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Untangling Genetic Risk for Alzheimer's Disease

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Abstract

Alzheimer's disease (AD) is a genetically heterogeneous neurodegenerative disorder caused by fully penetrant single gene mutations in a minority of cases, while the majority of cases are sporadic or show modest familial clustering. These cases are late-onset and likely result from the interaction of many genes and the environment. More than thirty loci have been implicated in AD by a combination of linkage, genome-wide association and whole genome/exome sequencing. We have learned from these studies that perturbations in endolysosomal, lipid metabolism and immune response pathways substantially contribute to sporadic AD pathogenesis. We review here current knowledge about functions of AD susceptibility genes, highlighting cells of the myeloid lineage as drivers of at least part of the genetic component in late-onset AD. Although targeted resequencing utilized for the identification of causal variants has discovered coding mutations in some AD-associated genes, a lot of risk variants lie in non-coding regions. Here we discuss the use of functional genomics approaches that integrate transcriptomic, epigenetic and endophenotype traits with systems biology in order to annotate genetic variants, and to facilitate discovery of AD risk genes. Further validation in cell culture and mouse models will be necessary to establish causality for these genes. This knowledge will allow mechanism-based design of novel therapeutic interventions in AD and promises coherent implementation of treatment in a personalized manner.

Keywords

Alzheimer's disease; functional genomics; genome-wide association studies; lipid metabolism; endolysosomal pathway; immune response

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Introduction

Alzheimer's disease (AD) is the most common form of neurodegeneration characterized clinically by the presence of short-term memory loss, impaired judgement and problem solving as well as changes in mood and behavior, together resulting in significant familial and social burden. Pathologically AD is characterized by the accumulation of extracellular amyloid-β (Aβ) plaques and hyperphosphorylation of tau protein aggregated in intraneuronal neurofibrillary tangles (1). There is also substantial neuronal loss in hippocampus and entorhinal cortex (2), and marked gliosis (3). AD affects approximately 5% of people over 65 years old and prevalence doubles with every 5 years of increasing age (4). Initial insight into the pathogenesis of AD came from genetic studies of early-onset familial forms that are caused by mutations in amyloid-β precursor protein (*APP*) (5), presenilin 1 (*PSEN1*) (6) and presenilin 2 (*PSEN2*) (7, 8) inherited as an autosomal dominant trait (9). These findings led to the proposal of "amyloid cascade hypothesis", which postulates that dysregulation of Aβ peptide production and degradation underlies the pathological and behavioral changes observed in AD patients (10). However, the majority of cases are late-onset AD (LOAD) and sporadic with an unknown cause. According to twin studies the heritability of AD is ~58% (11), suggesting that both genetic and non-genetic variation influence disease, e.g. environmental and epigenetic factors, somatic mutations. Indeed, a study of identical twins discordant for AD has shown reduced DNA methylation in temporal neocortex neuronal nuclei of the AD-affected twin (12).

Early-onset Alzheimer's Disease

The main factors influencing early-onset AD are coding mutations or copy number changes in genes that regulate Aβ production and degradation. Aβ is generated by sequential cleavage of APP by β- and γ-secretases. Overproduction of Aβ is a recognized AD risk factor observed in Down syndrome cases that possess chromosome 21 trisomy encompassing *APP* locus (9), and *APP* duplication cases because of copy number changes (13, 14). Most *APP* pathogenic mutations occur around the Aβ cleavage sites affecting APP processing by secretases, e.g. *APP-KM670/671NL* (15) or *APP-E682K* (16) at the β-secretase cleavage site, which increase Aβ production. Mutations in the Aβ sequence have the potential to affect its biophysical properties, such as hydrophobicity and aggregation rate, while C-terminal Aβ mutations at the γ-secretase site influence the Aβ42 to Aβ40 ratio (17). Mutations in *PSEN1* and *PSEN2* that form the active core of γ-secretase complex, affect endopeptidase- or carboxypeptidase-like activity, shifting production of Aβ40 and Aβ42 to longer and more neurotoxic species, e.g. Aβ43 in the case of *PSEN1-R278I* (18, 19) or *PSEN1-L435F* (20), which also shows a dramatic reduction in total Aβ production. Thus, the toxic dysfunction mechanism is used to describe AD-related genetic changes in γ-secretase (19). Indeed, evaluation of heterozygous null *PSEN1* mutation in genome-edited induced pluripotent stem cells (hiPSc)-derived human neurons shows reduced level of γ-secretase, but no effect on Aβ levels, supporting the toxic gain of function model (21). Interestingly, a study of 138 mutations in *PSEN1* concluded there is no correlation between Aβ42/Aβ40 ratio and AD age-at-onset, based on the *in vitro* assay used in this study, suggesting that Aβ levels may not be the sole driving factor and that other genetic and

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environmental factors contribute to disease progression (22). To date sequencing of β-secretase (*BACE1*) has not identified mutations that influence AD risk (23). However, two rare coding variants in α-secretase *ADAM10* (Q170H and R181G) have been reported in familial LOAD (24), and both mutations show impaired activity due to incorrect ADAM10 folding and elevated plaque load in APP transgenic mice (25).

Late-Onset Alzheimer's Disease

Genetic risk factors play a critical role in AD susceptibility. The "common disease – common variant" hypothesis proposes that a combination of multiple common variants and environmental factors underlie disease risk (26). Technological advances in high-throughput genotyping and sequencing allow testing of tens of thousands of control and patient samples that can be used to conduct genome-wide association studies (GWAS). GWAS report genetic variants and loci that are enriched in populations with a disease trait compared to unaffected individuals. Several GWAS of AD were performed (27–31) and later combined in a meta-analysis (32) reporting more than twenty AD susceptibility loci in European populations (33). *APOE* is the most significant risk factor confirmed in all studies across populations. While the largest GWAS have been performed in European populations, a GWAS in African Americans identified variants in *APOE* and *ABCA7* as genome-wide significant (34). A GWAS in Asian populations identified AD-associated genome-wide significant variants in or near *APOE* and *SORL1* (35). Given the vastly different sizes of the datasets in these GWAS, European cohorts remain the most powerful for gene discovery. Nevertheless, it is important to establish the contribution of these loci to risk in other populations. Half of identified susceptibility loci have minor alleles that are protective, thereby increasing AD age-at-onset. Furthermore, the known risk loci do not fully explain the genetic component of AD estimated at 58% based on twin studies (11).

Functional Genomics

GWAS are extremely useful as a way to identify association for multifactorial traits such as AD that have genetic and environmental components and don't rely on family history like linkage studies. However, large sample sizes are required and most signals have small effect. Furthermore, associations are reported for an index single nucleotide polymorphism (SNP) with the lowest *P* value, but in reality can be driven by any variant in the linkage disequilibrium (LD) block (33). Loci identified by GWAS often contain multiple genes that could all contribute to disease association, or only one of the genes in the locus could be causative. As a result labeling a gene within a locus in Manhattan association plots could be misleading (Figure 1), since it suggests assignment of causation, which is not possible based on GWAS alone.

It is the goal of functional genomics to make sense of genomic and transcriptomic data to uncover the mechanisms underlying SNP associations with disease. Foremost, association of genetic variation with AD endophenotypes that characterize disease progression and correlate with pathology can help prioritize SNPs that modify AD risk. As such, cerebrospinal fluid (CSF) levels of Aβ42 and tau/p-tau_{181}, and pathological traits in brain tissue such as plaque and tangle density, brain atrophy and cognitive impairment have been

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used as quantitative traits in genetic association analyses (36). For example, *APOE4* genotype is the strongest marker associated with accelerated grey matter atrophy as well as lower Aβ42 and higher tau/p-tau$_{181}$ levels in CSF (37–39).

Genomic regions where differences in gene expression are associated with SNP genetic variation are named expression quantitative trait loci (QTL) (40). Gene expression has been analyzed in normal human tissue (GTEx Consortium (41)), regional brain tissue (BRAINEAC database (42)) and prefrontal cortex of aging and demented people (ROS/MAP project (43, 44)). The disadvantage of these datasets is that tissues are not homogenous and thus underrepresented cell populations may be beyond the resolution of current datasets. Indeed, attempts to identify brain tissue eQTLs corresponding to AD GWAS loci have not produced compelling associations, with marginal results that do not replicate across datasets. Analyses of primary cell-type specific expression from the Immune Variation (ImmVar) project have shown that AD susceptibility alleles are enriched among eQTLs in monocytes, but not T cells (45). Based on this observation, evaluation of eQTLs in primary cells at baseline and under stimulated conditions in patient samples may help decipher the causal relationship between genetic and phenotypic variation.

Analyses of genomic sequence can provide information to categorize functional SNPs if found in regulatory regions (Figure 1), which include any of the elements involved in transcription and translation, such as enhancers, promoters, untranslated regions, introns, histone marks, etc. and lead to changes in chromatin state causing changes in expression or mRNA splicing captured by expression, splicing and methylation QTL (46, 47). AD-related methylation changes have been detected near known GWAS genes *ABCA7* and *BIN1* and novel genes *ANK1*, *RHBDF2*, *CDH23* and *RPL13* (48, 49). A study of chromatin state alterations in human samples found an upregulation of immune response genes and regulatory regions that are targeted by *SPI1*, a myeloid specific transcription factor (50). Furthermore, protein QTL can be used to map loci that affect protein abundance, which when coupled with GWAS can reveal networks of protein-protein interactions (51). Other epigenomic datasets are being generated by consortia such as PsychENCODE (52), the NIH ROADMAP Epigenomics (53), BLUEPRINT Epigenome (54), Accelerating Medicines Partnership for AD (AMP-AD) (55) and CommonMind (56), and will facilitate large-scale integrative functional genomics analyses.

A complementary approach to functional genomics uses systems biology to infer multi-scale networks, which are effective in identifying co-expressed gene modules enriched in functional categories. These gene modules can be used to generate hypotheses for further experimental testing. However, prior knowledge is rarely verified experimentally and annotations lack context- and cell-specific functions of each gene, thus prohibiting modeling of dynamic processes, such as disease progression. Analyses of networks in samples from AD patients versus control individuals revealed differentially regulated nodes of immune-related genes, governed by *TYROBP* (57), an adaptor protein DAP12 expressed in microglia that is required for TREM2 signaling. Whole genome sequencing in patients with sporadic early-onset AD has identified rare coding variants in *TYROBP* that perturb expression levels of TREM2 and TYROBP *in vitro* (58), confirming the significance of this module in AD risk. A proteomic study of cortical tissue from AD patients reported enrichment of AD

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GWAS candidates in microglial protein networks, supporting a causal role for myeloid cells in AD (59).

While GWAS enable the identification of common variants, usually with small effect size, other approaches are needed to identify rare variants. The most commonly used approaches are whole exome (WES) and whole genome sequencing (WGS). WGS provides the most comprehensive survey of the genome including regulatory regions not covered by WES. Like GWAS these studies may be performed in large unrelated cohorts, in isolated populations or in families. When studying rare variants, one advantage of families is that a rare variant discovered in one family member will be enriched in the remaining family members allowing analysis of segregation with disease. Most of the published WES/WGS studies have been relatively small and have focused on families/isolated populations. deCODE Genetics has used WGS in the Icelandic population to identify rare variants in *APP* (60), *TREM2* (61, 62) and *ABCA7* (63) that influence risk of AD. The *TREM2* (64, 65) and *ABCA7* (66, 67) findings have been widely replicated. Other studies using late onset AD families have identified *PLD3* (68), *UNC5C* (69) and *AKAP9* (70), but these await replication in larger cohorts.

Analysis of GWAS and gene expression data has highlighted four pathways enriched for AD association: cholesterol metabolism, immune response, regulation of endocytosis and protein ubiquitination (71). Below we discuss a selection of genes that fall into these categories reviewing experimental evidence for their contribution to AD (Table 1).

Lipid Metabolism

Apolipoprotein E (*APOE*) is the most important genetic AD risk factor influencing prevalence and age-at-onset. *APOE* association was originally identified from linkage studies and explains 15–20% of AD heredity. Two coding SNPs define six *APOE* genotypes – *ε2/ε2*, *ε2/ε3*, *ε3/ε3*, *ε2/ε4*, *ε3/ε4*, *ε4/ε4* listed from lowest to highest risk for AD (72, 73). *APOE* is the major apolipoprotein expressed in human brain primarily by astrocytes, is involved in cholesterol homeostasis and has been extensively studied in AD (74). *APOE* influences Aβ plaque load in an isoform-specific manner in APP transgenic mice, with highest amyloid-β deposition in human knock-in *APOE4* genotype lines compared to *APOE3* and *APOE2* (75). This effect can be explained by decreased Aβ clearance and/or facilitation of Aβ fibrillogenesis that is due to isoform-dependent differences, because APOE4 shows lower binding of Aβ and is degraded more rapidly through lipoprotein receptors (74, 76, 77). APOE contributes to synapse pruning by astrocytes (78) and together with Clusterin (*CLU*) is induced after injury in astrocytes and microglia promoting neuronal survival (74). *CLU* is primarily expressed in astrocytes and is involved in lipid transport, apoptosis and immune response. The minor allele of rs1113600, located in the intron of *CLU* is associated with reduced AD risk (28, 29), however, no eQTL was found in the locus (79).

CLU can bind Aβ and influence fibril formation *in vitro*. Deficiency of either *APOE* or *CLU* in APP transgenic mice does not affect Aβ deposition, but significantly reduces fibrillar Aβ in brain. Interestingly, double *APOE* and *CLU* knock-out increases plaque load, possibly

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through reduced clearance of Aβ in brain parenchyma corroborated by higher Aβ levels in CSF (80). The risk allele of rs1113600 has been associated with lower white matter integrity (81) and reduced connectivity between hippocampus and frontal cortex (82) in healthy individuals. Targeted resequencing of *CLU* in one study identified rare coding variations in the β-chain that were enriched in AD patients independent from rs1113600 association (83). These non-synonymous mutations and small insertion-deletions were subsequently shown to be associated with altered cellular localization and diminished extracellular secretion of CLU (84).

ATP-binding cassette transporter A7 (*ABCA7*) is universally expressed in brain and involved in lipid transport modulating lipid efflux. *ABCA7* is a highly replicated genetic risk factor for AD in individuals of European and African American ancestry (30, 32, 34). Deep sequencing in African Americans carrying risk and protective alleles at *ABCA7* led to the identification of a 44 base pair deletion in LD with the high risk allele, which results in a frameshift (85). Other analyses in European descent cohorts have identified several rare variants in *ABCA7* resulting in frameshift mutations and deletions, missense or splicing site variants that are enriched in AD cases, presumably leading to early stop codons and loss-of-function alleles (63, 86). Overexpression of *ABCA7* potentiates phagocytosis in macrophages (87) and decreases neuronal APP processing *in vitro* (88). *ABCA7* deficiency accelerates amyloid-β deposition in APP-J20 (89) or APP/PS1 (90) mouse models of amyloidosis without effect on cognition, and in humans loss-of-function alleles are associated with cortical and hippocampal atrophy (91).

Regulation of Endocytosis

Cellular trafficking has long been implicated in AD pathogenesis corroborated by the association of the sortilin-related receptor L (*SORL1*) with AD in case-control studies (92). LOAD GWAS identified rs11218343, a common variant in *SORL1* in European (32) and Asian populations (35). Rare variants in *SORL1* were also found in several families with autosomal dominant early-onset AD (93). Overexpression of *SORL1* in cell lines reduces Aβ production through increased retention of APP in the Golgi (94), while overexpression of the AD associated SORL1-G511R variant results in decreased binding and turnover of Aβ (95). Ablation of *SORL1* in APP/PS1 mice leads to increased plaque deposition, similar to the effect of *SORL1* KO on endogenous murine Aβ production (96).

Bridging integrator 1 (*BIN1*) participates in the endocytic trafficking of synaptic vesicles through membrane remodeling in neurons (97). The index SNP rs6733839 in the *BIN1* locus has been associated with AD risk in different populations (32, 98, 99). Fine-mapping of the *BIN1* locus identified rs59335482, a 3 base pair insertion ~28 kb upstream of *BIN1*, that is associated with higher AD risk, increased transcriptional activity *in vitro* using a luciferase assay, and higher *BIN1* levels (97). However, contrary evidence demonstrated that knock-down of BIN1 increases tau aggregation in neurons through an enlargement of Rab5-positive vesicles (100), and reduces lysosomal degradation of BACE1 thereby increasing Aβ production (101). Since BIN1 is largely expressed in mature oligodendrocytes and white matter (102), it is unclear how it could affect AD pathology in neurons.

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CD2-associated protein (*CD2AP*) is an adaptor and scaffolding protein, and its locus is an AD risk factor identified through association of an intronic variant rs10948363 (27, 30, 32). *CD2AP* affects APP endocytosis in neurons, but shows a mild effect on Aβ levels *in vitro* and no effect on plaque load in APP/PS1 mice with *CD2AP* haploinsufficiency (103, 104). *CD2AP* is expressed in endothelial cells and ablation of *CD2AP* in mice leads to reduced blood-brain barrier integrity, suggesting its contribution to AD may be APP independent (105).

Phosphatidylinositol-binding clathrin assembly gene (*PICALM*) is nominally associated with AD through protective variants rs10792832 and rs3851179, which are located 40 kb upstream of *PICALM*, which is the nearest gene (29, 32). Studies of *PICALM* in neuronal cells show that it regulates cleaved APP C-terminal fragment degradation via autophagosomes (106) and clathrin-mediated endocytosis of gamma-secretase (107). However, the effect on Aβ levels *in vitro* and amyloid-β load *in vivo* is variable and may depend on the level of *APP* overexpression (106, 108, 109). Interestingly, *PICALM* is reduced in brain endothelium from AD patients, and *PICALM* haploinsufficiency in an AD mouse model led to a reduction in Aβ clearance through the blood-brain barrier and concomitant increase in amyloid-β load (110).

These genes suggest a defect in synaptic function, further supported by the association of *MEF2C* and *PTK2B* loci with AD progression (32). MEF2C and PTK2B are reported to be involved in regulation of hippocampal synapses and long-term depression, respectively (111, 112), although the functional consequences of variation in these loci awaits validation. One should interpret these data with caution, as validation was mostly performed in cell lines or neurons in relation to APP and secretase trafficking. However, *SORL1*, *BIN1*, *CD2AP* and *PICALM* gene expression is substantial in microglia (113), and in the context of AD defective internalization of Aβ, APOE, CLU and tau has been reported.

Immune Response

The third group of genes involved in AD pathogenesis based on genetic studies belongs to immune system pathways. Complement receptor 1 (*CR1*) is a highly replicated AD risk factor associated with an intronic SNP rs6656401 but also in high LD with 2 other genes of the complement family (28, 32). CR1 is expressed on blood cells and microglia, and its function is to inhibit complement activation through C3b and C4b (114). The complement system is activated in AD (115), while in mice challenged with oligomeric Aβ it leads to increased synapse elimination by phagocytic microglia (116). Intragenic copy number variation in *CR1* is associated with AD risk, which functionally results in overproduction of the longer CR1 isoform increasing the number of C3b/C4b sites, which might explain the AD risk in this locus (117).

CD33 is a receptor for sialic acid-modified proteins that is found on myeloid and microglia cells and is involved in the anti-inflammatory immune response. Association of *CD33* with AD risk was identified by GWAS through rs3865444 located in the promoter region (27, 30). Although *CD33* was only suggestively associated with AD risk in the largest meta-analysis of AD GWAS (i.e. not genome-wide significant) (32), there is strong evidence from

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functional studies that changes in *CD33* expression affect Aβ levels *in vivo*. The exon 2 polymorphism rs12459419 in tight LD with rs3865444 has been proposed to be the causal variant modulating alternative splicing of *CD33* (118, 119). Increased inclusion of exon 2 in the presence of the rs3865444 risk allele produces full length CD33 with an IgV domain, which likely mediates sialic acid binding leading to receptor activation. At baseline *CD33* inhibited uptake of Aβ42 in mouse primary microglial cells and *CD33* ablation in APP/PS1 mice alleviated plaque pathology (120). In accordance, monocytes from rs3865444 risk allele carriers show an increase in *CD33* expression and reduced capacity to phagocytose Aβ, which correlates with an increase in brain Aβ load (121).

Inhibition of *CD33* signaling decreases surface expression of triggering receptor 2 on myeloid cells (*TREM2*) (122). Several studies have reported that rs75932628, which results in the *TREM2* missense variant (R47H), increases AD risk by about two-fold (61, 62). *TREM2* was first identified in Nasu-Hakola disease patients, a rare recessive disorder associated with an frontotemporal dementia-like syndrome (123), making *TREM2* a candidate gene for targeted sequencing in AD patients. *TREM2* expression is increased in response to brain injury in AD (124, 125) and is found on both resident microglia and infiltrating monocytes and macrophages (126). Variants in *TREM2* reduce transport and cell surface expression of the full length protein, thereby decreasing cell surface shedding and activity, which functionally results in a decrease of phagocytosis (127). *TREM2* is a pattern recognition receptor that binds phospholipids, such as phosphatidylserine exposed on cells undergoing apoptosis (128), as well as APOE- and Clusterin-containing lipoprotein particles (129, 130), promoting phagocytosis of Aβ complexed in these lipoprotein particles (131). Deficiency of *TREM2* in AD mouse models affects amyloid-β deposition in a temporal manner resolving pathology at early stages, but showing aggravated plaque load and impairment in microglia viability, proliferation and migration in aged mice (128, 132).

There are other loci supporting the role of microglial/myeloid cells in AD, however, more work is required to establish their functional significance in AD risk and disease progression. For example, the *MS4A6A* locus (rs983392 (32)) including 5 other members of the *MS4A* gene family, which are specifically expressed in microglia and regulate cell activation (133). The *HLA-DRB1*/*HLA-DRB5* locus containing 9 genes (rs9271192 (32)) of the major histocompatibility complex II family are involved in immunity. The *ZCWPW1* locus contains 7 genes (rs1476679 (32)) including *PILRA* and *PILRB* immune receptors involved in monocyte and neutrophil infiltration and response during inflammation (134, 135). A recent study that used the fine-mapping approach discussed above to dissect the causal gene in the *CELF1* locus, which includes 13 genes (rs10838725 (32)), reports identification of *SPI1* as a master regulator of endophenotypes and genes associated with AD (136). A large meta-analysis of exome chip data has also identified novel microglia-expressed genes associated with AD risk, *ABI3* and *PLCG2* (137).

Future Directions

Although some progress has been made in understanding the underlying pathogenic mechanisms associated with GWAS loci, this needs to be a major focus of future research using methods outlined in this review (Figure 1). If GWAS samples are enlarged, it is clear

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from other phenotypes that additional loci will be identified. Two recent large meta-analyses of AD samples have indeed identified additional risk loci (138, 139). A lesson from GWAS is that we need to be bold – large datasets will be needed to find strong evidence for individual genes/variants, identified in whole genome/exome sequencing projects. Although novel large effect size associations with AD provide valuable mechanistic insight into disease pathogenesis, association signals should be carefully assessed for the frequency, directionality and effect size that may change dependent on the methods used for patient stratification and variant identification by sequencing or genotyping.

Definitive verification of functional variants will come from *in vivo* and *in vitro* functional studies using mouse models and hiPSc-derived neurons, astrocytes and microglia cells that enable us to model sporadic AD in relevant cell types and predict therapeutic interventions based on mechanism. Functional non-coding AD variants can be tested using genome editing tools, such as CRISPR/Cas9 (140), that offer controlled genetic background to dissect the effect size. Evaluation of GWAS variants with protective effects may provide additional information about the pathways that can counteract disease. For example, a protective *APP-A673T* mutation identified in the Icelandic population leads to decreased Aβ production *in vitro* compared to *APP-A673V*, a LOAD mutant at the same amino acid that increases Aβ production (60). It remains to be shown if AD protective variants act by reversing detrimental phenotypes or boosting cell activity to counteract the pathology, which may have implications for designing AD therapies.

Conclusions

The application of functional genomics approaches will finally provide focus for researchers bombarded with the wealth of information from GWAS, transcriptome, proteome and metabolome studies in AD cohorts. Although we may not see an expansion of the number of GWAS common variants associated with AD, whole exome/genome sequencing in specific cohorts will lead the way for discovery of new AD-associated genes. Understanding the mechanisms underlying LOAD genes has shifted our attention from β-amyloid metabolism to other cellular pathways and the contribution of myeloid cell function in AD pathogenesis. Characterization of functional AD-associated variants will broaden our understanding of mechanisms underlying AD progression that is now studied in the context of cell-cell interactions of the brain. In the future it will be important to see how risk variants align to cell specific pathways and predict master regulators of protein hubs that are dysfunctional in AD in order to develop novel therapies.

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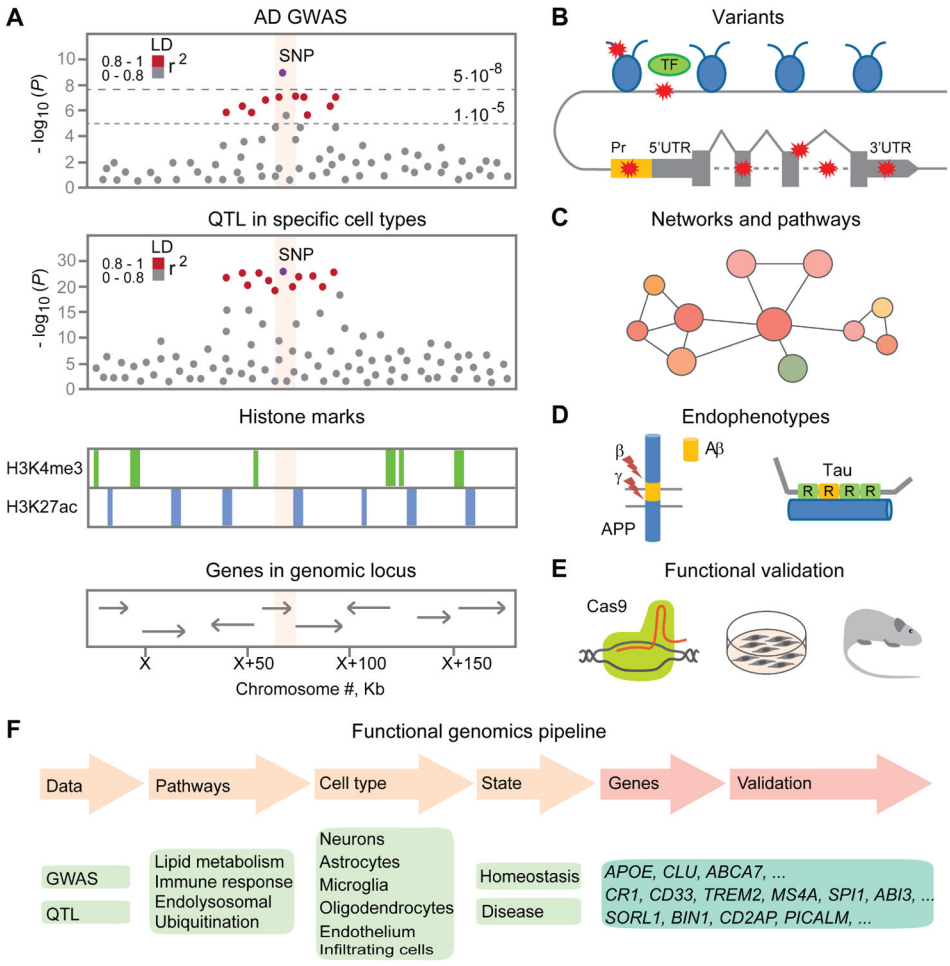

Figure 1. Schematic representation of a multidimensional approach for fine-mapping risk variants in Alzheimer's disease-associated loci

(**A**) An illustration of a locus-specific association signal from a genome-wide association study (GWAS) of Alzheimer's disease (AD), e.g. Manhattan association plot (top panel). Each dot represents a single nucleotide polymorphism (SNP), with the X-axis showing the chromosomal position and Y-axis showing the association *P* values on the -$\log_{10}$ scale. SNPs are colored (in red) by pairwise linkage disequilibrium (LD) pattern with the most strongly associated SNP. The regional association signal from a quantitative trait loci (QTL) study in specific cell populations (e.g., peripheral monocytes or macrophages) (middle panel), that may show expression, splicing and methylation QTL. The functional annotation of the genome with histone marks and the genomic position with the genes (bottom panel), e.g. H3K4me3 marks promoter regions, H3K27ac marks enhancer regions.

(**B**) Classification of SNPs in coding and non-coding regions by mechanism of action that may affect expression, splicing or protein function due to mutations, insertions and deletions. The coding variants change protein sequence. The non-coding variants may influence protein levels by modulating transcription factor binding at the intronic/distal enhancer or promoter regions, changing histone methylation and acetylation, splicing,

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miRNA, long noncoding RNA binding and stability, or structural variation. TF: transcription factor. UTR: untranslated region.

(**C**) Gene co-expression network and pathway analysis using large-scale transcriptomic or proteomic datasets.

(**D**) Endophenotypes relevant to AD: amyloid-β (Aβ) plaque load measured with positron emission tomography (PET) tracer, Aβ42 and tau/p-tau$_{181}$ levels in cerebrospinal fluid (CSF), neuropathological changes.

(**E**) Functional validation of genetic hits using genome editing tools (e.g. CRISPR/Cas9) in cell culture and mouse models.

(**F**) Integrating AD GWAS with functional genomics approaches can help prioritize candidate genes, biological pathways and cell types, which in turn can help generate novel hypothesis for experimental validation. Genes listed are discussed in the review.

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Table 1

Overview of genomic risk loci and genes with common and rare variants identified through Alzheimer's disease (AD) linkage studies, genome-wide association studies (GWAS) and whole genome /whole exome sequencing (WGS/WES)

Identification of a functional variant in a specific causal gene has only been established for a few loci. In those cases the putative gene and variant are named in the table with additional information about the cell type-specific gene expression and related biological processes. The odds ratio from a case-control study tells about the association of disease outcome with the presence of certain genotype: increased (risk) or decreased (protection) chances of getting the disease for an individual with one risk allele versus having no risk alleles. Adjacent genes were selected with dbSNP database by searching for the tagging SNP. Definitive conclusion about SNP association requires replication in independent datasets. QTL: quantitative trait loci.

Chr	SNPs	Closest gene name	Genes adjacent to the SNP	Putative gene with functional significance	Cell type-specific expression of putative gene	Biological processes	Odds ratio meta-analysis for minor allele with 95% confidence interval (CI) (common/ rare variant)	References
Loci								
Chr1	rs6656401	*CR1*	*CR2, CR1, CR1L*	*CR1* intragenic copy number variations leading to longer isoform overproduction	Microglia	Immune response and phagocytosis	1.18 (1.14–1.22)	(32)
Chr2	rs6733839	*BIN1*	*BIN1, CYP27C1*	*BIN1*3 base pair insertion	Ubiquitous	Endocytosis	1.22 (1.18– 1.25)	(32)
Chr2	rs35349669	*INPP5D*	*NEU2, INPP5D, ATG16L1*	/	/	/	1.08 (1.05– 1.11)	(32)
Chr5	rs2074612	*HBEG F*	*CYSTM1 , PFDN1, HBEGF*	/	/	/	1.08 (1.05– 1.11)	(138, 139)
Chr5	rs190982	*MEF2 C*	*TMEM161B, MIR9-2, LINC00461, MEF2C, MEF2C-AS1*	/	/	/	0.93 (0.90– 0.95)	(32)
Chr6	rs10948363	*CD2AP*	*TNFRSF21, CD2AP, ADGRF2, ADGRF4, OPN5*	/	/	/	1.10 (1.07– 1.13)	(32)
Chr6	rs9271192	*HLA-DRB1 / HLA-DRB5*	*C6orf10, BTNL2, HLA-DRA, HLA-DRB5, HLA-DRB6, HLA-DRB1, HLA-DQA1, HLA-DQB1, HLA-DQA2, HLA-DQB2, HLA-DOB*	/	/	/	1.11 (1.08– 1.15)	(32)
Chr7	rs11771145	*EPHA1*	*ZYX, EPHA1, EPHA-AS1, TAS2R62P, TAS2R60*	/	/	/	0.90 (0.88– 0.93)	(32)

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Chr	SNPs	Closest gene name	Genes adjacent to the SNP	Putative gene with functional significance	Cell type-specific expression of putative gene	Biological processes	Odds ratio meta-analysis for minor allele with 95% confidence interval (CI) (common/ rare variant)	References
Chr7	rs2718058	*NME8*	*GPR141 , NME8, SFRP4, EPDR1*	/	/	/	0.93 (0.90– 0.95)	(32)
Chr7	rs1476679	*ZCWP W1*	*GPC2, STAG3, PVRIG, GATS, SPDYE3 , PILRB, PILRA, ZCWPW1, MEPCE, C7orf61*	*PILRB* expression QTL	Microglia	Inflammatory signal transduction	0.91 (0.89– 0.94)	(32, 141)
Chr8	rs9331896	*CLU*	*EPHX2, CLU, SCARA3*	*CLU* rare coding variants and insertions / deletions	Astrocytes	Lipid metabolis m	0.86 (0.84– 0.89)	(32)
Chr8	rs28834970	*PTK2 B*	*TRIM35, PTK2B, CHRNA2*	/	/	/	1.10 (1.08– 1.13)	(32)
Chr10	rs7920721	*ECHDC3*	*USP6NL , ECHDC3, PROSE R2*	/	/	/	1.07 (1.04– 1.10)	(138, 139)
Chr11	rs10838725	*CELF1*	*DDB2, ACP2, NR1H3, MADD, MYBPC3, SPI1, SLC39A13, PSMC3, RAPSN, CELF1, PTPMT1, KBTBD4, NDUFS3, FAM180B, C1QTN F4, MTCH2, AGBL2, FNBP4, NUP160*	*SPI1* expression QTL	Myeloid cells	Myeloid lineage determination	1.08 (1.05– 1.11)	(32, 136)
Chr11	rs983392	*MS4A6A*	*OOSP2, MS4A3, MS4A2, MS4A6A , MS4A4E , MS4A4A , MS4A6E , MS4A7, MS4A14 , MS4A5, MS4A1*	*MS4A4A, MS4A6A* expression QTL	Microglia	Chemosensory receptors	0.90 (0.87– 0.92)	(32, 136)
Chr11	rs10792832	*PICAL M*	*CCDC83, PICALM , EED*	/	/	/	0.87 (0.85– 0.89)	(32)
Chr11	rs11218343	*SORL1*	*SORL1*	*SORL1* rare and common variants	Ubiquitous	Endocytosis and sorting	0.77 (0.72– 0.82) / 5.03 (2.02– 14.99)	(32, 142)
Chr14	rs17125944	*FERM T2*	*ERO1A, PSMC6, STYX, GNPNAT1, FERMT2*	/	/	/	1.14 (1.09– 1.19)	(32)
Chr14	rs10498633	*SLC24 A4 / RIN3*	*SLC24A4, RIN3, LGMN*	/	/	/	0.91 (0.88– 0.94)	(32)
Chr15	rs59685680	*SPPL2 A*	*USP8, USP50, TRPM7, SPPL2A*	/	/	/	0.92 (0.89– 0.95)	(138)
Chr15	rs74615166	*TRIP4*	*CSNK1G1, KIAA0101, TRIP4, ZNF609*	/	/	/	1.31 (1.19– 1.44)	(143)

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Chr	SNPs	Closest gene name	Genes adjacent to the SNP	Putative gene with functional significance	Cell type-specific expression of putative gene	Biological processes	Odds ratio meta-analysis for minor allele with 95% confidence interval (CI) (common/ rare variant)	References
Chr17	rs2632516	*BZRA P1-AS1*	*TSPOAP1, BZRAP1 -AS1, SUPT4H1, RNF43*	/	/	/	0.92 (0.91– 0.94)	(139)
Chr17	rs77493189	*SCIM P*	*ZFP3, ZNF232, USP6, ZNF594, SCIMP, RABEP1 , NUP88, RPAIN, C1QBP, DHX33*	/	/	/	1.11 (1.07– 1.15)	(138)
Chr18	rs8093731	*DSG2*	*DSG3, DSG2, DSG2-AS1*	/	/	/	0.73 (0.62– 0.86)	(32)
Chr19	rs4147929	*ABCA7*	*CNN2, ABCA7, HMHA1, POLR2E*	*ABCA7* rare and common loss-of-function mutations and deletions	Ubiquitous	Lipid metabolism and phagocytosis	1.15 (1.11–1.19) / 2.81 (1.89– 4.20)	(32, 86)
Chr19	rs429358 , rs7412	*APOE*	*APOE*	*ε4* genotype	Ubiquitous, major in astrocytes and microglia	Lipid metabolism and phagocytosis	~3–12	(72, 73)
Chr19	rs3865444	*CD33*	*SIGLEC9, SIGLEC7, CD33, SIGLECL1*	*CD33* alternative splicing of IgV domain	Microglia	Immune response and phagocytosis	0.94 (0.91– 0.96)	(32)
Chr20	rs7274581	*CASS4*	*AURKA, CSTF1, CASS4, RTFDC1, GCNT7*	/	/	/	0.88 (0.84– 0.92)	(32)
Genes								
Chr4	rs137875858	*UNC5 C*	*UNC5C*	UNC5C T835M	Neuron s	Response to neurotoxic stimuli, cell death	/	(69)
Chr6	rs75932628	*TREM2*	*TREM2*	TREM2 R47H	Microglia	Immune response and phagocytosis	~3–5	(61, 62)
Chr6	rs3747742	*TREML2*	*TREML2*	TREML2 S144G	Microglia	Immune response	0.91 (0.86– 0.97)	(144)
Chr7	rs144662445, rs149979685	*AKAP9*	*AKAP9*	AKAP9 I2546M, S3767L	Ubiquitous	Kinase signaling	3.61 (1.51– 9.00)	(70)
Chr15	rs61751103, rs145518263	*ADAM10*	*ADAM10*	ADAM10 Q170H, R181G	Ubiquit ous	APP processing to Aβ	/	(24)
Chr16	rs72824905	*PLCG2*	*PLCG2*	PLCG2 P522R	Microglia	Phospholipase signaling	0.68 (CI not reported)	(137)
Chr17	rs616338	*ABI3*	*ABI3*	ABI3 S209F	Microglia	Cell growth	1.43 (CI not reported)	(137)

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Chr	SNPs	Closest gene name	Genes adjacent to the SNP	Putative gene with functional significance	Cell type-specific expression of putative gene	Biological processes	Odds ratio meta-analysis for minor allele with 95% confidence interval (CI) (common/ rare variant)	References
Chr19	rs145999145	*PLD3*	*PLD3*	PLD3 V232M	Ubiquitous	Unknown	2.75 (2.05– 3.68)	(68)
Chr21	rs63750847	*APP*	*APP*	APP A673T	Ubiquitous	APP processing to Aβ	~0.2	(60)