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Review

The Genetics of Alzheimer Disease: Back to the Future

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Three decades of genetic research in Alzheimer disease (AD) have substantially broadened our understanding of the pathogenetic mechanisms leading to neurodegeneration and dementia. Positional cloning led to the identification of rare, disease-causing mutations in *APP*, *PSEN1*, and *PSEN2* causing early-onset familial AD, followed by the discovery of *APOE* as the single most important risk factor for late-onset AD. Recent genome-wide association approaches have delivered several additional AD susceptibility loci that are common in the general population, but exert only very small risk effects. As a result, a large proportion of the heritability of AD continues to remain unexplained by the currently known disease genes. It seems likely that much of this "missing heritability" may be accounted for by rare sequence variants, which, owing to recent advances in high-throughput sequencing technologies, can now be assessed in unprecedented detail.

Introduction

Alzheimer disease (AD) is the most common neurodegenerative disease and one of the most common diseases in the industrialized world. Clinically it is defined by a slowly progressing loss of cognitive functions, ultimately leading to dementia and death. Neuropathologically it is characterized by the aggregation and deposition of misfolded proteins, in particular aggregated β-amyloid (Aβ) peptide in the form of extracellular senile (or neuritic) "plaques," and hyperphosphorylated tau (τ) protein in the form of intracellular neurofibrillary "tangles" (NFTs). These pathognomonic changes are often accompanied by abundant microvascular damage, including vascular amyloid deposits, and pronounced inflammation of the affected brain regions.

Genetically, AD is usually divided into two forms: (1) familial cases with Mendelian inheritance of predominantly early-onset (<60 years, early-onset familial AD [EOFAD]), and (2) so-called "sporadic" cases with less apparent or no familial aggregation and usually of later onset age (≥60 years, late-onset AD [LOAD]). It needs to be emphasized that this traditional dichotomization is overly simplistic as there are cases of early-onset AD without evidence for Mendelian transmission while, conversely, LOAD is frequently observed with a strong familial clustering, sometimes resembling a Mendelian pattern. While EOFAD is caused by rare and highly penetrant mutations in three genes (see below), the genetics of LOAD is more complex. Current thinking posits that susceptibility for LOAD is conferred by numerous genetic risk factors of relatively high frequency but low penetrance and therefore small effect size (see below). While LOAD is also sometimes referred to as "sporadic AD," it is important to emphasize that up to 60%–80% of this form of AD is genetically determined (Gatz et al., 2006). Still, environmental and epigenetic factors likely make an important contribution in determining an individual's risk, although the precise nature and mechanisms underlying this nongenetic component remain largely elusive, in part because they are difficult to assess experimentally (see also the review by Traynor and Singleton [2010] in this issue of *Neuron*).

In this review, we provide a historical as well as quantitative summary of genetic research in AD. Systematic evaluation of the aggregated association evidence accumulated in the field to date (Figure 1) reveals a pronounced distinction between results from candidate gene versus genome-wide approaches: during the course of only three years, genome-wide association studies (GWAS) in AD have yielded more reproducible and consistent—and thus likely more relevant—findings than three decades of candidate-gene-driven research. Accordingly, in this article we will not focus on results from the candidate gene era (which have been extensively reviewed in the past, e.g., Bertram and Tanzi, 2008; Avramopoulos, 2009), but rather on the most recently implicated GWAS loci in AD, in particular those that showed evidence for genome-wide significant association either in individual studies or as a result of systematic meta-analyses. In addition, we discuss the potential relevance of these loci to AD pathogenesis and provide an outlook of the promises and limitations of future genetic studies in AD.

Positional Cloning Led to the Discovery of Three Early-Onset Familial AD Genes

Similar to most other Mendelian diseases, early progress in deciphering the genetics of AD was afforded by studying large, multigenerational pedigrees suffering from very early-onset forms of the disease (EOFAD). Assessing coinheritance of specific genetic markers in genetic linkage analyses provided a rough estimate of the most likely location of the underlying disease gene, which was subsequently identified by means of "positional cloning," i.e., a more or less systematic mutational screening of DNA segments

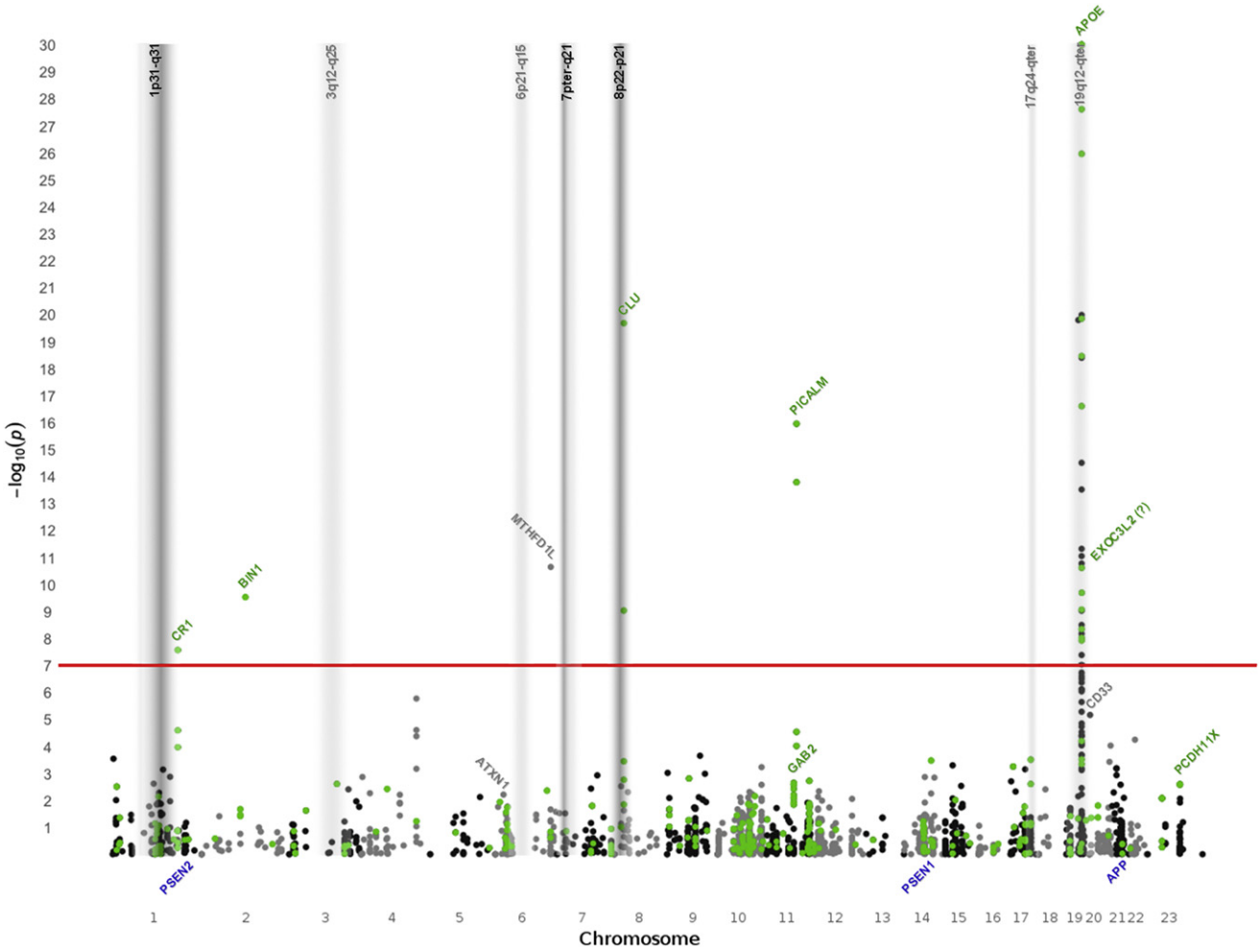

Figure 1. Manhattan Plot of Currently Published Genetic Association Findings in AD

Displayed are −log(10) p values (y axis) of all polymorphisms (n = 2033) with published genetic data currently available on the AlzGene database (http://www.alzgene.org; current on September 27, 2010), listed in genomic order (x axis). Green dots represent p values resulting from random-effects allele-based meta-analyses of ≥ four independent data sets using either genotype summary data or effect size estimates provided in the original publications. Black/gray dots represent either single-study p values or the results of meta-analyses on < four independent data sets. Red horizontal line indicates one common threshold for genome-wide significance ($p = 1 \times 10^{-7}$). Note that p values at the *APOE* locus actually go below 1×10^{-50} and are truncated here for display purposes. Vertical columns represent approximate locations of LOAD linkage findings (based on a "narrow definition" of diagnosing AD) as reported in a recent meta-analysis of LOAD linkage studies (Butler et al., 2009). Dark columns represent regions that showed "genome-wide suggestive," while light columns showed "genome-wide nominal" evidence for linkage. Genes in blue font represent the approximate locations of the currently known EOFAD genes. Data from both resources were scaled to represent the NCBI36/hg18 build of the human reference genome. The plot was generated in R using "qqman" (http://gettinggeneticsdone.blogspot.com/).

close to the linkage peak. This approach led to the identification of three distinct genetic loci—*APP* (amyloid precursor protein) on chromosome 21q, *PSEN1* (presenilin 1) on 14q, and *PSEN2* (presenilin 2, a homolog of *PSEN1*), on 1q—that cause AD with high penetrance in mutation carriers (reviewed in Tanzi and Bertram, 2005). Currently, more than 200 distinct disease-causing mutations are known across these genes and several more are discovered each year (for an up-to-date overview see the AD & FTD Mutation Database [Cruts and Van Broeckhoven, 1998], http://www.molgen.ua.ac.be/admutations/). Concurrent with a wealth of functional and molecular genetic data, the identification of these EOFAD genes has significantly informed our understanding of the pathogenetic mechanisms underlying neurodegeneration in AD, which in most instances proved to be connected to an abnormal production of the Aβ peptide, although some were also found to influence Aβ clearance or aggregation (Murakami et al., 2003; Tsubuki et al., 2003).

Aβ is cleaved from APP by the subsequent action of two enzymes, β- and γ-secretase (Cole and Vassar, 2008; Steiner et al., 2008). Interestingly, the catalytic center of γ-secretase is encoded by the EOFAD genes *PSEN1* and *PSEN2*. This convergence of genetic and molecular evidence has given support to the "amyloid hypothesis," which postulates that the abnormal production of Aβ is the initial step in triggering the pathophysiological cascade that eventually leads to AD (Glenner and Wong, 1984; Hardy and Higgins, 1992; reviewed in Tanzi and Bertram, 2005), and that other neuropathological hallmarks of AD—hyperphosphorylated τ-protein and neurofibrillary

Table 1. Overview of All Published GWAS in AD

GWAS	Design	Population	No. SNPs	No. AD GWAS (Follow-up)	No. CTRL GWAS (Follow-up)	"Featured" Genes
Grupe et al., 2007	Case-control	USA & UK	17,343	380 (1428)	396 (1666)	***APOE***, *ACAN, BCR, CTSS, EBF3, FAM63A**, GALP, GWA_14q32.13, GWA_7p15.2, LMNA, LOC651924, MYH13, PCK1, PGBD1, TNK1, TRAK2, UBD*
Coon et al., 2007; Reiman et al., 2007	Case-control	USA, Netherlands#	502,627	446 (415)	290 (260)	***APOE***, ***GAB2***
Li et al., 2008	Case-control	Canada & UK	469,438	753 (418)	736 (249)	***APOE***, *GOLM1, GWA_15q21.2, GWA_9p24.3*
Poduslo et al., 2009	Family-based & Case-control	USA	489,218	9 (199)	10 (225)	*TRPC4AP*
Abraham et al., 2008	Case-control	UK‡	561,494	1082 (-)	1239 (1400)	***APOE***, *LRAT*
Bertram et al., 2008	Family-based	USA	484,522	941 (1767)	404 (838)	***APOE***, ***ATXN1***, ***CD33***, ***GWA_14q31***
Beecham et al., 2009	Case-control	USA^	532,000	492 (238)	496 (220)	***APOE***, *FAM113B*
Carrasquillo et al., 2009	Case-control	USA●	313,504	844 (1547)	1255 (1209)	***APOE***, ***PCDH11X***
Lambert et al., 2009	Case-control	Europe‡	~540,000	2035 (3978)	5328 (3297)	***APOE***, ***CLU (APOJ)***, ***CR1***
Harold et al., 2009	Case-control	USA & Europe●‡	~610,000	3941 (2023)	7848 (2340)	***APOE***, ***CLU (APOJ)***, ***PICALM***
Heinzen et al., 2009 (CNV)	Case-control	USA^	n.g.	331 (-)	368 (-)	***APOE***, *CHRNA7*
Potkin et al., 2009	Case-control	USA (ADNI)†	516,645	172 (-)	209 (-)	***APOE***, *ARSB, CAND1, EFNA5, MAGI2, PRUNE2*
Seshadri et al., 2010	Case-control	Europe & USA●‡#	~2,540,000	3006 (6505)	22604 (13532)	***APOE***, ***BIN1***, ***CLU (APOJ)***, ***EXOC3L2***, ***PICALM***
Naj et al., 2010	Case-control	USA & Europe†#^	483,399	931 (1338)	1104 (2003)	***APOE***, ***MTHFD1L***

Modified after content on the AlzGene website (http://www.alzgene.org; current on September 27th 2010). Studies are listed in order of publication date (determined by PubMed-ID number). "Featured Genes" are those genes/loci that were declared as "associated" in the original publication, although criteria for declaring association may vary across studies; genes underlined and in bold font were reported to show experiment-wide "genome-wide significant" association; in many studies, surrogate marker were used for *APOE*. Numbers of "AD Cases" and "Controls" refers to sample sizes used in initial GWAS screening, whereas "Follow-up" refers follow-up data sets (where applicable); please consult AlzGene website for more details on these studies. Symbols (●, ‡, #, †, ^) indicate sample overlap across studies with identical symbols. **This locus was originally named "*THEM5*."

tangles, vascular damage, and inflammation—are consequences, rather than causes, of the disease process. The amyloid hypothesis has also informed the search for and functional interpretation of genetic factors in LOAD (see below). While the three currently known EOFAD genes explain a large proportion of the Mendelian forms of AD, they do not explain all, making it likely that additional EOFAD-causing genes exist, which—if identified—could provide novel and valuable insights into the pathogenesis of AD.

Candidate Gene Studies in AD

The fact that most currently known EOFAD genes cause AD by an abnormal production of the Aβ peptide led to the formulation of other Aβ-centered hypotheses in the search for the genetic causes of LOAD (e.g., with potential effects on Aβ production, aggregation, or clearance; Table 2). One of the first such "candidate genes" assessed for genetic association with AD was *APOE* (encoding apolipoprotein E [apoE]) on chromosome 19q13. Since the *APOE*-containing chromosomal region was implied by means of genetic linkage analysis before any bona fide association studies were conducted (Pericak-Vance et al., 1991), *APOE* was a candidate gene on both functional *and* positional grounds, a convergence that has (re)emerged for some of the most recently implicated LOAD genes (Figure 1). The original discovery that the ε4 allele of a 3 allele haplotype (composed of ε2, ε3, and ε4 alleles, which show different biochemical properties at the protein level) leads to a dose-dependent increase in AD risk of ~4-fold as compared to noncarriers (Strittmatter et al., 1993) has been replicated in essentially all independent follow-up studies (Bertram et al., 2007). The association between increased risk for AD and ε4 continues to be—by a margin—the lead association finding even in modern-days genetic studies of LOAD (Table 1). In contrast to ε4, the rarer ε2 allele appears to exert "protective" effects (or "healthier aging") when inherited with the ε3 allele as compared to homozygous ε3 allele carriers (Corder et al., 1994; Gerdes et al., 2000), a finding that has been consistently replicated, albeit at lower statistical significance (Farrer et al., 1997).

Functionally, apoE-dysfunction has been connected to several pieces in the puzzle of Aβ-centered AD hypotheses (Table 2), and a detailed discussion of the wealth of in vitro and in vivo evidence supporting its role in AD pathogenesis is beyond the scope of this review (for recent reviews, see Kim et al., 2009a, and Vance and Hayashi, 2010). However, despite the broad molecular evidence that apoE protein is involved in AD-specific pathways, it is interesting that genetic variation in the *APOE* gene has also been associated with risk for numerous other neuropsychiatric disorders including Parkinson disease (Lill et al., 2010b; Williams-Gray et al., 2009) and multiple sclerosis (Lill et al., 2010a), as well as a number of cardio- and cerebrovascular diseases (Peck et al., 2008; Willer et al., 2008), age-related macular degeneration (Bojanowski et al., 2006), and longevity (Sebastiani et al., 2010; Gerdes et al., 2000). It should be noted, however, that different alleles are associated with disease risk across different phenotypes and none of these associations is nearly as well established as *APOE*'s effects on AD risk. Functionally, all of the aforementioned putative associations could at least partially relate to apoE's pivotal role in lipid and cholesterol metabolism (Zhang et al., 1992).

The nonspecific nature of the *APOE*-AD association has prompted several investigators to propose that the actual AD-predisposing effect may be exerted by other genes/proteins in the chromosomal interval containing *APOE* (Takei et al., 2009; Roses et al., 2010). The latter study identified a polymorphic poly-T variant in *TOMM40* (encoding translocase of outer mitochondrial membrane 40 homolog; which maps only ~2,000 bp proximal of *APOE*), of which "long" poly-T repeats are associated with a younger onset age even in ε3 allele carriers as compared to "short" poly-T repeats. If confirmed in independent data sets, this finding could explain why the ε4 allele in *APOE* does not account for all of the genetic variance attributed to the chromosome 19q13 region in LOAD. In addition, it was recently proposed that genetic variants in or near the *EXOC3L2* (exocyst complex component 3-like 2) gene (~300 kb distal of *APOE*) may have an effect on AD risk that is independent of ε4 (Seshadri et al., 2010). Unfortunately, systematic assessment of the possible genotype-phenotype correlations in the chromosomal interval containing *APOE* is aggravated by the fact that the SNPs defining the ε2/3/4 haplotypes are only poorly covered by current genome-wide microarrays, necessitating manual regenotyping in most instances (Thompson et al., 2009).

The early success of the candidate gene approach in AD has spurred a large number of genetic association studies assessing other loci of potential relevance based on functional hypotheses (mostly Aβ-centered). The outcomes have been inconsistent; of the nearly 700 candidate AD genes investigated over the past 30 years, only few show significant risk effects when data from all available studies are combined (Bertram and Tanzi, 2008) (for an up-to-date overview see the AlzGene Database). Noteworthy examples include associations seen with common variants in *ACE* (angiotensin-converting enzyme; Kehoe et al., 1999), *ADAM10* (disintegrin and metalloproteinase domain-containing protein 10; Kim et al., 2009b), *CHRNB2* (cholinergic receptor, nicotinic, beta 2; Cook et al., 2004), *DAPK1* (death-associated protein kinase 1; Li et al., 2006), *IL8* (interleukin 8; Li et al., 2009), *MTHFR* (methylenetetrahydrofolate reductase; Chapman et al., 1998), *OTC* (ornithine carbamoyltransferase; Bensemain et al., 2009), *SORL1* (sortilin-related receptor; Rogaeva et al., 2007), and *TF* (transferrin; van Rensburg et al., 1993), which all show modest genetic effects but only modest statistical support in random-effects meta-analyses. While the modest effect sizes exerted by these candidate loci (i.e., odds ratios [OR] between ~1.15 and 1.5) are quite typical for genetically complex diseases, their statistical support (i.e., p values between ~0.0001 and 0.01) is orders of magnitude below (i.e., less significant) that observed for loci that have recently emerged from GWAS in AD (i.e., p values $<< 1 \times 10^{-7}$, Figure 1). This can be attributed to a number of reasons likely acting in combination, including type-I error, small sample size, and different sources of bias (Ioannidis, 2005). It is interesting to note that the candidate gene approach was substantially more successful in identifying robust disease associations in some other disorders, such as Parkinson disease (PD), where the lead susceptibility signals (*SNCA* [α-synuclein], *MAPT* [microtubule-associated protein tau], *LRRK2* [leucine-rich repeat kinase 2], *GBA* [glucosidase, beta, acid]) were already established with genome-wide significance years before the GWAS era (Lill et al., 2010b; Ross and Farrer, 2010). Another surprising difference between AD and PD is that two of the genes established to cause autosomal-dominant forms of PD (*SNCA* and *LRRK2*) also show unequivocal and highly significant risk effects on non-Mendelian ("idiopathic") PD, while no such correlation appears to exist in AD for *APP*, *PSEN1*, and *PSEN2* (AlzGene database). Furthermore, *MAPT* (encoding τ-protein, the abnormal deposition of which represents a neuropathological hallmark for AD but not PD) is highly significantly associated with risk for PD ($p = 3.6 \times 10^{-21}$), but currently not AD.

Genome-wide Association Studies in AD

One of the main limitations of the candidate gene approach is its focus on a preconceived functional and/or positional hypothesis. Until recently, this approach was aggravated by technical limitations, as it was both laborious and expensive to develop multiplex genotyping assays that allowed for investigation of more than a few markers at a time. In the last five years, however, the advent of microarray technology has revolutionized genetics research, and it is now possible to assess several hundreds of thousands (or via in silico genotyping, or imputation, several millions) of single-nucleotide polymorphisms (SNPs) in one experiment. Usually, SNPs on these microarrays are interspersed at high density throughout all chromosomes, effectively allowing one to perform genome-wide association testing in a largely hypothesis-free manner, e.g., as GWAS. While genome-wide screening has distinct advantages, massive multiple testing is a critical issue and substantially more rigorous criteria are required to declare an association as being "significant" on an experiment-wide level. Several thresholds to declare genome-wide significance have been proposed, with p values usually ranging between 5×10^{-7} and 5×10^{-8} (Ioannidis et al., 2009; McCarthy et al., 2008), although there are other ways to determine study-specific genome-wide significance (Ionita-Laza et al., 2007).

Several GWAS have been performed in AD to date (Table 1). All but one have seen *APOE* as the by far most significant finding

(with p values down to $\sim 1 \times 10^{-160}$; Harold et al., 2009), but over three dozen other loci beyond *APOE* have been implicated (Table 1). Of these, only a few were reported to show study-specific genome-wide significance in at least one report, and only these loci will be discussed in chronological order in the remainder of this section.

The first genome-wide significant finding was reported for *GAB2* (GRB2-associated binding protein 2) by Reiman et al. (2007), who found this effect to be most pronounced in carriers of the ε4 allele at *APOE*. This finding has been met with mixed replications (e.g., Chapuis et al., 2008; Sleegers et al., 2009) and currently shows a p value of 2.2×10^{-3} in the ongoing AlzGene meta-analyses. It also showed nonsignificant (p value ~0.15) effect sizes in the same direction as in the original report in a large subsequently published GWAS (Harold et al., 2009), although no further details were given. Functionally, GAB2 protein may be involved in the production of Aβ as it binds to Grb2 (growth factor receptor-bound protein 2), which in return can bind APP and both presenilins (Nizzari et al., 2007). Other data also suggest a potential involvement in tau phosphorylation and NFT formation (Reiman et al., 2007).

The second genome-wide significant association signals were reported by Bertram et al. (2008), in a family-based GWAS for *ATXN1* (ataxin 1), *CD33* (siglec 3), and an as yet uncharacterized locus on chromosome 14 (GWA_14q31.2). While for the latter, current AlzGene meta-analyses show no support of independent replication in case-control data sets (e.g., Bettens et al., 2009), only insufficient data exist to merit meta-analyses for the other two loci (although Harold et al. [2009] reported no evidence for association with either of these genes). Functional genetic experiments suggest that differences in *ATXN1* expression can modulate Aβ levels in vitro, an effect that appears to be mediated via β-secretase cleavage of APP (Zhang et al., 2010). *CD33* belongs to the family of sialic acid-binding, immunoglobulin-like lectins that are believed to promote cell-cell interactions and to regulate the functions of cells in the adaptive and innate immune systems (Crocker et al., 2007; von Gunten and Simon, 2006), both involved in contributing to the inflammatory reactions observed in the brains of AD patients. In this context it is interesting to note recent data suggesting that Aβ could function as an antimicrobial peptide that may have a normal function in the innate immune system (Soscia et al., 2010).

In 2009, several AD GWAS were published suggesting the presence of additional AD susceptibility genes. First, Carrasquillo et al. (2009), highlighted *PCDH11X* (protocadherin 11 X-linked), currently the only GWAS signal on the X chromosome. Just like several other loci discussed in this section, independent replication of this finding has been inconsistent (e.g., Beecham et al., 2010). Some protocadherins have been proposed as γ-secretase substrates (Haas et al., 2005), and it remains to be seen whether or not PCDH11X competes with APP for γ-secretase.

Later that year, two large GWAS from the UK (Harold et al., 2009) and France (Lambert et al., 2009) were published back-to-back highlighting three novel AD genes, i.e., *CLU* (clusterin; a.k.a. apolipoprotein J), *CR1* (complement component (3b/4b) receptor 1), and *PICALM* (phosphatidylinositol binding clathrin assembly protein). All three of these loci have since received overwhelming support from independent follow-up studies (Carrasquillo et al., 2010; Jun et al., 2010; Schjeide et al., in press) and currently rank at the very top of the AlzGene meta-analyses, directly following *APOE*. All three loci show genome-wide significant association in allelic meta-analyses combining all available data with p values ranging from 2.1×10^{-20} (*CLU*; rs11136000), 2.7×10^{-8} (*CR1*; rs3818361), and 1.1×10^{-16} (*PICALM*; rs3851179; Figure 1). In addition, there are several other SNPs in each of these loci showing highly significant association (p values $< 1 \times 10^{-5}$) with AD risk, leaving essentially no doubt that variants in these or nearby genes represent genuine AD susceptibility loci. Furthermore, it is interesting to note that—like *APOE*—two of these novel AD loci map in or close to regions showing strong evidence for LOAD linkage in a recent meta-analysis of genome-wide linkage studies (Butler et al., 2009), i.e., *CLU* on chromosome 8p21 and *CR1* on 1q32.2 (Figure 1). Despite their strong statistical support, it should be emphasized that the effect sizes exerted by these loci are collectively low (allelic ORs ~1.15 for all three loci), which is much less than for *APOE* ε4 (allelic OR ~4) or other established neurodegenerative disease loci (e.g., ORs > 1.3 for three of the established Parkinson susceptibility loci—*SNCA*, *MAPT*, and *LRRK2*—which were all confirmed by different GWAS; Pankratz et al., 2009; Simón-Sánchez et al., 2009; Satake et al., 2009).

Functionally, the novel loci implicated by Harold et al. (2009) and Lambert et al. (2009) may exert their effects in a number of ways (Table 2). Clusterin is a ~75 kDa chaperone molecule that is expressed in all tissues, including the CNS. The main associated SNP (rs11136000) lies deeply intronic with no known or implied functional effect. In addition to possibly being involved in clearance and aggregation of Aβ, clusterin has also been reported to be involved in Aβ fibrillization (DeMattos et al., 2002, 2004), regulation of brain cholesterol and lipid metabolism, and the inhibition of neuronal apoptosis/potentiation of neuroprotection (Nuutinen et al., 2009). CR1 is the main receptor of the complement C3b protein, a key inflammatory protein activated in AD (Khera and Das, 2009; Wyss-Coray et al., 2002). In vitro and in vivo experiments suggest that complement activation can protect against Aβ-induced neurotoxicity and may reduce the accumulation/promote the clearance of amyloid and degenerating neurons (Rogers et al., 2006; Wyss-Coray et al., 2002). PICALM plays a role in clathrin-mediated endocytosis (Tebar et al., 1999), synaptic transmission, and the removal of apoptotic cells (Harel et al., 2008; Yao et al., 2005). With respect to AD it is interesting that the C-terminal fragment of APP generated by β-secretase cleavage undergoes clathrin-mediated endocytosis before being cleaved by γ-secretase (Koo and Squazzo, 1994). It is therefore possible that dysfunctional PICALM protein could interfere with this process, but this notion has not been supported by preliminary in vitro studies (Wu et al., 2009). Furthermore, brain-expressed PICALM protein is predominately expressed in endothelial cells, where it could play a role in Aβ transport into the bloodstream (Baig et al., 2010). The hypothesis that PICALM may be involved in Aβ clearance is also supported by recent data indicating that—like *APOE* ε4—the *PICALM* risk allele is associated with reduced levels of Aβ in the cerebrospinal fluid of AD patients and control individuals (Schjeide et al., in press).

Table 2. Potential Mechanisms Linking Genome-wide Association Findings to AD Pathogenesis

	APOE	*ATXN1*	*BIN1*	*CD33*	*CLU*	*CR1*	*GAB2*	*PCDH11X*	*PICALM*
Aβ-production		Zhang et al., 2010	Wigge et al., 1997; Pant et al., 2009				Nizzari et al., 2007	Haas et al., 2005	Tebar et al., 1999
Aβ-aggregation	Kim et al., 2009a; Moir et al., 1999				DeMattos et al., 2002; Thambisetty et al., 2010				
Aβ-clearance	Kim et al., 2009a; Holtzman et al., 1999		Wigge et al., 1997; Pant et al., 2009		Zlokovic et al., 1996; DeMattos et al., 2004	Wyss-Coray et al., 2002; Rogers et al., 2006			Tebar et al., 1999; Baig et al., 2010
τ-phosphorylation							Reiman et al., 2007		
Synaptic transmission								Senzaki et al., 1999; Blanco et al., 2000	Yao et al., 2005; Harel et al., 2008
Inflammation	Kim et al., 2009a			Crocker et al., 2007; von Gunten and Simon, 2006	Xie et al., 2005	Wyss-Coray et al., 2002; Khera and Das, 2009			
Cerebrovascular events	Kim et al., 2009a								

Schematic overview of the potential functional impact of GWAS findings and their reported or suggested potential involvement in a number of pathogenetic pathways of relevance to AD. Only signals mapping to known genes and reported to show genome-wide significance for association with AD in at least one study are included in this table. References listed in intersecting cells point to a selection of both primary and review publications on the proposed pathomechanisms (only 1–2 representative publications are selected per example). Note that some pathomechanisms (e.g., Aβ-degradation; τ-aggregation) have currently not been linked to any of the proposed GWAS loci; conversely, no hypotheses or data with respect to the potential impact of *EXOC3L2* or *MTHFD1L* on AD pathogenesis have been published to date which is why they are not listed in this table.

In 2010, two further GWAS were published suggesting the existence of three additional AD susceptibility loci. The first (Seshadri et al., 2010) resulted from a large collaborative effort that also included the GWAS data from four of the five aforementioned studies (Table 1). In addition to replicating the association between *CLU* and *PICALM*—which was not unexpected given that a large proportion of samples overlapped with the GWAS that originally implicated these genes—this study highlighted two potential additional AD risk factors, i.e., *BIN1* (bridging integrator 1; originally implicated at subgenome-wide significance by Harold et al. [2009]) and *EXOC3L2* (exocyst complex component 3-like 2), or a locus nearby on chromosome 19q13.32. Combining all available data, both genes currently display highly significant association with AD risk on AlzGene with p values around 3.0×10^{-10} and 2.1×10^{-10}, respectively (Figure 1), and allelic ORs in the order of ~1.15. *BIN1* (also known as amphiphysin II) encodes several isoforms of an adaptor protein involved in receptor-mediated endocytosis (Pant et al., 2009; Wigge et al., 1997), which—as hypothesized for PICALM—could have an effect on Aβ production and/or the clearance of Aβ from the brain. In addition, rare, homozygous mutations in *BIN1* have been found to cause recessive centronuclear myopathy, a condition characterized by muscle weakness and abnormal centralization of nuclei in muscle fibers (Nicot et al., 2007). The disease-causing effect is probably triggered by abrogating BIN1's interaction with dynamin 2, which has also been associated with risk for LOAD in candidate gene analyses (Aidaralieva et al., 2008), albeit inconsistently. The biological function of the protein encoded by *EXOC3L2* remains largely elusive. It should be emphasized, however, that the ~100 kb region harboring the risk-associated variant (rs597668) on chromosome 19q13.32 contains several other genes (e.g., *NKPD1* [NTPase, KAP family P loop domain containing 1], *TRAPPC6A* [trafficking protein particle complex 6A], *BLOC1S3* [biogenesis of lysosomal organelles complex-1, subunit 3], *MARKL1* [MAP/microtubule affinity-regulating kinase 4], and *MARK4* [MAP/microtubule affinity-regulating kinase 4]), which could also represent the functional correlates underlying this association. It is also noteworthy that the associated SNP only maps ~300 kb distal to the *APOE* region, so it remains to be seen whether these two regions are genetically/functionally related. It also is quite possible that rs597668 is merely "tagging" the association with *APOE* and does not actually represent a novel AD locus in its own right.

The latest addition to the set of GWAS-derived putative LOAD loci is *MTHFD1L* (methylenetetrahydrofolate dehydrogenase [NADP+ dependent] 1-like), recently reported to show genome-wide significant association with AD risk in ~5,000 individuals (Naj et al., 2010). In contrast to most other AD GWAS findings, the risk allele at the associated SNP (rs11754661) appears to confer relatively large effect sizes, i.e., allelic ORs ~2, which translates into nearly doubling the risk for AD in carriers of the minor allele. The study was an extension of this group's earlier GWAS (Beecham et al., 2009), which had already previously implicated this locus at genome-wide *suggestive* significance. As such, it was included in auxiliary analyses of the GWAS by (Harold et al., 2009), who reported no evidence of association with SNP rs11754661 in their sample (OR = 1, p = 0.98), despite excellent power to detect the proposed OR of ~2.

In summary, GWAS have substantially reshaped the landscape of LOAD genetics during the course of only three years. Currently, the most promising findings relate to the identification of variants in or near *BIN1*, *CLU*, *CR1*, and *PICALM* whose status as novel AD risk loci have been confirmed by extensive and independent replication data. Other GWAS loci, such as *ATXN1*, *CD33*, *EXOC3L2*, *GAB2*, *MTHFD1L*, and *PCDH11X*, should be considered more provisional until further replication data become available. While fine-mapping and biochemical studies are still needed to identify the sequence variants underlying the currently observed genetic associations and to confirm and characterize their presumed molecular effects, nearly all of the newly reported GWAS loci have been linked to Aβ metabolism in one or more ways (Table 2). In particular, this relates to Aβ-aggregation or clearance of Aβ from the brain either directly or indirectly, e.g., via effects on the immune system response to Aβ-related toxicity. However, these potential, Aβ-centered functional connections are still preliminary in most instances, and further research is needed to clarify whether or not other pathways are affected by these loci. Furthermore, it can be expected that several additional AD susceptibility variants will be identified in future genome-wide efforts using higher-density microarrays in combination with substantially increased sample sizes, alternative phenotype definitions (e.g., "endophenotypes" such as neuroimaging or CSF biomarker levels), and via systematic data-integration and meta-analysis efforts. It remains to be seen whether these findings will reveal hitherto unrecognized, novel pathogenetic mechanisms beyond those related to the metabolism of APP and Aβ.

Back to the Future: Beyond GWAS and the Search for Causal Variants

Despite the enthusiasm revolving around the novel GWAS findings, it should not be forgotten that, individually, the risk effects exerted by the new GWAS loci are small, i.e., they confer a mere ~0.10-fold to 0.15-fold increase or decrease in AD risk in carriers versus noncarriers of the associated alleles, compared to a nearly 4-fold increase in AD risk related to the presence of the *APOE* ε4 allele. Although to date no precise estimate exists regarding the proportion of LOAD heritability explained by the combined effects of *APOE* and the confirmed GWAS loci, it appears reasonable to assume that this proportion does not exceed 50%. This is the upper bound of explained heritability in other complex diseases for which—unlike AD—significant association has been demonstrated for *several* common loci of large effect (i.e., ORs > 2 to > 3), such as age-related macular degeneration (Chen et al., 2010; Manolio et al., 2009). In other words, a substantial proportion of the heritability for LOAD likely remains unexplained by the currently known susceptibility genes. The "missing heritability" in these traits has been coined as the "dark matter" of GWAS, in the sense that "one is sure it exists, can detect its influence, but simply cannot see it (yet)" (Manolio et al., 2009).

There are four main areas likely to account for the missing heritability in AD: (1) common variants that are inappropriately tagged by any of the existing microarrays; (2) common variants

that are tagged by existing microarrays but exert even smaller effects than the ones already identified (i.e., ORs < 1.1) and can only be identified in *very large* sample sizes (for AD this would require between ~25,000 and > 100,000 combined cases and controls for allele frequencies ranging between 0.5 and 0.05, respectively, to be detected at genome-wide significance, i.e., p values $\sim 1 \times 10^{-7}$); (3) copy-number variants and structural chromosomal changes (while these can be resolved to a certain degree on existing GWAS microarrays, this has only rarely been carried out for AD to date, see Table 1); (4) rare sequence variants (e.g., with minor allele frequencies << 5%) conferring both small and large effects. While the former three issues can be addressed by upcoming GWAS, current microarray technology is not designed for de novo identification or the reliable measurement of rare sequence variants. As a matter of fact, owing to this inherent limitation, most GWAS analysis pipelines explicitly exclude rare variants (e.g., MAFs < 5% or < 1%) prior to analysis. Thus, the identification of the presumed disease-associated rare variants will require deep resequencing in suitable data sets, either small scale (i.e., restricted to specific loci, e.g., previously associated GWAS regions, similar to what has been done for years in positional cloning and candidate gene experiments) or large scale (e.g., whole exome, or whole genome). The genetics community has already begun to construct a comprehensive catalog of rare sequence variants in the human genome by applying large-scale resequencing using recently developed, massively parallel (so called "next-generation") sequencing techniques, e.g., as part of the 1000 Genomes project (http://www.1000genomes.org/). For the most part, however, this will not alleviate the need to actually directly test these variants in sufficiently large collections of affected and unaffected individuals in disease-centered discovery projects.

As for all previous eras of human genetic research (i.e., positional cloning, candidate gene, GWAS), the specific disease-causing or disease-modifying effects can only be established following in-depth functional genetic characterization of the associated variants, followed by validation in patient materials and/or relevant animal models. While this molecular evidence has proven immensely difficult to attain for most common variants of small effect, the functional characterization of the rare, EOFAD-causing mutations in *APP*, *PSEN1*, and *PSEN2* has been pivotal for our understanding of AD pathogenesis. This can be attributed to the fact that most EOFAD mutations engender amino acid changes with clear functional consequences on Aβ metabolism. In contrast, common disease-associated variants often lie in genomic regions of no obvious functional consequence, e.g., gene deserts, or deep within introns. Based on the sheer number of potentially functional coding region variants to emerge from deep resequencing efforts over the coming years, much of the progress in the field will depend on the development of appropriate and efficient in silico and in vitro high-throughput pipelines to study variant-activity relationships in a systematic manner.

It goes beyond the scope of this review to provide a detailed account of the various available approaches for the generation and analysis of large-scale resequencing data aimed at identifying rare variants linked to disease. However, several landmark, proof-of-principle projects have already been completed that can be regarded as initial reference (reviewed in Manolio et al., 2009; McClellan and King, 2010). These studies succeeded not only to identify novel disease-causing variants of Mendelian diseases in genes previously unlinked to the specific traits (Bilgüvar et al., 2010; Gilissen et al., 2010; Ng et al., 2010), but also to "resolve" the complex patterns that typically emerge from GWAS approaches (Dickson et al., 2010; Johansen et al., 2010; Nejentsev et al., 2009), although most of these findings still await functional genetic confirmation and characterization. It does not seem too far-fetched to expect that in AD, as well, such efforts will revolutionize our understanding of the true genetic forces underlying disease susceptibility, possibly more so than GWAS have begun to expand our knowledge about the genetic basis of LOAD beyond *APOE*.

A continuing challenge in the coming years will be to efficiently distinguish between findings that likely reflect genuine genetic effects versus those that are simply due to chance. For common variants, i.e., those typically assayed in candidate gene or GWAS approaches, several guidelines have already been suggested (e.g., Chanock et al., 2007; Little et al., 2009; Khoury et al., 2009) that essentially amount to demonstrating genome-wide significance upon combining results from all available data sets (e.g., via meta-analysis) in the absence of significant heterogeneity or bias. For variants with only insufficient support (e.g., those showing nominal association but lacking power to achieve genome-wide significance), intermediate measures have been proposed that may help to assess the "solidity" of a finding until sufficient data are available (e.g., using Bayesian analyses to estimate the odds that a finding is "real" [Wellcome Trust Case Control Consortium, 2007; Wakefield, 2007], or grading its "epidemiologic credibility" [Ioannidis et al., 2008]). For rare variants, the situation is more complex for a number of reasons, including the need for very large sample sizes (owing to the low allele frequencies), or confounding due to allelic heterogeneity (i.e., different alleles in the same gene that contribute independent risk effects). Several approaches have been suggested to overcome these issues, e.g., to pool variants within the same coding regions (Price et al., 2010) or to measure general "mutational load" in case versus control subjects (International Schizophrenia Consortium, 2008). A crucial factor in this context will be to distinguish between rare variants with disease-specific effects from neutral coding changes, e.g., by means of high-throughput functional assays and/or by studying pedigrees rather than unrelated cases and controls to prove cosegregation with disease status.

Conclusions

Three decades of genetic research in AD have substantially broadened our understanding of the pathogenetic mechanisms leading to neurodegeneration and dementia. Initially, genetic linkage analysis followed by positional cloning identified the major causes underlying EOFAD by pinpointing rare, disease-causing mutations in *APP*, *PSEN1*, and *PSEN2*. Candidate gene approaches, sometimes informed by genetic linkage results, have led to the discovery of *APOE* as the single most important risk factor for LOAD. Recently, GWAS have delivered several additional susceptibility loci that are common in the

general population, but only exert very small genetic effects. Several other common risk-factor genes can be expected to emerge from GWAS in the coming years. Collectively, however, a large proportion of the heritability of AD will continue to remain unexplained by the variants invoking these association signals. It seems likely that much of the "missing heritability" may be caused by rare sequence variants in genes that predispose to both early- and late-onset forms of AD.

For the first time in the history of human genetics research, the genetic basis of AD and other heritable diseases can be assessed in unprecedented detail and efficiency owing to recent advances in large-scale sequencing technologies. Our initial understanding of the etiology of AD began with the identification of rare causal mutations in EOFAD. As we commence to engage in large-scale resequencing projects, we may very well find ourselves "back to the future" by discovering rare causal variants in genes that were initially associated with AD based on common SNPs appearing to exert only small effects on disease risk.

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