Published in final edited form as:

Future Neurol. 2015 December ; 10(6): 547–558. doi:10.2217/fnl.15.42.

Novel susceptibility loci for Alzheimer's disease

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Abstract

Late-onset Alzheimer's disease (AD), a highly prevalent neurodegenerative disorder characterized by progressive deterioration in cognition, function and behavior terminating in incapacity and death, is a clinically and pathologically heterogeneous disease with a substantial heritable component. During the past 5 years, the technological developments in next-generation high-throughput genome technologies have led to the identification of more than 20 novel susceptibility loci for AD, and have implicated specific pathways in the disease, in particular intracellular trafficking/endocytosis, inflammation and immune response and lipid metabolism. These observations have significantly advanced our understanding of underlying pathogenic mechanisms and potential therapeutic targets. This review article summarizes these recent advances in AD genomics and discusses the value of identified susceptibility loci for diagnosis and prognosis of AD.

Keywords

Alzheimer's disease; genetics; genomics; gene

At present, approximately 5.5 million persons in the USA are affected with late-onset Alzheimer's disease (AD) [1]. Due to the aging of the society, it is expected that this figure will quadruple by the year 2050 [2,3]. Key clinical symptoms are a progressive decline in cognition, function and behavior that unavoidably results in full incapacity and death. Pathological manifestations in brain include intracellular deposits of hyperphosphorylated tau protein in the form of neurofibrillary tangles, and extracellular β -amyloid protein ($A\beta$) in diffusible oligomers and insoluble plaques which is generated by amyloidogenic processing of the amyloid precursor protein (APP) by β - and γ -secretases. To date, available drugs do not effectively prevent the disease and only marginally influence severity and progression of the disease. Heritabilities of up to 80% indicate that the disease has a substantial genetic component [4,5]. Identifying genes and gene networks associated with the disease is expected to disclose causative pathogenic mechanisms, pinpoint proteins and pathways for drug development, and inform the development of genetic testing methods for identifying persons at risk.

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Financial & competing interests disclosure

The author has no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Early identification of *APP*, *PSEN1* & *PSEN2* AS AD risk genes

Early studies of large families multiply affected by early-onset AD (onset age: 30–50 years) yielded the discovery of autosomal dominant mutations in the *APP*, *PSEN1* and *PSEN2* genes [6–8]. The identification of these genes led to the formulation of the ‘amyloid cascade hypothesis,’ which suggests the enhanced generation of A β generated through sequential cleavage of the amyloid precursor protein by β - and γ -secretase, as a key pathogenic mechanism in AD. A β peptides can aggregate, leading to toxic A β oligomers and amyloid plaque formation. To date, 33 pathogenic mutations in *APP*, 185 mutations in *PSEN1* and 13 mutations in *PSEN2* have been identified (www.molgen.ua.ac.be/ADMutations/).

Apolipoprotein E

The first gene to be unequivocally established as a susceptibility gene for the common, late onset form of AD was *APOE* in 1993 [9,10], a protein involved in lipid-transport. In humans, three isoforms exist which are encoded by the *APOE* 2,3 and 4 alleles. The differences among the three isoforms, which lie at amino acid residues 112 and 158, have major effects on the structure and function of *APOE*. The primary source of *APOE* in the brain are the astrocytes and to lesser extent microglia and neurons [11]. *APOE* traffics lipids generated through neurodegeneration to cells requiring them for membrane repair, proliferation, or remyelination. In addition, *APOE* affects glutamate receptor function and synaptic plasticity by modulating *APOE* receptor recycling in neurons [12].

The *APOE* ϵ 4 allele is linked to a higher risk of AD, lower memory performance, and decreases the age at onset of AD in a dosage-dependent manner [9,13–16]. It has been estimated that in non-Hispanic whites, *APOE* may account for as much as 20–50% of LOAD risk [17,18]. In minority populations, reports on the effect of *APOE* on AD risk have been inconsistent [19], although the largest GWAS performed to date in African– Americans strongly suggests an increased risk of LOAD for *APOE* ϵ 4 carriers [20]. In the brains of AD patients, *APOE* ϵ 4 dosage correlates strongly with burden of A β , A β oligomers and plaque accumulation [21,22]. This notion is supported by *in vitro* studies demonstrating that the *APOE* ϵ 4 isoform binds A β peptides more vigorously than *APOE* ϵ 3 [23] and leads to increased A β aggregation [24,25], and by studies demonstrating that knockout *APOE* $^{-/-}$ mice develop less nonfibrillar A β deposits [26,27]. hAPP transgenic mice expressing human *APOE* ϵ 3 or *APOE* ϵ 4 also demonstrate a gene dose- and isoform-dependent *APOE* effect on A β accumulation [28]. *APOE* is also an A β chaperone involved in A β clearance. Finally, there is also evidence that *APOE* affects tau metabolism. While *APOE* ϵ 4 stimulates tau phosphorylation leading to preneurofibrillary tangles, *APOE* ϵ 3 may inhibit this [29]. Alternatively, as a major cholesterol transporter, *APOE* may exert its effects through effects on cholesterol trafficking in brain. In animal models, high cholesterol levels have been associated with changes in *APP* metabolism [30,31] and an increased A β load [32,33].

The *APOE* gene is located in a linkage disequilibrium (LD) block harboring *APOE* and two additional genes, the translocase of outer mitochondrial membrane 40 homolog (*TOMM40*) and Apolipoprotein C-I (*APOC1*). A previous study [34] reported an association between a variable length poly-T polymorphism (‘poly-T’) at rs10524523 in the *TOMM40* gene

encoding and risk for AD and age of onset of AD in a small sample of non-Hispanic whites (n = 34). However, more recent studies of this polymorphism in a larger samples of non-Hispanic whites failed to confirm the original findings after adjusting for the effect of *APOE*ε4 [35] and showed that *APOE* alleles ε2, ε3 and ε4 account for essentially all the inherited AD risk associated with this LD block [36].

Novel susceptibility loci associated with late-onset AD

Recent genome-wide association studies (GWAS) focusing on very common gene variants (minor allele frequency [MAF] > ~5%) have identified an additional twenty susceptibility loci.

Most of these studies were performed in non-Hispanic White individuals of European ancestry. The first set of GWAS reported *CLU*, *PICALM*, *CR1* and *BIN1* as susceptibility loci [37–39]. The strongest effect size after *APOE* was observed for the associated SNPs at the *BIN1* locus on chromosome 2q14.3. The gene encoding BIN1 (Bridge Integrator 1, also called Amphiphysin 2) is highly expressed in the brain. Increasing evidence suggests that BIN1 affects AD risk by modulating tau pathology [40]. In addition, BIN1 may modulate AD risk by its involvement in several other pathways implicated in AD etiology, in particular clathrin-mediated endocytosis and intracellular trafficking, but also immune response, calcium homeostasis and apoptosis [41]. The Phosphatidylinositol binding clathrin assembly protein encoded by the *PICALM* gene may be involved in APP processing through its role in clathrin-mediated endocytosis. In line with this notion, *PICALM*, which is expressed in neurons, colocalizes with APP in endocytic vesicles [42], *PICALM* levels are upregulated in the cortex of Tg2576 AD mice compared with wild-type mice [43] and modulation of *PICALM* expression *in vitro* and *in vivo* changes the extent of Aβ production [42]. *CLU*, located on chromosome 8p21.1, encodes a stress-activated apolipoprotein involved in apoptosis, complement regulation, lipid transport, membrane protection and cell–cell interactions [44]. In AD brains, clusterin mRNA expression is elevated. Clusterin can be isolated from amyloid deposits and influences Aβ clearance, amyloid deposition and neuritic toxicity [45,46]. *CR1*, located on chromosome 1q32 in a cluster of complement-related proteins, encodes the CR1 protein, a component of the complement response. Neuroinflammation and dysregulation of the immune response is a central feature of AD, in line with the notion that elevated complement cascade activity may exacerbate AD pathology [47]. In addition, clearance of plasma Aβ42 is dependent on C3b binding to CR1 [48].

The second series of large GWAS studies identified *ABCA7*, *CD33*, the *MS4A4A/MS4A4E/MS4A6E* cluster, *CD2AP* and *EPHA1* as AD susceptibility loci [20,49]. The *ABCA7* gene, which was also identified in the largest GWAS to date performed in African Americans [20], encodes a member of the superfamily of ATP-binding cassette (ABC) transporters which traffic substrates across cell membranes and are largely involved in lipid transport and homeostasis. *ABCA7* has been shown to directly mediate phagocytosis, membrane trafficking of APP and amyloid processing [50]. *CD33*, also called Siglec-3, is a transmembrane glycoprotein expressed on myeloid progenitor cells, mature monocytes and macrophages, that contains an extracellular immunoglobulin (Ig) V-set sialic-acid binding

domain, an extracellular Ig C2-set domain and cytosolic immunoreceptor tyrosine-based inhibitory motifs. CD33 has been implicated in various immune system-related cellular functions including inhibition of cellular proliferation and activation [51]. In addition, CD33 has been reported to play a role in clathrin-independent receptor-mediated endocytosis [52]. *CD33* expression [53] and CD33-positive microglia [54] is increased in AD brains. The larger CD33 isoform modulates A β uptake [54] and A β phagocytosis are inhibited in microglial cells expressing *CD33* [54]. CD33-positive immunoreactive microglia is also positively correlated with insoluble A β 42 and plaque burden in brains with AD [54]. The *MS4A* locus on chromosome 11q12–13 contains a cluster of several genes spanning ~800 kb including *MS4A4A*, *MS4A4E* and *MS4A6E*. Although the members of this cluster are poorly characterized, it is clear that these genes share common structural features and similar intron/exon splice boundaries [55]. Most of the encoded proteins contain four membrane-spanning regions, N- and C-terminal cytoplasmic domains, as well as extracellular domains and an intracellular domain. MS4A genes are predominantly expressed in hematopoietic cells, but have also been found in other tissues including brain [56]. For some members including MS4A1, MS4A2 and MS4A4B, a role in immune response has been demonstrated. In addition, MS4A1 and MS4A2 may be involved in the regulation of calcium hemostasis [57,58]. Due to their similar structure and sequence homology other members of the MS4A cluster are likely to share overlapping functional properties. *CD2AP* encodes CD2 associated protein, which is a scaffolding protein involved in regulation of the actin cytoskeleton. *CD2AP* interacts directly with filamentous actin and a variety of cell membrane proteins through multiple actin binding sites, SH3 domains and a proline-rich region containing binding sites for SH3 domains [59], resulting in critical roles in endocytosis and intracellular trafficking. In addition, CD2AP may modulate tau toxicity [60]. The protein encoded by *EPHA1*, Ephrin receptor A1, belongs to the ephrin receptor subfamily of the protein-tyrosine kinase family. This family of proteins is highly conserved across species, is expressed in the brain and is mediating developmental events, particularly in the nervous system. Ephrin receptors mediate axonal guidance, neural plasticity, and modulate the MAPK pathway and response at glutamatergic synapses [61–63].

Finally, the largest GWAS to date, performed by the International Genomics of Alzheimer's Project (IGAP) which performed a mega-meta-analysis on 74,046 non-Hispanic white subjects [64], confirmed *CRI1*, *BIN1*, *CD2AP*, *EPHA1*, *CLU*, *MS4A6A*, *PICALM*, *ABCA7* and *CD33* at genome-wide significance and identified in addition *HLA-DRB5/HLA-DRB1*, *PTK2B*, *SORL1*, *SLC24A4/RIN3*, *DSG2*, *INPP5D*, *MEF2C*, *NME8*, *ZCWPW1*, *CELF1*, *FERMT2* and *CASS4* [65]. Most cluster in the specific pathways identified by the earlier GWAS, that is, immune response (*HLA-DRB5/DRB1*, *INPP5D*, *MEF2C*), APP processing (*SORL1*, *CASS4*), Tau pathology (*CASS4*, *FERMT2*), cell migration (*PTK2B*) and lipid transport and endocytosis (*SORL1*) strongly reinforcing the importance of these pathways in LOAD etiology. Of note, *SORL1* (sortilin-related receptor, L [DLR class] 1) had previously been demonstrated to modulate trafficking and processing of APP in a candidate gene approach [65]. In addition, consistent with the notion of a complex disease, the findings of this study further strengthen the evidence for additional pathways including hippocampal synaptic function (*MEF2C*, *PTK2B*); cytoskeletal function and axonal transport (*CELF1*, *NME8*, *CASS4*); regulation of gene expression and posttranslational modification of

proteins, microglial and myeloid cell function (*INPPD5*), and APP (*SORL1* and *CASS4*) and tau (*CASS4* and *FERMT2*) pathology.

The threshold for genome-wide significance applied in these studies was the commonly used p-value cut off of $p = 5 \times 10^{-8}$ [66]. As common in complex disease, all of these variants identified by GWAS have very small effect sizes ($1.0 < OR < 1.3$), and the proportion of heritability explained is modest. In addition, as opposed to *APOE*, which has known coding variants directly associated with AD, most of these variants identified in the recent large-scale GWASs fall *outside* coding regions [64]. The absence of known direct effects on the genes makes it difficult to interpret how these variants contribute to AD etiology. For most of the GWAS variants, it remains unclear whether they directly contribute to AD or are in LD with other disease causing variant(s). GWAS variants located outside of coding regions may play regulatory roles, such as altering expression, DNA methylation, or splicing [67]. For example, the ENCODE project reported that up to 54% of noncoding variants detected by GWAS lie in regulatory regions [68,69]. In order to minimize the risk of false positive findings, replication in independent datasets and functional validation is critical.

In order to elucidate how the variants identified by GWAS influence AD etiology, several studies have made an effort to examine transcription and splicing of the genes nearest the GWAS variants with the strongest association. Expression of a *CLU* isoform influenced by the GWAS variant rs11136000 increases of *CLU* protein secretion in individuals with significant AD neuropathology [70]. Increased CD33 expression is associated with AD and inhibits microglial A β uptake [53,54]. Increased copy number variants (CNVs) located within the *CR1* gene are significantly associated with AD [71]. *SORL1* harbors an intronic polymorphism associated with decreased expression in AD [72,73], and harbors functional mutations resulting in perturbed trafficking of APP (discussed below) [74]. In a recent study in three independent AD cohorts (176 patients from 124 Caribbean Hispanics families, 120 patients and 33 unaffected individuals from the 129 NIA-LOAD Family Study; and 263 unrelated Canadian individuals of European ancestry) [75], targeted sequencing of the GWAS loci revealed an excess burden of deleterious coding mutations in *ABCA7* and *BIN1*.

Rare variants associated with AD

As commonly seen in GWAS of complex disease, the common variants identified in the AD GWAS, summarized above, have replicable associations with small effects on AD risk, but are likely not the underlying causative functional variants. Functional variants with larger effects on risk are expected to include primarily low-frequency coding variants not detected by GWAS. Advances in next-generation sequencing techniques enable the assessment of entire exomes and genomes providing the potential to identify rare pathogenic mutations. Recent studies using next-generation sequencing have identified several rare and low-frequency functional variants conferring strong effects on AD risk. The most common methods to implicate rare or low-frequency variants identified by sequencing are showing segregation with disease status in multiplex families, association analyses of single variants in samples of independent cases and controls and burden tests (which collapse rare variants in a defined genomic region into a single variable) combined with bioinformatics annotation. However, similar to common variants identified by GWAS, replication of rare variants in

independent datasets and functional validation is critical in order to minimize the risk of false positives.

APP

A recent whole genome sequence (WGS) study in 1795 Icelanders identified a coding mutation in the *APP* gene that protects elderly without cognitive impairment against cognitive decline and AD. This mutation (A673T), causing a substitution in the vicinity of the aspartyl protease beta-site in APP, leads to a significant reduction in A β production, in line with the notion that decreasing β -secretase processing of APP may be protective. Follow up studies determining the frequency of this variant in elderly from the United States showed that it is extremely rare in US cohorts indicating that it may be primarily restricted to Icelandic and Scandinavian populations [76–79].

GWAS loci

As discussed above, in a recent study in three independent AD cohorts (176 patients from 124 Caribbean Hispanics families, 120 patients and 33 unaffected individuals from the 129 NIA-LOAD Family Study; and 263 unrelated Canadian individuals of European ancestry) [75], targeted sequencing of the GWAS loci revealed an excess burden of deleterious coding mutations in *ABCA7* and *BIN1*. *ABCA7* sequence analyses of various independent cohorts identified loss of function mutations suggesting that a (partial) loss-of-function of *ABCA7* could be a potential pathogenetic mechanism in AD [80,81]. This notion is supported by an independent study demonstrating that *ABCA7* loss of function alters amyloid processing [50]. Sequence variants in *BIN1* were also identified in a cohort of Han Chinese [40].

TREM2

In 2013, two companion papers [82,83] implicated a rare missense variant, rs75932628, predicted to cause a R47H substitution in *TREM2* encoding the triggering receptor expressed on myeloid cells 2 protein, to increase AD risk at a magnitude similar to that of *APOE ϵ 4*. These findings were subsequently confirmed in various populations, in part with variants within different regions of the gene conferring AD risk [84–89]. In addition, R47H has been associated with history of parental late-onset AD and earlier maternal age-at-onset [90], early-onset AD [91], autosomal recessive Nasu-Hakola disease (polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy) [92,93], frontotemporal lobar degeneration [94,95], Parkinson's disease [96] and sporadic amyotrophic lateral sclerosis [97]. *TREM2* encodes a membrane protein acting as a receptor signaling complex with the TYRO protein tyrosine kinase binding protein. The encoded protein functions activates immune responses in macrophages and dendritic cells and is involved in chronic inflammation by triggering the production of constitutive inflammatory cytokines. Expression of *TREM2* correlates with cortical levels of A β . Compromised function of *TREM2* is believed to negatively affect clearance of cell debris and A β in AD, while a prompting effect of *TREM2* on cytokine levels might trigger an inflammatory response and neuronal death.

PLD3

In a WES study in 14 multiplex LOAD families Cruchaga *et al.* [98] identified a rare genetic variant (Val232Met) in the gene encoding phospholipase D3 (*PLD3*) that segregated with disease status in two families and doubled risk for AD in several independent case control datasets. Burden analyses further suggested that several variants across the gene increase AD risk. PLD3 belongs to the family of PLD proteins, which has six members (*PLD1* to *PLD6*) that catalyze the hydrolysis of phosphatidylcholine resulting in free choline and phosphatidic acid. Phosphatidic acid and its derivate diacylglycerol play key roles in several pathways implicated in AD including cytoskeleton formation, membrane trafficking and receptor-mediated endocytosis, as well as exocytosis, cell growth and cell differentiation.

There is some evidence for an interaction of PLD with components of the amyloid cascade [99]. In AD brains, PLD activity is reduced [100], A β 42 peptide internalized via formyl-peptide receptor-like-1 (FPRL1) activates PLD in cultured rat astrocytes and microglia [101] and A β 25–35 triggers PLD activity in human neuroblastoma cells [102]. PLD1 negatively regulates A β production by interacting with the cytoplasmic domain of presenilin-1 [103]. In addition, PLD1 stimulates trafficking and accumulation of presenilin-1 at the cell surface [104]. Overexpression of APP elevates PLD2 activity in P19 mouse embryonic carcinoma [105] in cultured neurons oligomeric A β enhances PLD2 activity [106]. PLD3 is poorly characterized. In the study by Cruchaga *et al.* [98] PLD3 overexpression significantly reduced APP, A β 42 and A β 40, while knockdown of PLD3 had inverse effects. Several subsequent studies seeking to replicate these associations failed [107–109] or observed only marginal significance [110]. While several factors, in particular low statistical power and population stratification present challenges in replication of associations for low-frequency and rare variants and may explain these inconsistencies, additional data are required to clarify the proposed role, if any, of rare variants in *PLD3* on AD risk.

SORL1

SORL1 (sortilin-related receptor, L[DLR class] A-type repeats containing) is one of five members of the vacuolar protein sorting-10 domain-containing receptor family. *SORL1* has been demonstrated to be involved in the intracellular trafficking of APP [111], sorting it into the recycling pathway and redirecting it away from the endosome–lysosome pathway thereby reducing APP cleavage into A β 40 and 42. Following the identification of *SORL1* as an AD susceptibility gene in candidate gene and GWAS in various ethnic groups, a recent family- and cohort-based genetic association study in Caribbean Hispanic multiplex families and cases and controls, and a cohort of non-Hispanic White cases and controls identified two disease-associated rare variants (rs117260922-E270K and rs143571823-T947M) and a common variant (rs2298813-A528T) *SORL1*. Transfected cell lines for all three variants showed increased A β 40 and A β 42 secretion, and all variants increased the level of APP at the cell surface, suggesting failed sorting into the recycling pathway.

MAPT & GRN

Mutations in *MAPT* encoding the microtubule-associated protein tau and *GRN* encoding progranulin are the major genetic causes of frontotemporal lobar degeneration (FTLD). *MAPT* mutations often occur in the microtubule-binding domain and lead to alterations in

microtubule dynamics or increase 4R tau production. The >70 GRN mutations known to date reduce progranulin levels or result in loss of function. Sequence variants in both genes have been associated with sporadic and familial late-onset AD [112–116]. While in some of these cases there was clinical evidence of underlying AD pathology, such as a positive PIB-PET scan, a CSF profile consistent with AD, or temporoparietal atrophy on MRI, in almost all of these studies autopsy confirmation of the clinical diagnosis was lacking. Thus, it has been repeatedly suggested that these mutation carriers with a clinical AD diagnosis likely have mixed FTLN and AD neuropathology, and that genetic variants in these genes are restricted to the FTLN spectrum [113,116]. Genomic studies with clinicopathologic data are needed to clarify these issues.

Rare variants in other genes

Additional studies have reported rare variants in the cholesterol and phospholipid regulator, ATP-binding cassette transporter, *ABCA1*, nicastrin [117], *AKAP9* [118], *NOTCH3* [119] and the alpha-secretase disintegrin and metalloproteinase domain-containing protein 10 (*ADAM10*) [120,121]. Also these associations need to be validated by further studies.

Structural variants—There is increasing evidence that also structural variants such as deletions, duplications, insertions, inversions or translocations contribute substantially to the etiology of complex diseases. Due to the complexity of sequence alterations caused the mechanistic links between structural variants and phenotypes of interest are often difficult to assess and prove. Apart from the CNVs described in *CR1* [71] and a ~470 kb duplication on chromosome 15q11.2 encompassing five genes (*TUBGCP5*, *CYFIP1*, *NIPA2*, *NIPA1* and *WHAMML1*) [122], no structural variants have been reported in AD. However, this is a rapidly developing field and continues to be an area of study.

Discussion

Over the past 5 years, GWAS have identified more than 20 common variants associated with late-onset AD. In addition, recent NGS studies have led to the identification of several rare functional variants exerting large effects on AD risk. In summary, these findings implicate specific pathways in AD etiology, in particular immune response and inflammation, cell migration, APP and tau pathology, hippocampal synaptic function, cytoskeletal function and axonal transport, regulation of gene expression and posttranslational modification of proteins, and microglial and myeloid cell function.

These studies indicate significant locus heterogeneity in the late-onset form of AD. In addition, they confirm that in addition to common variants, rare or low-frequency coding variants not detected by GWAS contribute to AD risk. This observation of a considerable role of rare variants supports the notion that common diseases are more similar to Mendelian diseases than is suggested by the common disease – common variant hypothesis, which argues that common diseases are primarily caused by common variants [123]. It is likely that a significant part of the genetic contribution to late-onset AD is due to rare variants with moderate to strong impact on disease risk. It is also likely that variants with largest effect sizes will have significant functional consequences. This rare, functional variant model is in line with the observed inconsistencies in AD linkage studies. Variants that increase the risk

of disease by less than about fourfold are expected to generate inconsistent linkage peaks [124]. Then available linkage evidence for AD also is in line with high locus heterogeneity. Common variants may act as modifiers of the effects of rarer variants [125].

Despite these recent advances in AD genomics, a significant part of the genetic contribution to AD remains unexplained. In the largest GWAS performed to date [64], the identified common variants outside the *APOE* locus have population attributable risks (PAR) of 1–8%. While the effects sizes of some of the novel variants identified by this study may be biased by winners curse (i.e., an upward bias in the estimated effect of a newly identified allele on disease risk when the study lacks sufficient statistical power), this calculation suggest that the continuous quest for identification of additional causative sequence variants critical. Additional common variants could be identified by GWAS if the sample sizes are increased, additional ethnic groups are studied or ‘borderline’ associations (such as those with $p > 5 \times 10^{-8}$ and $p \leq 1 \times 10^{-7}$) are more closely investigated. The identification of rare variants remains challenging due to several factors including population stratification and low statistical power due to low minor allele frequencies. A potential solution that has been employed in the above-mentioned sequencing studies and has proven to be successful is to perform WES or whole-genome-sequencing in a highly selected population at increased risk for disease such as multiplex families, followed by a combination of genotyping and deep resequencing of the variant or gene of interest in large numbers of cases and controls. Extreme-trait designs such as focusing on early-onset AD or subjects at the extreme ends of memory function, can also increase the *a priori* power to identify causative sequence variants. The fact that disease-associated genomic regions or variants within genes differ between ethnic groups which may reflect allelic heterogeneity with potentially diverse mechanisms of action, underscores the importance of investigating different ethnic populations for disease risk variant discovery. Sequencing of pools of individuals (Pool-seq) provides a cost-effective alternative to sequencing individuals separately [126]. Advantages of identifying rare or low frequencies variants include a higher effect size and easier functional follow-up.

Future perspective

The significant decrease in costs of high-coverage whole genome sequencing in combination with the rapid increase in the sequencing capacity of existing platforms now allows cost-effective whole genome sequencing studies permitting the examination of the entire genome, including structural variants. Combined with the significant advances in other high-throughput sequencing strategies, such as ChIP-seq, Chem-seq or RNA-seq, including analysis of cell free RNA, advanced analysis methods such as haplotype resolving strategies or analysis of noncoding RNA, and functional studies, it is expected that within the next few years, additional functional sequence variants modifying risk for AD will be identified. It is expected that the large-scale sequencing studies under way, such as the Alzheimer’s disease Sequencing Project (ADSP), will further characterize the molecular pathways underlying AD and identify pathway networks. This network characterization is expected to increase treatment efficacy and reduce adverse effects of available drugs, accelerate the identification of determinants of drug resistance, and identify novel targets for treatment and prevention. With regard to personal genomics, it is hoped that such analyses will provide specific

enough information about AD risk to influence lifestyle choices and employ relatively noninvasive monitoring programs (e.g., imaging), develop effective genetic testing methods and design effective personalized therapies. In clinical settings, next-generation sequencing analysis of a patient's genome can help to design the treatment regimen.

Acknowledgments

The author was supported by NIH grants K23AG034550, P50 AG08702, UF1AG047133 and R01 AG034189.

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EXECUTIVE SUMMARY

- 5.5 million persons in the USA are affected with late-onset Alzheimer's disease (AD); this figure will quadruple by the year 2050.
- Existing drugs only marginally affect disease severity and progression.
- Heritabilities of up to 80% indicate a substantial genetic component. Identifying AD susceptibility genes will disclose causative pathogenic mechanisms, pinpoint proteins and pathways for drug development and inform development of genetic testing methods for identifying persons at risk.

Early identification of *APP*, *PSEN1* & *PSEN2* AS AD risk genes

- The discovery of autosomal dominant mutations in the *APP*, *PSEN1* and *PSEN2* genes resulting in early-onset AD led to the formulation of the 'amyloid cascade hypothesis,' suggesting that the enhanced generation of A β generated through cleavage of APP by β - and γ -secretase is a key pathogenic mechanism in AD.

Apolipoprotein E

- Early linkage studies implicated the *APOE ϵ 4* allele as strongest susceptibility gene in late-onset AD.

Novel susceptibility loci associated with late-onset AD

- Recent GWAS studies have identified additional ~20 common variants in *ABCA7*, *BIN1*, *CASS4*, *CD2AP*, *CD33*, *CELF1*, *CLU*, *CR1*, *EPHA1*, *FERMT2*, *HLA*, *INP55D*, *MEF2C*, *MS4A6A*, *NME8*, *PICALM*, *PTK2B*, *SLC2A4*, *SORL1* and *ZCWPW1* conferring small effects on disease risk.

Rare variants associated with AD

- Recent next generation sequencing studies implicated rare sequence variants with large effects on AD risk in *TREM2*, *SORL1*, *PLD3* and possibly *ABCA7*, *BIN1*, *MAPT*, *GRN*, *ABCA1*, *nicastatin*, *ADAM10*, *AKAP9* and *NOTCH3*.

Discussion

- Further large-scale genomic studies in various populations are needed to identify the additional disease-associated loci.
- Functional studies are needed to clarify the molecular mechanisms through which identified sequence variants affect AD risk.

Future perspective

- The ongoing genomic studies are expected to further disentangle the underlying molecular pathways and have the potential to identify novel targets for drug development and improved personalized medicine.