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Review

Alzheimer's Disease Genetics: From the Bench to the Clinic

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Alzheimer's disease (AD) is a clinically heterogeneous neurodegenerative disease with a strong genetic component. Several genes have been associated with AD risk for nearly 20 years. However, it was not until the recent technological advances that allow for the analysis of millions of polymorphisms in thousands of subjects that we have been able to advance our understanding of the genetic complexity of AD susceptibility. Genome-wide association studies and whole-exome and whole-genome sequencing have revealed more than 20 loci associated with AD risk. These studies have provided insights into the molecular pathways that are altered in AD pathogenesis, which have, in turn, provided insight into novel therapeutic targets.

Alzheimer's Disease

Alzheimer's disease (AD) is defined clinically by a gradual decline in memory and other cognitive functions and neuropathologically by gross atrophy of the brain and the accumulation of extracellular amyloid plaques and intracellular neurofibrillary tangles.

Genetic, biochemical, and neuropathological data suggest that aggregation of β-amyloid (Aβ) is central to initiating AD pathogenesis (Hardy and Selkoe, 2002). Aβ is a proteolytic fragment of the amyloid precursor protein (APP) (Thinakaran and Koo, 2008), generated as a result of sequential cleavage by β- and γ-secretases. Deposition of extracellular amyloid plaques is followed by the accumulation of neurofibrillary tangles in neuronal cell bodies and associated processes (Braak and Braak, 1991). Neurofibrillary tangles are composed of hyperphosphorylated tau aggregates. The presence of neurofibrillary tangles in AD brains is strongly correlated with neuronal dysfunction and disease progression (reviewed in Holtzman et al., 2011). The amyloid cascade hypothesis posits that changes in APP and/or Aβ homeostasis lead to the aggregation of Aβ and deposition in plaques and that these events are sufficient to initiate the cascade of pathologic and clinical changes associated with AD, including the aggregation of tau protein in neurofibrillary tangles.

Linkage Studies

Dominantly inherited mutations in *β-amyloid precursor protein* (*APP*), *presenilin 1* (*PSEN1*), and *presenilin 2* (*PSEN2*) cause early-onset Alzheimer's disease (AD). These genes as well as *APOE* were identified through genetic linkage studies in families.

APP

APP is located at chromosome 21q21 and encodes a ubiquitously expressed type 1 transmembrane protein. *APP* is alternatively spliced to produce three transcripts: APP695, APP751, and APP770. The APP695 isoform is the major *APP* isoform that is expressed in neurons, while the APP751 isoform is highly expressed in astrocytes (Yoshikai et al., 1990). Proteolytic processing of APP leads to the production of fragments through the nonpathogenic and the amyloidogenic pathways. The majority of APP is proteolyzed by α- and γ-secretases, leading to cleavage of APP within the Aβ domain. The result is nonpathogenic fragments: sAPPα and α-C-terminal fragment (CTF) (Thinakaran and Koo, 2008). Alternatively, APP can be cleaved through sequential proteolytic cleavage by β- and γ-secretases to generate Aβ peptides, sAPPβ and β-CTF. Cell-surface APP is internalized, allowing Aβ to be generated in the endocytic pathway and secreted into the extracellular space (Thinakaran and Koo, 2008).

Dominant mutations in *APP* account for approximately 14% of early-onset autosomal dominant cases of AD (reviewed in Guerreiro et al., 2012), with more than 30 mutations described (http://www.molgen.vib-ua.be/ADMutations). Two recessive *APP* mutations, A673V and E693Δ, also reportedly cause early-onset AD (reviewed in Guerreiro et al., 2012). The majority of mutations in APP cluster in the region that is adjacent to or within the Aβ domain; however, early genetic studies only sequenced the exons in *APP* that encode the Aβ sequence (exons 16 and 17), leaving the possibility that variants may exist elsewhere in *APP* that cause or increase risk for AD.

Mutations in *APP* have revealed many important aspects of the molecular mechanisms underlying AD pathogenesis. The Swedish mutation (KM670/671NL) increases plasma Aβ levels by 2- to 3-fold by altering β-secretase cleavage efficiency (Mullan et al., 1992). Duplications of *APP* and the surrounding sequence are also associated with early-onset AD. Families carrying these duplications exhibit classic AD and cerebral amyloid angiopathy (CAA) (reviewed in Guerreiro et al., 2012). The frequency of these copy number mutations among autosomal dominant cases of AD varies based on the population: 18% Japanese, 8% French, 2% Dutch, but none in Swedish/Finnish early-onset familial cases (reviewed in Guerreiro et al., 2012). Additionally, individuals with Down syndrome, which results from trisomy of chromosome 21, develop AD neuropathology (reviewed in Guerreiro et al., 2012). Individuals with partial trisomy of chromosome 21, which does not include the *APP* gene, fail to develop AD neuropathology (reviewed in Guerreiro et al., 2012). Thus, excess Aβ production is sufficient to cause AD. Several *APP* mutations cluster at or after the C-terminal portion of the Aβ domain. These mutations alter γ-secretase function, leading to a shift in APP processing that increases

the highly amyloidogenic Aβ42 fragment while reducing the Aβ40 fragment. The result is an altered Aβ42/Aβ40 ratio without a change in total Aβ levels (Bergmans and De Strooper, 2010). Because Aβ42 is more prone to aggregate than Aβ40, these findings suggest that Aβ aggregation is a critical component of AD pathogenesis. The Arctic mutation (E693G) occurs within the Aβ domain (reviewed in Guerreiro et al., 2012). While this mutation fails to alter absolute Aβ levels or the Aβ42/Aβ40 ratio, the Arctic mutation probably increases the aggregation rate of the mutant peptide (Nilsberth et al., 2001). The Dutch mutation (E693Q) also occurs in the Aβ domain and reportedly results in accelerated Aβ aggregation (reviewed in Guerreiro et al., 2012). Individuals carrying the Dutch mutation develop hereditary cerebral hemorrhage with amyloidosis, which is characterized by predominant vascular Aβ deposition with diffuse plaques in the parenchymal tissue (reviewed in Guerreiro et al., 2012). These mutations provide further evidence that Aβ aggregation is a critical process in AD pathogenesis. Genetic changes that lead to altered APP processing and Aβ accumulation may produce variable neurological and neurovascular phenotypes.

PSEN1 and PSEN2

PSEN1 is located at chromosome 14q24.3, while its homolog, *PSEN2*, is located at chromosome 1q31-q42. *PSEN1* and *PSEN2* are structurally similar integral membrane proteins that contain nine transmembrane domains with a hydrophilic intracellular loop region (reviewed in Guerreiro et al., 2012). PSEN1 and PSEN2 are critical components of the γ-secretase complex, which cleaves APP into Aβ fragments. PSEN1 and PSEN2 localize in the endoplasmic reticulum and Golgi apparatus, where they play an important role in protein processing (De Strooper, 2003; Kovacs et al., 1996). As many as 185 dominant, pathogenic mutations have been identified in *PSEN1*, accounting for approximately 80% of early-onset familial AD cases (reviewed in Guerreiro et al., 2012) (http://www.molgen.vib-ua.be/ADMutations). To date, 13 dominant, pathogenic mutations have been identified in *PSEN2*, which account for approximately 5% of early-onset, familial AD cases (reviewed in Guerreiro et al., 2012) (http://www.molgen.vib-ua.be/ADMutations). *PSEN1* and *PSEN2* mutations are distributed throughout the protein, with some clustering occurring in the transmembrane domains (Guerreiro et al., 2010). Dominantly inherited mutations in *PSEN1*, as well as *APP*, have also been identified in late-onset AD cases with a strong family history of disease (see discussion below). These families may carry additional genetic variants that delay age at onset of the normally fully penetrant disease mutation. Additional variants have been described in *PSEN1* and *PSEN2* that are nonpathogenic or whose pathogenicity remains unclear. Recent evidence suggests that some of these variants, such as *PSEN1* E318G (Benitez et al., 2013) and *PSEN2* R62H (Cruchaga et al., 2012a), may represent risk factors for AD (described below).

PSEN1 and PSEN2 with nicastrin, anterior pharynx-defective-1 (APH-1), and presenilin enhancer 2 (PEN2) form the γ-secretase complex, which catalyzes the cleavage of many membrane proteins (Wakabayashi and De Strooper, 2008). Elegant kinetic studies by Chávez-Gutiérrez and colleagues have demonstrated that familial AD mutations in *PSEN1* and *PSEN2* affect γ-secretase function by three mechanisms (Chávez-Gutiérrez et al., 2012). First, there are variable inhibitory effects on the initial endoproteolytic cleavage step that releases the intracellular domain of APP. Second, there is a premature release of intermediary substrates of APP during the consecutive carboxypeptidase-like γ-secretase cleavage, leading to the generation of longer Aβ peptides. Finally, there is an effect on the cleavage site, leading to preferential cleavage of APP at position 49–50 or 51–50. These three mechanisms provide an explanation of the historical observations that *PSEN1* and *PSEN2* mutations lead to altered Aβ42/Aβ40 ratios.

ADAM10

Recently, rare coding variants in *ADAM10* were found to cosegregate in seven late-onset AD families (Kim et al., 2009b). ADAM10 is the major α-secretase involved in cleavage of the APP ectodomain (Jorissen et al., 2010; Kuhn et al., 2010; Postina et al., 2004). *ADAM10* risk variants, Q170H and R181G, increase Aβ levels in vitro (Kim et al., 2009b). In Tg2576 mice, *ADAM10* Q170H and R181G disrupt α-secretase activity and shift APP processing toward amyloidogenic cleavage, yielding increased plaque load (Suh et al., 2013). These genetic and biological findings provide further support for the hypothesis that alteration of APP processing and Aβ generation is sufficient to cause AD.

APOE

Apolipoprotein E (*APOE*) is located at chromosome 19q13.2 and encodes a pleiotropic glycoprotein. *APOE* is highly expressed in liver, brain, and macrophages (Siest et al., 1995), where it plays a role in mobilization and redistribution of cholesterol (Mahley, 1988). APOE has also been implicated in neuronal growth and repair, nerve regeneration, immune response, and activation of lipolytic enzymes (Mahley, 1988; Mahley and Rall, 2000). *APOE* occurs as three possible isoforms that differ based on two amino acid residues (112 and 158): *APOE*ε2, *APOE*ε3, and *APOE*ε4. *APOE*ε3 is the most common *APOE* isoform, occurring in approximately 72% of the population. Family-based methods originally identified genetic linkage between AD and the region of chromosome 19 that contains the *APOE* gene (reviewed in Guerreiro et al., 2012). *APOE*ε4 increases risk in familial and sporadic early- and late-onset AD, increasing risk 3-fold for heterozygous carriers and increasing risk 8- to 10-fold for homozygous carriers (reviewed in Guerreiro et al., 2012). *APOE*ε4 also has a dose-dependent effect on age at onset. Interestingly, *APOE*ε2 decreases risk for late-onset AD and delays age at onset (reviewed in Guerreiro et al., 2012). Rare coding variants that affect risk for AD may also occur in *APOE* (Kamboh et al., 1999; Medway et al., 2014); however, deep sequencing of the *APOE* gene in large data sets has not been performed. It is also possible that *APOE* variants exist in regulatory regions that impact *APOE* expression, which could, in turn, influence plaque accumulation. In mouse models, APOE expression levels influence amyloid plaque accumulation (Kim et al., 2011a).

The role of APOE in AD is complex. APOE binds to Aβ, influencing the clearance of soluble Aβ and Aβ aggregation (Castellano et al., 2011; Kim et al., 2009a). APOE4 binds to Aβ more rapidly than APOE3, resulting in accelerated fibril formation (Sanan et al., 1994; Strittmatter et al., 1993). APOE also regulates Aβ metabolism indirectly by interacting with low-density lipoprotein receptor-related protein 1 receptors (Verghese et al., 2013). In vivo, APOE influences the amount and structure of

intraparenchymal Aβ deposits in an isoform-dependent manner (Fagan et al., 2002; Holtzman et al., 2000). Thus, the major risk gene associated with AD probably influences Aβ metabolism as a mechanism of pathogenicity.

Genome-Wide Association Studies

Case/Control Studies

Only 50% of individuals with AD carry an *APOE*ε4 allele, suggesting that other genetic factors must contribute to risk for disease. Before 2005, the general strategy used to identify novel risk genes was to perform an analysis of one or more polymorphisms in a candidate gene. Despite hundreds of studies, few reported genetic associations were replicated across studies, most likely due to type 1 error (false-positive associations in the discovery data set) (see http://www.alzgene.org) (Bertram et al., 2010). The main reason for this is that other common genetic risk factors probably have a much smaller impact on risk than *APOE*. As a result, these studies lacked power to detect novel risk genes because the sample sizes used in earlier studies were too small. Since 2005, several technological advances have transformed studies of the genetics of complex traits such as AD (Wray et al., 2008). The first advance was the development of cheap and comprehensive genome-wide arrays, allowing for the simultaneous evaluation of millions of SNPs in thousands of samples, genome-wide association studies (GWASs). The first GWAS for AD used modest sample sizes (500–2,000 samples) and most studies only detected genome-wide significant evidence of association for *APOE* (reviewed in Bertram et al., 2010). The first studies to use thousands of AD cases and elderly nondemented controls successfully generated replicable associations for several new genetic risk factors including *clusterin* (*CLU*), *phosphatidylinositol-binding clathrin assembly protein* (*PICALM*), *complement receptor 1* (*CR1*), *bridging integrator protein 1* (*BIN1*), and *sialic acid–binding immunoglobulin* (*Ig*)*–like lectin* (*CD33*) (Bertram et al., 2008; Harold et al., 2009; Lambert et al., 2009; Seshadri et al., 2010) (Table 1). Two subsequent studies that each included more than 8,000 cases and a similar number of controls identified additional loci with genome-wide significant evidence for association ($<5 \times 10^{-8}$): *membrane-spanning 4A gene cluster* (*MS4A4A*, *MS4A6A*, *MS4A6E*), *CD2-associated protein* (*CD2AP*), *Ephrin receptor A1*(*EPHA1*), and *ATP-binding cassette transporter* (*ABCA7*) (Hollingworth et al., 2011; Naj et al., 2011) (Table 1).

Several studies have now demonstrated a clear relationship between the size of the study and the number of genome-wide significant signals across a range of traits (Ball, 2013; Lambert et al., 2013). Recently, a meta-analysis of GWAS data from 74,046 individuals from four large consortia confirmed all of the previous associations except *CD33* and reported 12 new susceptibility loci for AD. The signals are close to the genes: *Cas scaffolding protein family member 4* (*CASS4*), *CUGBP*, *Elav-like family member 1* (*CELF1*), *Desmoglein 2* (*DSG2*), *Fermitin family member 2* (*FERMT2*), *Major histocompatibility complex class II*, *DR beta 5-1* (*HLA-DRB5-DRB1*), *Inositol polyphosphate-5-phosphatase* (*INPP5D*), *myocyte enhancer factor 2C* (*MEF2C*), *NME/NM23 family member 8* (*NME8*), *Protein tyrosine kinase 2 beta* (*PTK2B*), *solute carrier family 24* (*SLC24A4*)*-Ras and rab interactor 3 (RIN3*), *sortilin-related receptor L*, *A repeats containing* (*SORL1*), *zinc finger*, and *CW type with PWWP domain 1* (*ZCWPW1*) (Lambert et al., 2013) (Table 1). However, as of today, it is not clear whether variants in these genes or neighboring genes are responsible for the initial association. It is likely that as sample sizes continue to grow, additional risk loci will be revealed. However, until those studies are performed, it is difficult to predict which GWAS signals with p values greater than 10^{-8} will be "real" AD risk genes.

These studies employed samples of European descent but two additional GWASs have examined African-American and Asian (Korean and Japanese) case-control series. In addition to *APOE*, the most significant finding in the African-American study of 5,896 cases and controls was with SNPs in *ABCA7*, which reached genome-wide significance and exhibited a somewhat larger effect than in European samples (Reitz et al., 2013). Other loci seen in Europeans showed p values < 0.05 (*BIN1*, *CR1*, *EPHA1*, and *CD33*). The study in Japanese and Koreans used a multistage strategy to identify a novel locus, *SORL1*, which was replicated in a large European American cohort (Lambert et al., 2013; Miyashita et al., 2013). *PICALM* and *BIN1* SNPs also showed association in the Asian case-control series. Together, these studies demonstrate that some of these loci show similar effects across populations (e.g., *BIN1*), while others appear to have a bigger impact in some populations (e.g., *ABCA7*). As larger data sets become available for other populations, population-specific loci and those that are shared across populations will become more fully resolved.

The common SNPs identified in these GWASs alter risk by 10%–15%, suggesting that the effect of these risk alleles is much smaller than that of *APOE*ε4 unless they are tagging rare alleles of larger effect. For the most part, the functional alleles responsible for each of these associations remain to be determined. For some of the GWAS loci, there are several genes within the associated region, any one of which may contain the functional risk variant (see Table 1). However, many of these AD risk loci have putative functions in the immune system (*CLU*, *CR1*, *ABCA7*, *CD33*, and *EPHA1*); four are involved in processes at the cell membrane, including endocytosis (*PICALM*, *BIN1*, *CD33*, and *CD2AP*); and three are involved in lipid biology (*APOE*, *CLU*, and *ABCA7*) (Table 1; Figure 1). Although some of the new genes appear to be involved in Aβ metabolism (for example, *CLU* and *PICALM*), the fact that others probably influence inflammation, endocytosis, and lipid biology suggests that targeting specific components of these pathways may lead to novel directions for drug discovery and treatment. It is also possible that some of the new genetic discoveries are not targeting the nearby gene but actually targeting noncoding RNAs. If so, this could also provide new insights.

Approaches for identifying new risk factors associated with other aspects of the late-onset AD phenotype include genetic studies for elucidating the rate of disease progression, age at onset, cerebrospinal fluid (CSF) or plasma biomarker measurements, and functional and structural imaging measures.

Neuroimaging

Genetic studies using twin and family-based designs have demonstrated that many brain-derived traits, including

Table 1. Functional Classes of AD Genes

SNP	Gene	MAF	Odds Ratio	Known Function	Related AD Risk Genes	Potential Effects on APP and Tau	Pathways	Gene Expression
–	*APP*	–	–	Neurite outgrowth, adhesion, and axonogenesis	–	Cleavage yields Aβ	APP processing	–
–	*PSEN1*	–	–	Component of catalytic subunit of gamma-secretase complex; proteolytic cleavage of integral membrane proteins	–	Cleaves APP	APP processing	–
–	*PSEN2*	–	–	Component of catalytic subunit of gamma-secretase complex; proteolytic cleavage of integral membrane proteins	–	Cleaves APP	APP processing	–
–	*APOE*	–	–	Mediates binding, internalization, and catabolism of lipoproteins	SORL1	Aβ clearance	Lipid metabolism	–
–	*ADAM10*	–	–	Proteolytic cleavage of integral membrane proteins	–	Cleaves APP	APP processing	–
rs6656401	*CR1*[a]	0.197	1.18	Mediates cellular binding of immune complexes that activate complement	–	Aβ clearance	Immune Response	Increased
rs6733839	*BIN1*[b]	0.409	1.22	Regulation of endocytosis of synaptic vesicles	SLC24A4 and RIN3	Mediates tau toxicity	Synapse function	Increased
rs10948363	*CD2AP*	0.266	1.1	Scaffold molecule regulating actin cytoskeleton	INPP5D and CASS4	Mediates tau toxicity	Synapse function and endocytosis	No change
rs11771145	*EPHA1*[c]	0.338	0.9	Brain and neural development; angiogenesis, cell proliferation, and apoptosis	–	–	Immune response and neural development	No change
rs9331896	*CLU*	0.379	0.86	Chaperone; regulation of cell proliferation	PTK2B	Aβ clearance	Immune response and lipid metabolism	Increased
rs983392	*MS4A6A*[d]	0.403	0.9	Signal transduction	–	–	Immune response	No change
rs10792832	*PICALM*[e]	0.358	0.87	AP2-dependent clathrin-mediated endocytosis	–	APP trafficking and Aβ clearance	Synapse function and endocytosis	No change
rs4147929	*ABCA7*[f]	0.19	1.15	Lipid homeostasis; phagocytosis of apoptotic cells by macrophages	–	Aβ clearance	Immune response and lipid metabolism	Increased
rs3865444	*CD33*	0.307	0.94	Mediates sialic acid-dependent binding to cells	–	Aβ clearance	Immune response	Increased
rs9271192	*HLA-DRB5–HLA-DRB1*[g]	0.276	1.11	Immunocompetence and histocompatibility	–	–	Immune response	–
rs28834970	*PTK2B*	0.366	1.1	Induction of long term potentiation in hippocampus	CLU	–	Synapse function and neural development	–
rs11218343	*SORL1*	0.039	0.77	APOE receptor; binds LDL and RAP and mediates endocytosis of the lipids to which it binds	APOE	APP trafficking	Lipid metabolism, synapse function, and endocytosis	Decreased

(*Continued on next page*)

Table 1. *Continued*

SNP	Gene	MAF	Odds Ratio	Known Function	Related AD Risk Genes	Potential Effects on APP and Tau	Pathways	Gene Expression
rs10498633	*SLC24A4* and *RIN3*	0.217	0.91	Brain and neural development (SLC24A4); stimulates and stabilizes GTP-Rab5 in protein transport from plasma membrane to early endosome (RIN3)	BIN1	–	Neural development, synapse function, and endocytosis	–
rs8093731	*DSG2*[h]	0.017	0.73	Mediates cell-cell junctions between epithelial and other cell type	–	–	–	–
rs35349669	*INPP5D*	0.488	1.08	Negative regulator of myeloid cell proliferation and survival	CD2AP	–	Immune response	–
rs190982	*MEF2C*	0.408	0.93	Controls synapse formation during activity-dependent refinement of synaptic connectivity and facilitates hippocampal-dependent learning and memory	–	–	Neural development, synapse function, and immune response	–
rs2718058	*NME8*[i]	0.373	0.93	Ciliary functions	–	–	–	–
rs1476679	*ZCWPW1* and *NYAP1*[j]	0.287	0.91	Epigenetic regulation (ZCWPW1); brain and neural development (NYAP1)	–	–	Neural development	–
rs10838725	*CELF1* and *MADD*[k]	0.316	1.08	Regulates pre-mRNA splicing (CELF1); long-term neuronal viability (MADD)	–	Mediates tau toxicity	Neural development	–
rs17125944	*FERMT2*	0.092	1.14	Actin assembly and cell shape and mediator of angiogenesis	–	Mediates tau toxicity	Cytoskeleton and axonal transport	–
rs7274581	*CASS4*[l]	0.083	0.88	Docking protein in tyrosine-kinase signaling involved in cell adhesion and spreading	CD2AP	–	Cytoskeleton and axonal transport	–
rs75932628	*TREM2*	0.049	2.55	Immune response, triggers production of inflammatory cytokines	–	Aβ clearance	Immune response	–
rs145999145	*PLD3*	0.06	2.75	Unknown	–	APP trafficking and cleavage	Unknown	Increased

[a]*CR2*, *CR1L* also located within GWAS locus.
[b]*CYP27C1* also located within GWAS locus.
[c]*LOC285965*, *TAS2R60* also located within GWAS locus.
[d]*MS4A3*, *MS4A2*, *MS4A6A*, *MS4A4A*, *MS4A6E* also located within GWAS locus.
[e]*EED* also located within GWAS locus.
[f]*CNN2*, *POLR3E*, *GPX4* also located within GWAS locus.
[g]*HLA-DRB6*, *HLA-DQA1*, *HLA-DQB1*also located within GWAS locus.
[h]*GSG2* also located within GWAS locus.
[i]*GPR141* also located within GWAS locus.
[j]*PMS2P1*, *PILRB*, *PILRA*, *C7ORF61*, *C7ORF47*, *MEPCE* also located within GWAS locus.
[k]*SLC39A13*, *PSMC3*, *NDUFS3*, *KBTBD4*, *PTPMT1*, *MTCH2*, *AGBL2*, *FNBP4*, *NUP160* also located within GWAS locus.
[l]*C20ORF43*, *CSTF1* also located within GWAS locus.

structural, functional, and metabolic measures, are significantly heritable (Koten et al., 2009; Thompson et al., 2001). Imaging phenotypes have been used for gene discovery using GWAS approaches and to better understand the phenotypes associated with known genetic risk factors.

The most widely studied candidate gene in imaging studies of AD is *APOE*. *APOE*ε4 carriers demonstrate accelerated loss of gray matter with age (Lu et al., 2011), decreased connectivity in the default mode network (Damoiseaux et al., 2012), increased hippocampal atrophy (Potkin et al., 2009), increased amyloid

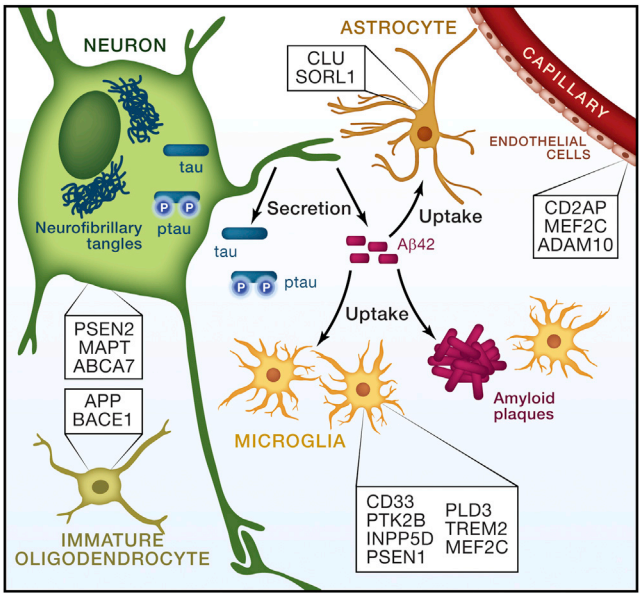

Figure 1. Cell-Type Expression of Alzheimer's Disease Risk Genes May Influence AD Pathogenesis

The major cell types present in the brain are depicted. Genes are listed in the cell type in which they are most highly expressed (B. Barres, personal communication). GWAS loci that contain multiple genes were excluded from this figure, as it remains unclear which gene is responsible for the signal (CR1, BIN1, PICALM, HLA, SLC24A4, DSG2, ZCWPW1, CELF1, FERMT2, CASS4).

deposition, as detected by Aβ ligands (PIB and florbetapir) (Jagust et al., 2012; Ramanan et al., 2014), decreased glucose metabolism (Reiman et al., 2005), and increased numbers of cerebral microbleeds, associated with higher levels of cerebral amyloid angiopathy (Yates et al., 2014). Several studies have shown differences in brain structure and metabolism among individuals carrying *APOE*ε4 (Chen et al., 2010; Schraml et al., 2013) and other AD risk alleles in cognitively normal adults decades before the onset of AD (Biffi et al., 2010; Bralten et al., 2011; Braskie et al., 2011; Erk et al., 2011; Rajagopalan et al., 2013). The late-onset AD GWAS SNP in *BIN1* has been associated with entorhinal cortex thickness (Biffi et al., 2010), while GWAS SNPs in *CR1* have been associated with brain amyloid burden (Thambisetty et al., 2010) and episodic memory decline in healthy and cognitively impaired elders (Keenan et al., 2012), and several *PICALM* SNPs were associated with entorhinal cortex thickness (Biffi et al., 2010; Furney et al., 2011). The Alzheimer's disease Neuroimaging Initiative (ADNI) has made both imaging and genetic data publicly available on 1,650 individuals. As a result, more than 100 genetic studies have been published using this data (reviewed in Shen et al., 2014). A recent GWAS of florbetapir PET signal reported genome-wide significant association with *APOE* and *butyryl cholinesterase* (*BCHE*) SNPs, previously associated with BCHE activity (Ramanan et al., 2014). GWAS of hippocampal atrophy implicated additional risk genes, such as *FRMD6* (Shen et al., 2014), that have subsequently been shown to be associated with disease in AD cohorts (Hong et al., 2012; Sherva et al., 2014), demonstrating the value of using these endophenotypes to augment genetic studies. The Enhancing Neuroimaging Genetics through Meta-Analysis (ENIGMA) Consortium was formed in 2009 to enhance the replicability of GWAS findings for neuroimaging phenotypes (Thompson et al., 2014). Together with the CHARGE consortium, this group has meta-analyzed tens of thousands of individuals with GWAS and MRI scans. These meta-analyses have provided compelling evidence for novel loci influencing hippocampal volume and intracranial volume (Stein et al., 2012).

Fluid Biomarkers

Several studies have examined the genetics of both plasma and cerebrospinal fluid biomarkers. Most studies have focused on Aβ or tau/ptau, two well-studied and validated AD biomarkers (reviewed in Craig-Schapiro et al., 2009). Like the case-control studies, early biomarker studies examined candidate genes and found strong evidence for association between CSF Aβ and *APOE* genotype, providing further evidence that *APOE* influences AD through an Aβ-dependent mechanism (Kauwe et al., 2009; Kim et al., 2011b). Several other candidate genes from case-control GWAS have been examined in the fluid biomarker data sets, e.g., variants in *SORL1*, *CLU*, and *MS4A4A* have been associated with CSF Aβ levels (Alexopoulos et al., 2011; Elias-Sonnenschein et al., 2013), while others found no evidence for association (Kauwe et al., 2011). Subsequent studies have taken a genome-wide approach. However, GWAS of CSF Aβ have not observed genome-wide significant evidence of association for any locus other than *APOE* (Kim et al., 2011b). This suggests that *APOE* genotype explains a large proportion of the variance in Aβ levels and that no other loci have similarly large effects. It is also likely that some of the problems in these analyses may be due to measurement problems for the Aβ phenotype (reviewed in Mattsson et al., 2013). A recent GWAS of plasma Aβ in over 3,500 individuals failed to observe any genome-wide significant signals including *APOE* (Chouraki et al., 2014). It is unclear whether this reflects differences in systemic versus CNS regulation of Aβ levels by *APOE* or evidence of differences in measurement of Aβ across studies that can decrease the power of a meta-analysis or both.

In contrast, CSF tau/phospho-tau studies have demonstrated some success. Initial studies using a candidate gene approach reported association between phospho-tau 181/total tau and SNPs in *PPP3R1*, the regulatory subunit of calcineurin (Cruchaga et al., 2010). The same SNPs were reported to be associated with rate of cognitive decline in this study (Cruchaga et al., 2010) and in an independent cohort (Peterson et al., 2014). Several studies have also reported association between *MAPT* SNPs and CSF tau levels (Kauwe et al., 2008; Laws et al., 2007). Initial GWAS of CSF tau levels reported association with the *APOE* region of chromosome 19 but did not identify novel associations at a genome-wide significant level (Han et al., 2010; Kim et al., 2011b). The largest published CSF GWAS examined 1,269 individuals and reported four genome-wide significant loci in *APOE*, between *GEMC1* and *OSTN* on chromosome 3, at 9p24.2 within *GLIS3* and at 6p21.1 within the *TREM* gene cluster (Cruchaga et al., 2013). The *APOE* association with CSF tau levels was significant even after inclusion of CSF Aβ levels as a covariate, suggesting that *APOE* genotype may influence tau levels by both Aβ-dependent and Aβ-independent mechanisms. SNPs in *APOE* and those between *GEMC1* and

OSTN also showed association with AD risk, tangle pathology and rate of cognitive decline (Cruchaga et al., 2013). Cell and animal studies provide some support for Aβ-independent effects of APOE on tau metabolism (Mahley and Huang, 2012; Wolf et al., 2013).

While Aβ and tau are the most well studied AD fluid biomarkers, several other proteins implicated in AD GWASs have also been examined in genetic studies of AD endophenotypes including CSF and plasma levels of APOE and CLU. SNPs in *CLU* associated with AD risk were also associated with clusterin protein levels in plasma in several studies (Schürmann et al., 2011; Xing et al., 2012). CSF APOE levels appear to exhibit strong genetic control with 72% of the variability in CSF APOE levels explained by SNPs on a GWAS chip (Cruchaga et al., 2012b). The single largest effect was explained by *APOE*ε4 genotype, which explained 8% of the variance. No other genetic variant reached the genome-wide significance threshold, but nine additional variants exhibited a p value $< 10^{-6}$. Pathway analysis indicated that these loci are involved in lipid metabolism.

Sequencing Studies

The most recent GWAS for late-onset AD combined several large data sets and identified more than 20 loci associated with AD risk (Lambert et al., 2013). However, each of these new loci only account for a small percentage of the genetic variance that contributes to AD susceptibility. Thus, a large proportion of the genetic heritability for AD has not been explained by GWAS.

It has been hypothesized that low-frequency (MAF 1%–5%) and rare variants (MAF < 1%) may explain the missing genetic heritability. As was the case with linkage and GWAS, initial sequencing efforts were focused on candidate genes in small populations until the arrival of next-generation sequencing technology, which allowed for low-cost, high-throughput sequencing and ushered in the era of whole-exome and whole-genome sequencing. Even with these new advances, the first studies using this technology were also focused on the pathogenic AD genes. Subsequent genome-wide studies led to the identification of novel variants and genes.

One powerful approach to identify AD risk variants with large effect sizes has been to study families with a strong history of late-onset AD. Studies sponsored both by NIMH and by NIA have invested in the collection and characterization of large family data sets with at least two late-onset AD cases (Wijsman et al., 2011; Bertram et al., 2008).

Resequencing of Pathogenic AD Genes

Although pathogenic mutations in *APP*, *PSEN1*, and *PSEN2* were originally associated with early-onset, familial AD, mutations in these genes have also been reported in families with late-onset AD (Devi et al., 2000; Jayadev et al., 2010; Kauwe et al., 2007; Larner et al., 2007; Tomaino et al., 2007). Mutation screening efforts in *APP* have been historically limited to exons 16 and 17 in early-onset AD families (reviewed in Guerreiro et al., 2012). Screening for *APP* mutations in exons 16–17 in 40 late-onset AD families identified one pathogenic *APP* mutation (E693G) with incomplete penetrance in a single family (reviewed in Guerreiro et al., 2012). Screening for mutations in *PSEN1* has been performed mainly in early-onset AD families, although a small number of late-onset AD cases (>60 years; <100 families) were also screened leading to the report of some late-onset AD families carrying mutations in *PSEN1* (Arango et al., 2001; de Silva et al., 2001; Devi et al., 2000; Kauwe et al., 2007; Larner et al., 2007; Rogaeva et al., 2001; Scacchi et al., 2007; Taddei et al., 2002).

No systematic screen of *APP*, *PSEN1*, and *PSEN2* in late-onset AD families was published until 2012. Twelve missense, nonsense, and splice-site variants were found in 28 families (6.3% of the screened families), including known pathogenic variants in *PSEN1* in seven families (Cruchaga et al., 2012a). Likely pathogenic variants, defined as novel variants that segregated with disease status, were found in *APP*. Similar results were reported in an Ibero-American cohort (Jin et al., 2012), where two known and one novel likely pathogenic mutation in *PSEN1* were found in three of 176 cases. Together, these studies suggest that the impact of mutations in *APP*, *PSEN1*, and *PSEN2* in late-onset AD families is higher than previously estimated, affecting between 1.5% and 2% of all AD families.

Protective APP Variants

It is well known that *APOE*ε4 is associated with increased AD risk and that *APOE*ε2 decreases AD risk, demonstrating that different variants within the same gene may have opposing effects on disease risk. However, most genetic studies of AD have focused on identifying variants that increase AD risk. In fact, until recently, no study had reported a protective variant in *APP*, *PSEN1*, or *PSEN2*. Jonsson et al. reported that rs63750847 (*APP* A673T) was associated with reduced risk for AD (odds ratio [OR] = 0.23) in the Icelandic population (Jonsson et al., 2012). Interestingly, this mutation occurs at the same amino acid residue as a recessive mutation reported to cause early-onset AD (*APP* A673V) (Di Fede et al., 2009). This mutation is near the proteolytic cleavage site of BACE1 (position 2 in the Aβ peptide) and results in impaired BACE1 cleavage of APP in the A673T carriers and reduction of Aβ40 and Aβ42 in vitro (Jonsson et al., 2012). This study provides a proof of principle for the hypothesis that reducing the BACE1 cleavage of APP may protect against AD.

Risk Variants in APP, PSEN1, and PSEN2

In addition to identifying known and likely pathogenic mutations in *APP* and *PSEN1*, the resequencing studies have revealed additional variants that may modify AD risk. Thus far, all of the genetic variants in these genes have been classified as pathogenic or not pathogenic. However, it is likely that rare variants in *APP*, *PSEN1*, and *PSEN2* could also act as risk factors for AD. For example, the *PSEN2* variants, R62H and R71W, failed to show perfect segregation with AD status but were found to be associated with a lower age at onset. The hypothesis that variants in *APP*, *PSEN1*, and *PSEN2* may serve as risk factors for AD is further supported by a later study in which next-generation sequencing technologies and biomarker levels were combined to identify additional risk variants (Benitez et al., 2013). *PSEN1 E318G* (rs17125721) was previously classified as nonpathogenic because it does not segregate with disease status in some families (Lindquist et al., 2009; Sandbrink et al., 1996). This coding variant was strongly associated with CSF total Tau and pTau181 levels (Benitez et al., 2013). Analyses in case-control series demonstrated that in *APOE*ε4 carriers, *PSEN1-E318G* is associated with a 10-fold increase in risk of developing AD and an earlier age at onset of AD (Benitez et al.,

2013). Together, these results suggest that some variants in *PSEN1* and *PSEN2* are risk factors for AD rather than fully penetrant, causative mutations.

The identification of novel variants in genes that are associated with disease may present as a challenge in the clinic. Several groups have developed guidelines for determining whether a novel variant is truly pathogenic (Guerreiro et al., 2010). These guidelines examine segregation and association data. Variants that occur in three or more cases in a single family are classified as pathogenic. Variants occurring in two cases in a single family are probably pathogenic. For variants identified in only one case, variants are classified as probable if present in three cases and absent in at least 100 controls, possible if present in two cases and absent in at least 100 controls, and not pathogenic/risk factor if present in controls. Probable pathogenicity is further confirmed by demonstration of conservation between PSEN1 and PSEN2, presence of pathogenic mutations at the same residue, fitting the helix rule, and altering Aβ levels.

Impact of Dementia Genes in Clinical AD

Mutations in *progranulin* (*GRN*), *microtubule-associated protein tau* (*MAPT*), and the hexanucleotide expansion repeat in *C9ORF72* are established causes of familial frontotemporal dementia (FTD) (DeJesus-Hernandez et al., 2011; Huey et al., 2006; Van Deerlin et al., 2007). However, several studies have reported individuals carrying these mutations presenting with clinical symptoms that are indistinguishable from AD (Brouwers et al., 2007; Cortini et al., 2008; Kelley et al., 2010; Lindquist et al., 2008; Ludolph et al., 2009; Momeni et al., 2009; Rademakers et al., 2003, 2007; Reed et al., 1997). Among those that have come to autopsy, all had a confirmed diagnosis of FTD. Recent studies in which these genes were screened in large clinical series of AD cases suggest that the frequency of pathogenic mutations in *GRN*, *MAPT*, and *C9ORF72* in clinical AD is similar to the frequency of pathogenic mutations in *APP*, *PSEN1*, and *PSEN2* (Cruchaga et al., 2012a; Jin et al., 2012; Wojtas et al., 2012). Overall, more than 67 *GRN* and *MAPT* mutation carriers with AD-like symptoms of dementia have been described (Brouwers et al., 2007, 2008; Carecchio et al., 2009; Cortini et al., 2008; Kelley et al., 2010; Lindquist et al., 2008, 2009; Momeni et al., 2009; Ostojic et al., 2004; Rademakers et al., 2003, 2007; Tolboom et al., 2010), 80% of which have a clear family history of dementia and 72% showed an age at onset $\geq$60 years. These data suggest that there is substantial heterogeneity in the clinical presentation associated with FTD mutations and that clinical cohorts of AD are frequently contaminated with cases of FTD misdiagnosed as AD.

New AD Risk Genes: TREM2

Using whole-exome sequencing and whole-genome sequencing strategies, two groups simultaneously reported a low-frequency variant (*TREM2* R47H; rs75932628) in triggering receptor expressed on myeloid cells 2 protein (*TREM2*) that was associated with an increase in AD risk (Guerreiro et al., 2013a; Jonsson et al., 2013). While there has been some disagreement as to the degree to which *TREM2* R47H increases AD risk, a meta-analysis of all of the reports describing an association between *TREM2* R47H and risk for AD reported that carrying the *TREM2* R47H variant increases risk for AD by 3.4-fold (Guerreiro and Hardy, 2013). Guerreiro et al. described several rare variants in *TREM2* that were observed more frequently in cases than controls, suggesting that there may be multiple rare variants in *TREM2* that increase risk for AD. This is supported by a recent study showing that *TREM2* R47H and *TREM2* R62H are associated with AD risk (Cuyvers et al., 2014). Several studies also suggest that *TREM2* R47H could be associated with Parkinson's disease, frontotemporal dementia, and amyotrophic lateral sclerosis (Guerreiro et al., 2013b; Lattante et al., 2013; Rayaprolu et al., 2013), but this remains controversial. In previous studies, rare homozygous loss-of-function mutations in *TREM2* were associated with an autosomal recessive form of early-onset dementia, polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy (PLOSL) (Paloneva et al., 2003). Subsequent studies found *TREM2* mutations in three patients with frontotemporal-like dementia without any bone-associated symptoms (Guerreiro et al., 2013b). These observations highlight a recurring theme in neurodegenerative diseases: that heterozygous and homozygous mutations in the same gene can lead to clinically distinct disorders (Hruska et al., 2008; Singleton et al., 2010; Smith et al., 2012; Swan and Saunders-Pullman, 2013).

TREM2 is a type one transmembrane receptor protein expressed on myeloid cells including microglia, monocyte-derived dendritic cells, osteoclasts, and bone-marrow-derived macrophages (reviewed in Colonna, 2003). TREM2 transduces its intracellular signaling through TYROBP. Although the natural ligands of TREM2 remain speculative, upon ligand binding TREM2 associates with TYROBP to mediate downstream signaling. In the brain, TREM2 is primarily expressed on microglia and has been shown to control two signaling pathways: regulation of phagocytosis and suppression of inflammation reactivity (reviewed in Colonna, 2003). The identification of coding variants in *TREM2* that increase risk for AD supports the role of the immune response and inflammation in AD pathogenesis. However, the mechanism by which these variants increase risk for AD remains unknown. Coding variants in other genes in the *TREM*-gene family may also modify AD risk as is the case for a common protective variant in *TREML2* (Benitez et al., 2014).

New AD Risk Genes: PLD3

Applying a family-based design to identify novel AD risk genes revealed several rare, missense, and synonymous variants in *phospholipase D3* (*PLD3*) associated with AD risk (Cruchaga et al., 2014). Whole-exome sequencing identified one variant in *PLD3* (*PLD3* V232M; rs145999145), which segregated with disease status in two of 14 independent NIA-LOAD families. Follow-up studies in several case-control series demonstrated that this variant is associated with a 2- to 3-fold increase in risk for AD. Resequencing of *PLD3* in over 4,000 AD cases and controls revealed a significant excess of coding variants in cases compared to controls in both European and African American samples.

PLD3 is a nonclassical phospholipase. Little is known regarding the function of PLD3 or its role in the brain. Previous studies have reported a link between PLD1 and PLD2, classical phospholipases, and APP metabolism (Cai et al., 2006a, 2006b). In cell models of APP processing, PLD3 also influences APP metabolism such that overexpression leads to lower Aβ levels, while knockdown of PLD3 leads to increased levels of Aβ

(Cruchaga et al., 2014). Additional studies will be needed to elucidate the specific function of PLD3 in the brain and the pathogenic mechanism associated with the risk variants.

Disease Mechanisms Implicated by Studies of Genetic Risk Factors for AD

Early-onset AD mutations in *APP*, *PSEN1*, and *PSEN2* lead to altered production or ratios of Aβ isoforms in the brain, while *APOE* influences Aβ clearance and aggregation; both observations support the hypothesis that Aβ levels are critical for disease pathogenesis. GWASs have now identified polymorphisms in or near more than 20 genes that are associated with AD risk (Table 1). The identification of common variants that have small effects on AD risk has created a broader picture of the processes and pathways involved in AD risk including lipid metabolism, the inflammatory response, and endocytosis (Figure 1). Whole-genome/exome sequencing studies have also identified risk alleles in *TREM2* and *PLD3*. The identification of rare variants in the population that have moderate to large effects on AD risk will be most valuable in identifying pathways that are central to disease pathogenesis. The majority of the genes recently identified affect Aβ production and clearance, highlighting the importance of this pathway in AD pathogenesis.

Lipid Metabolism

APOE and several recent late-onset AD GWAS genes are involved in lipid metabolism: *CLU*, *ABCA7*, and *SORL1* (Harold et al., 2009; Hollingworth et al., 2011; Lambert et al., 2009, 2013; Naj et al., 2011) (Table 1). APOE probably affects Aβ metabolism directly by binding to Aβ or indirectly by binding to lipoprotein receptors that play a role in Aβ clearance (reviewed in Holtzman et al., 2012). ABCA7 may influence AD risk via its ability to transfer cholesterol to APOE and modulate Aβ levels or by clearing Aβ aggregates (Chan et al., 2008; Kim et al., 2013). Clusterin (Apolipoprotein J) also plays a role in Aβ clearance. APOE- and Clusterin-deficient APP transgenic mice exhibit earlier and more extensive Aβ deposition than control mice (DeMattos et al., 2004). Genes involved in lipid metabolism share a potential role in clearance of highly amyloidogenic Aβ from the brain. So polymorphisms in these genes that modify efficiency of Aβ clearance could have deleterious effects in the brain.

Immune Response

Neuroinflammation and dysregulation of the immune response is a central feature of AD (reviewed in Holtzman et al., 2011). Common variants have been identified in several genes that are associated with late-onset AD in GWASs: *CR1*, *CD33*, *MS4A*, *CLU*, *ABCA7*, *EPHA1*, *HLA-DRB5-DRB1*, and *INPP5D* (Bertram et al., 2008; Harold et al., 2009; Hollingworth et al., 2011; Lambert et al., 2009, 2013; Naj et al., 2011) (Table 1). Rare coding variants were also identified in *TREM2* in sequencing studies of late-onset AD cohorts (Guerreiro et al., 2013a; Jonsson et al., 2013). Elevated complement cascade activity could exacerbate AD pathology; thus, individuals with CR1 variants that dampen the complement response may be at lower risk for developing AD pathology (Liu and Niu, 2009; Shen et al., 2001). CD33 may play a role in Aβ clearance and other neuroinflammatory pathways mediated by microglia in the brain (Griciuc et al., 2013). TREM2 may influence neurodegeneration, possibly through clearance of protein aggregates or via neuroinflammatory mechanisms (Colonna, 2003). Interestingly, TREM2, like APP, is cleaved by γ-secretase (Wunderlich et al., 2013). Extensive evidence from human studies and mouse models demonstrates that amyloid plaques are surrounded by activated immune cells. Interestingly, transcriptomics of unique cell populations in the mouse brain reveal that one-third of late-onset GWAS genes are differentially expressed at high levels in microglia (B. Barres, personal communication). Thus, polymorphisms in immune response genes that exacerbate or dampen the immune system's response to accumulating Aβ may contribute to AD pathogenesis.

Endocytosis

Endocytosis is critical for normal processing of APP, which is central to AD pathogenesis. Furthermore, synaptic activity and neurotransmitter release is disrupted in AD (reviewed in Holtzman et al., 2011). Genes associated with endocytosis and synaptic function were identified in several late-onset AD GWAS: *BIN1*, *PICALM*, *CD2AP*, *EPHA1*, *SORL1*, *RIN3*, *MEF2C*, and *MADD* (Harold et al., 2009; Hollingworth et al., 2011; Lambert et al., 2009; Lambert et al., 2013; Naj et al., 2011) (Table 1). APP, Aβ, and APOE are internalized through the endosomal-lysosomal pathway, in which BIN1 plays a critical role (Chapuis et al., 2013; McMahon et al., 1997). PICALM-mediated Aβ generation and clearance may influence accumulation of Aβ in AD brains (Xiao et al., 2012). CD2AP is required for synapse formation (Dustin et al., 1998), where it associates with CBL, endophilin, and synaptojanin. EPHA1 plays roles in cell and axonal guidance and synaptic plasticity (Lai and Ip, 2009). SORL1 directs APP to endocytic pathways for recycling (Rogaeva et al., 2007) and plays an important role in Aβ generation (Schmidt et al., 2007; Spoelgen et al., 2006). Indeed, small molecules that increase the stability and function of the retromer complex reduce the amount of APP in the endosomes and reduce Aβ levels, suggesting that this could be a possible therapeutic avenue (Mecozzi et al., 2014). In cell and animal models of AD, synaptic activity alters Aβ (Cirrito et al., 2005, 2008); thus, polymorphisms in genes that influence synaptic function and normal processing of APP and Aβ are likely to contribute to AD pathogenesis.

Tau

While all of the AD genes identified by linkage studies and the majority of genes identified by GWASs and sequencing studies have been implicated in pathways related to APP and Aβ metabolism, some genes are also implicated in tau metabolism. Tau is the central component of neurofibrillary tangles, which are highly predictive of AD progression. Recent CSF GWASs have demonstrated that *APOE* genotype produces an Aβ-independent effect on CSF tau levels, suggesting that APOE could influence tau accumulation in the brain (Cruchaga et al., 2013). Decreased expression of the *Drosophila* ortholog of BIN1 is associated with decreased tau-mediated toxicity in a *Drosophila* model (Chapuis et al., 2013). Similarly, the *Drosophila* orthologs to CD2AP, FERMT2, and CELF1 were identified as modifiers of tau-mediated toxicity (Shulman et al., 2014). There is increasing evidence that tau is released from the cell and that this release may be modified by synaptic activity (Karch et al., 2012, 2013; Pooler et al., 2013; Yamada et al., 2014). Coupled with in vitro and in vivo evidence of spreading of tau aggregates, these

observations lead us to hypothesize that genetic variants that alter synaptic function, such as *BIN1*, *CD2AP*, *PTK2B*, *MEF2C*, *NYAP1*, and *MADD*, may influence tau release and spreading of tau pathology in the brain (Table 1).

Cell-Type-Specific Expression of LOAD Risk Genes

Our understanding of the role that APP processing plays in AD pathogenesis has been informed largely by the familial AD genes *APP*, *PSEN1*, and *PSEN2*. For these genes, there is a clear link between altered Aβ production by neurons and development of disease. However, with the identification of additional common risk loci by GWASs and rare risk variants by WES and WGS, we are gaining a more complete picture of the important role that supporting cells play in AD pathogenesis (Figure 1). Many genes that fall within the GWAS loci are highly expressed in microglia and astrocytes (Figure 1). In an unbiased approach, we mined a gene expression data set from specific cell types isolated from mouse brains, which included astrocytes, neurons, oligodendrocytes, microglia, and endothelial cells (B. Barres, personal communication). In searching LOAD risk genes identified by GWAS and sequencing methods, we found that many of these genes were most highly expressed in cells other than neurons. In Figure 1, we list genes based on the cell type in which they are most highly expressed in the mouse brain. Considering the expression pattern of these genes in the brain may be very meaningful in determining the mechanisms underlying disease. For example, clearance of Aβ by microglia and astrocytes may play a more important role in LOAD than Aβ production by neurons.

Future Directions for Genetic Studies

Substantial progress has been made during the last 5 years toward understanding the genetic architecture of AD, because of the technological advances in genotyping and sequencing that have made it feasible to genotype or sequence thousands of individuals. GWASs have now identified more than 20 loci that influence risk for AD. It is clear from these studies that with the exception of *APOE*, common risk alleles for AD have modest effects on risk individually but they have pointed to a number of important disease pathways. A recent study indicates that common variants explain 33% of total phenotypic variance. *APOE* alone explained 6% and other known markers 2%, meaning more than 25% of phenotypic variance remains unexplained by known common markers (Ridge et al., 2013). It is expected that studies using even larger sample sizes will identify additional loci, most likely with smaller effect sizes. Identification of additional loci will probably implicate further biological pathways in disease risk, but it is unlikely that GWAS SNPs will be used to predict individual risk for AD. In other disorders where even larger samples have been genotyped, more risk loci have been identified, suggesting that there is some value to continuing to increase the number of samples with GWAS data. While very large sample sizes have been examined for people of European descent, this is not true for other populations. Large GWASs in these populations may identify genes not detected in Europeans. In addition, other AD-related phenotypes including rate of progression of AD, rate of cognitive decline, and age at onset of AD have yet to be examined in the largest data sets.

An alternative approach to increasing sample size is to use gene-based or pathway-based analyses to identify the genes, that while not genome-wide significant are overrepresented among the SNPs with low p values in GWASs. This approach has identified additional significant genes that further implicate immune regulation, energy metabolism, and protein degradation in AD risk (Jones et al., 2010). Another approach has compared GWAS data across related disorders to identify common underlying genes demonstrating a genetic link between cardiovascular disease and AD risk (Liu et al., 2014; Moskvina et al., 2013). A challenge for the field remains follow-up of the GWAS loci to identify the specific functional alleles and the specific mechanisms underlying the GWAS signals.

We anticipate that similar success in identifying novel genes will be observed as large-scale sequencing projects begin to yield results. Early studies, in small discovery data sets, have already identified several promising genes that modify AD risk (Cruchaga et al., 2014; Guerreiro et al., 2013a; Jonsson et al., 2013). So far these studies have largely focused on whole-exome sequencing and have identified coding variation that alters risk for AD by as much as 2- to 3-fold. This has the advantage of making functional follow-up of the genes identified through sequencing substantially easier than those identified in GWASs. Several groups are currently performing whole-exome sequencing or whole-genome sequencing in unrelated AD cases and controls and in families multiply-affected by AD. It is anticipated that whole-exome sequencing or whole-genome sequencing will be available on more than 20 thousand well-characterized samples before the end of 2014. Both the GWAS data and the sequencing data will be available through public repositories such as NIAGADS and dbGaP. Combining this data with other large data sets such as transcriptomics from human brain tissue or induced pluripotent stem cell-derived neurons and glia will provide further insight into the disease pathways, and regulatory nodes within these pathways that may provide druggable targets for future therapies. An early application of this integrative network-based approach used brain tissue from over 1,600 AD cases and nondemented individuals to identify an immune- and microglia-specific module that is dominated by genes involved in pathogen phagocytosis (Forabosco et al., 2013; Zhang et al., 2013). This module contains TREM2 (identified by WES) but shows that TYROBP (a.k.a. DAP12, which binds to TREM2) is a key regulator of the network and is upregulated in AD brains. Mouse microglia overexpressing intact or truncated TYROBP revealed expression changes that significantly overlapped the human brain TYROBP network. Interestingly, LOAD GWAS genes MS4A4A, MS4A6A, and CD33 were also identified in the module that contains TYROBP (Zhang et al., 2013). The causal network structure was able to predict response to gene perturbations and thus presents a useful framework to test models of disease mechanisms underlying AD.

Using Genetics to Provide Insights into Clinical Research

The AD field as a whole has struggled to move drugs and potential druggable targets from mouse models into effective therapies. In part, this may be due to the fundamental study design

and patient recruitment of Phase 3 clinical studies (Bateman et al., 2011; Moulder et al., 2013). The majority of Phase 3 clinical trials have used individuals with mild to moderate dementia and a clinical diagnosis of late-onset AD, which is known to be a heterogeneous disease. Recent studies have highlighted that many people enrolled in these clinical trials were amyloid negative based on neuroimaging data and therefore unlikely to respond to an antiamyloid treatment (Vellas et al., 2013). A drug's impact on halting or slowing disease progression is hard to assess when power is drastically reduced by the presence of non-AD cases among the patients enrolled in the study. A second problem with these trials has been that a drug that targets Aβ may not be effective in symptomatic AD cases because Aβ deposition occurs in the preclinical phase of the disease and thus reversal of Aβ deposition after clinical symptoms appear may have no impact on disease progression. Identifying individuals with presymptomatic disease would require large-scale screening in most populations.

One way to address these issues is to focus trial design on individuals who will develop AD, with a known age at onset and disease progression. Dominantly inherited AD cases meet these criteria since mutation carriers will all develop AD and usually do so with an age at onset similar to that observed in their parents and other family members. Second, because the mutations in these families cause disease by increasing Aβ production, a therapy designed to remove Aβ or inhibit Aβ production is directly targeting the disease mechanism. The Dominantly Inherited Alzheimer Network (DIAN) was initiated 6 years ago to recruit families that carry *APP*, *PSEN1*, and *PSEN2* mutations. Observational studies in these families have demonstrated that biomarker changes can occur 15–20 years prior to the expected age at onset of AD (Fagan et al., 2014; Potter et al., 2013), providing a large window of opportunity for presymptomatic clinical trials. Two clinical trials in presymptomatic, biomarker-positive mutation carriers have started within the last year. The DIAN study is testing two anti-Aβ antibodies while the Alzheimer's disease Prevention Initiative is testing a different Aβ monoclonal antibody in presymptomatic individuals from the Colombian kindred carrying the *PSEN1* E280A mutation. Initial outcomes in these trials will be a change in fluid and imaging biomarkers, with follow-up studies assessing clinical and cognitive outcomes (Moulder et al., 2013). For the first time, these clinical trials will provide a true test of the β-amyloid cascade hypothesis, more than 20 years after the initial hypothesis. This is perhaps fitting given that the hypothesis arose around initial genetic discoveries in these and other families.

Conclusion

The identification of common and rare variants that contribute to AD risk has provided many new opportunities to understand the mechanisms underlying AD. GWASs and next-generation sequencing studies have revealed genes that fall into several common pathways that were previously known to be important in AD pathogenesis: lipid metabolism, immune response, and endocytosis/synaptic function. As whole-genome and whole-exome sequencing studies in large data sets are completed, many more genes will likely be added to this list. As we gain a more complete picture of the genes and pathways dysregulated in AD, we will be able to develop better, more targeted therapeutics.

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