated with increased risk of AD especially through performing genome-wide association studies (GWAS). In this review we give a comprehensive overview of the GWAS studies in AD performed so far, providing a novel overview of several GWAS approaches, from case–control studies, to copy number variations (CNV) analysis to quantitative-trait association studies. Furthermore, we also give emphasis on novel avenues of research, such as genome-wide analysis of CNV or low-frequency rare functional gene variants.

2. Genome-wide association studies in AD

Detailed identification and characterization of single-nucleotide polymorphisms (SNPs) in the human genome (Sachidanandam et al., 2001) and development of novel high-throughput SNP genotyping technologies enabled a more comprehensive insight into the genetic

basis of common, complex diseases. In contrast to candidate gene studies, which do not allow results beyond the scope of the initial hypothesis, GWAS allow for simultaneous testing of a very large number of genetic markers. The studies usually involve analysis of tens of thousands of genetic markers in thousands of individuals, in a mostly hypothesis-free manner (Bertram and Tanzi, 2009). The genetic markers utilized in the GWAS consist of SNPs chosen based on their ability to cover common variation in the human genome (McCarthy et al., 2008). More recent microarray technology also allows for assessment of CNVs, namely deletions or multiplications of genomic DNA at certain chromosomal regions.

Implementation of the GWAS approach has yielded a large number of genome-wide significant and replicated findings in many geneti-cally complex diseases (Ioannidis et al., 2009). However it has been shown that most common variants individually or in combination confer relatively small increments in risk (1.1–1.5-fold) and explain only a small proportion of heritability (Hindorff et al., 2009). GWAS in AD have so far yielded much less reproducible results, when compared to studies of other complex diseases, with the exception of the APOE locus, whose association with AD was identified in all but one study, and always found to be orders of magnitude more significant than any of the newly implicated genes to date (Bertram and Tanzi, 2009). Collective data overview and systematic meta-analysis of the association studies carried out in AD can be found at the AlzGene website. AlzGene represents the most comprehensive electronic database of the genetic association studies published in the field of AD, including GWAS (Bertram et al., 2007). The overview of the late onset AD GWAS results can be found in Table 1.

2.1. The study of Grupe et al. (2007)

The first published GWAS of late onset AD used a select set of more than 17,000 SNPs from 11,211 genes, chosen according to likelihood of being functional polymorphisms (Grupe et al., 2007). In the first stage of the study, genotyping was performed on a screening sample consisting of 380 AD cases and 396 control subjects from UK. Follow-up studies of the promising markers were performed in four independent cohorts from UK and USA, totaling more than 3000 subjects. Among the loci identified, APOE-related SNPs were the only ones to exhibit genome-wide significance. Besides APOE, 16 more loci were identified as nominally significant, namely ACAN, BCR, CTSS, EBF3, FAM63A, GALP, GWA_14q32.13, GWA_7p15.2, LMNA, LOC651924, MYH13, PCK1, PGBD1, TNK1, TRAK1 and UBD. Although none of these replicated in more than two of the five tested samples groups, four SNPs were especially interesting, namely GALP, TNK1, PCK1 and GWA_14q32.13, exhibiting significant p-values across all samples. Subsequent replication studies have shown mixed results regarding confirmation of the identified loci, with GWA_14q32.13, TNK1 and GALP showing the strongest association. Galanine and galanine-like peptides (GALP) have been implicated in inhibition of long term potentiation in the hippocampus, as well in suppression of cholinergic neurotransmission (Lang et al., 2007), and have been shown to be overexpressed in the AD brains. On the other hand, tyrosine kinase, non receptor 1 (TNK1) has been shown to enable tumor necrosis factor α induced necrosis, providing a possible mechanisms for increased neuronal death in AD patients.

2.2. The studies of Coon et al. (2007) & Reiman et al. (2007)

The second study utilized the approach of genotyping more than 500,000 SNPS on the Affymetrix 500K platform (Coon et al., 2007). The analysis was performed on a sample of 664 neuropathologically confirmed AD cases and 422 controls from the US. Initial analysis showed that APOE was the only signal to reach genome-wide significance after Bonferroni correction for multiple testing. In an effort to identify additional statistically significant data, the authors focused