

Current status on Alzheimer disease molecular genetics: from past, to present, to future

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Linkage studies, candidate gene and whole-genome association studies have resulted in a tremendous amount of putative risk genes for Alzheimer's disease (AD). Yet, besides the three causal genes—amyloid precursor protein and presenilin 1 and 2 genes—and one risk gene apolipoprotein E (*APOE*), no single functional risk variant was identified. Discussing the possible involvement of rare alleles and other types of genetic variants, this review summarizes the current knowledge on the genetic spectrum of AD and integrates different approaches and recent discoveries by genome-wide association studies.

INTRODUCTION

It was estimated that, in 2010, 35.6 million people worldwide will be living with a diagnosis of dementia. This number of diagnosed dementia patients is projected to nearly double every 20 years, leading to 65.7 million patients in 2030 (1). Also, it is known that the actual number of dementia patients is much higher since several more people live with dementia but never receive a clinical diagnosis. These numbers indicate that dementia is rapidly becoming a major threat to healthcare in our societies.

Alzheimer's disease (AD) is by far the most common form of dementia with prevalence estimates ranging from 4.4% in persons aged 65 years to 22% at ages 90 and older (2). Increased age and a positive family history of dementia are the two major risk factors of AD. AD is clinically characterized by insidious onset and progressive impairment of memory and other cognitive functions (3), ultimately resulting in complete dependency and death of the patient. As clinical AD symptoms overlap substantially with other disorders of the central nervous system [such as frontotemporal dementia [FTLD (4)]], a definite diagnosis of AD can only be obtained after pathological brain examination. The key features of AD brains are neuronal and synapse loss, extracellular plaques composed of amyloid- β ($A\beta$) peptides and intraneuronal neurofibrillary tangles consisting of hyperphosphorylated tau protein (5), although other lesions such as TDP-43 immunoreactivity and ischaemia are common observations.

In general, two subgroups are recognized upon the age at which the first clinical symptoms become apparent; early-onset AD (onset age <65years) and late-onset AD (onset age >65years). Although most patients develop AD at later age, it is mainly the research performed on the rare autosomal-dominant early-onset form of AD that provided valuable insights into disease pathogenesis. Fully penetrant (causal) mutations leading to early-onset familial AD were identified within three genes; the amyloid precursor protein gene (*APP*) and the two presenilin genes (*PSEN1* and *PSEN2*). While the heritability for the more common late-onset form of AD is predicted to be as high as 80% based on twin studies (6), over the last decades only the apolipoprotein E gene (*APOE*) has been irrefutably recognized as a major risk factor for late-onset AD (7). Nonetheless, *APOE* ϵ 4 does not account for all genetic variation in AD (8,9). The complex late-onset form is most likely caused by multiple genetic and environmental susceptibility factors. High-throughput genomic association studies on extensive populations have opened up new avenues in detecting susceptibility factors for late-onset AD. Recently, three novel risk genes have been identified [*CLU*, *CR1* and *PICALM1* (10,11)].

MONOGENIC FORMS OF AD: CONSTITUTION OF $A\beta$ CASCADE

Since the identification of the first *APP* missense mutation in hereditary cerebral hemorrhages with amyloidosis (HCHWA-D)

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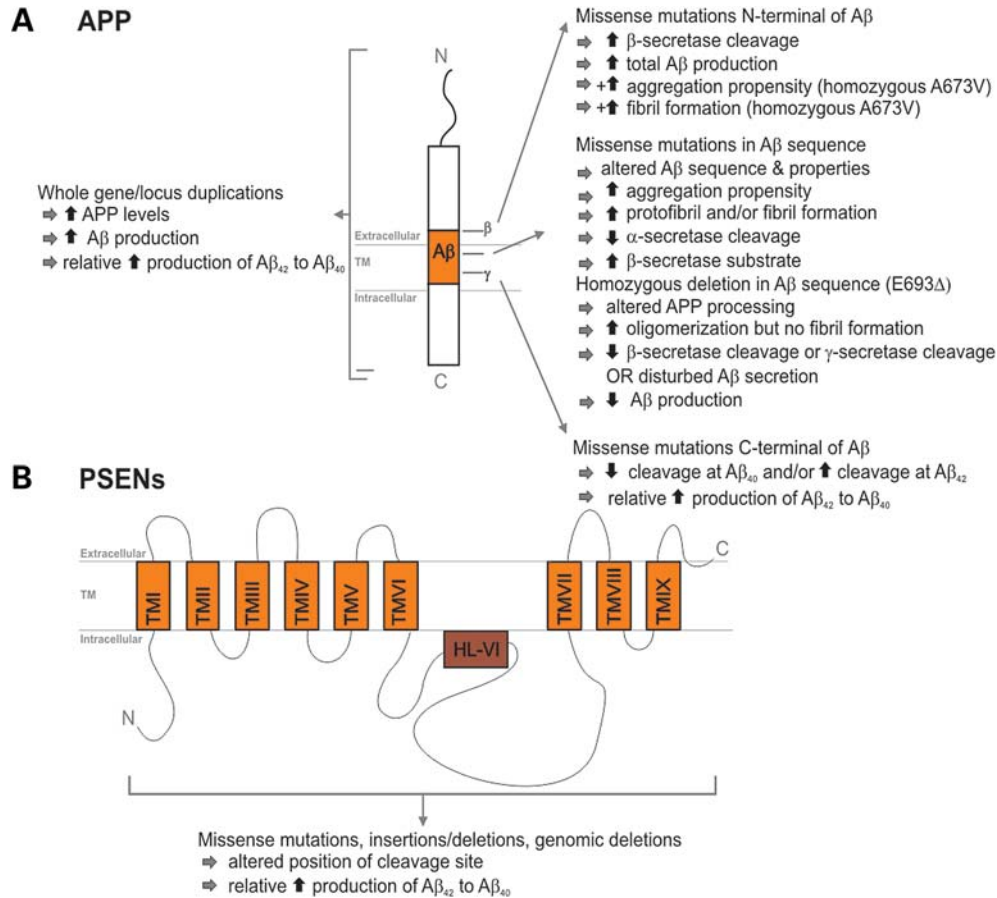


Figure 1. Effect of different causal *APP* and *PSEN1* mutations on APP processing and Aβ generation. (A) Schematic presentation of the APP protein structure. Right, the effect of *APP* mutations on APP processing is given according to their location relative to the Aβ peptide. The additional effects of the N-terminal recessive mutations A673V and E693Δ are indicated by the '+' symbol. Left, the effect of whole *APP* gene or locus duplications is depicted. (B) Schematic presentation of the PSEN protein structure. Boxes represent the transmembrane regions that are separated by hydrophilic loops. The effect of different mutations scattered throughout the protein is summarized.

(12), 23 *APP* missense mutations have been identified in 77 AD families (for up-to-date information, see AD Mutation Database, <http://www.molgen.vib-ua.be/ADMutations/>). *APP* mutations account for <0.1% of AD patients (13). Following the amyloidogenic pathway in neurons, APP is proteolytically cleaved by β-secretase and subsequently by γ-secretase generating full-length Aβ₄₀ or Aβ₄₂ (14,15). Interestingly, all missense mutations influence APP processing since they are positioned in or near the Aβ coding exons (*APP* exons 16 and 17) (AD Mutation Database) (Fig. 1). In addition to dominant *APP* mutations, two recessive mutations causing disease only in the homozygous state were identified: a trinucleotide deletion E693Δ segregating in one Japanese family proportionally decreased Aβ₄₀ and Aβ₄₂ with no change in their ratio (16), and A673V in one other family (Fig. 1) (17). Although very rare, it does suggest that other disease-causing mutational mechanisms can occur in this well-studied gene, which might explain at least some seemingly sporadic patients with early-onset AD. Additionally, the mutation spectrum extended to *APP* locus duplications underscoring the importance of *APP* gene dosage in AD. Duplicated *APP* regions containing several genes (18–20) or *APP* only (21) were clinically linked to early-onset AD often with extensive cerebral amyloid

angiopathy (22). Depending on the ethnic population under study, *APP* duplications accounted for <2–18% of autosomal-dominant early-onset AD families (19,21,23,24).

At present, 178 different AD-related mutations in 393 families have been identified in *PSEN1*, while only 14 mutations in 23 families were detected in *PSEN2* (<http://www.molgen.vib-ua.be/ADMutations/>). The majority of *PSEN* mutations are single-nucleotide substitutions, but small deletions and insertions have been described as well (AD Mutation Database). Mutations are scattered over the protein with some clustering within the transmembrane domains and the hydrophilic loops surrounding these domains (25). *PSENs* are functionally involved in the γ-secretase-mediated proteolytic cleavage of *APP* (26). Mutations in *PSENs* impair this cleavage, resulting in an increased Aβ₄₂/Aβ₄₀ ratio, by either an increase in Aβ₄₂ as shown in plasma and fibroblast media of *PSEN* mutation carriers (27,28) or by a decrease in Aβ₄₀, suggesting a loss-of-function mechanism rather than a gain-of-function [(29,30) Fig. 1].

Summarized, all three causal AD genes lend support to a common pathogenic AD pathway, stating a pivotal role for Aβ. According to this amyloid hypothesis, neurodegenerative processes are the consequence of an imbalance between Aβ

production and A β clearance (31–33), suggesting that other genes involved in these pathways might be risk factors as well.

COMPLEX FORMS OF AD: CONTINUOUS SEARCH FOR NEW RISK GENES

The genetic complexity of late-onset AD is underscored by the detection of only one consistent susceptibility factor; *APOE* ϵ 4 (34). Yet, genetic linkage and association studies over the last 20 years led to a plethora of putative risk genes (for up-to-date information; see AlzGene; <http://www.alzgene.org/>) (35). Approaches differed from hypothesis-driven (gene or pathway-based) to hypothesis-free (genome-scale) genetic studies. In a hypothesis-free setup, linkage scans on late-onset AD families and affected sib-pairs (ASP) identified candidate regions on several chromosomes. Particularly chromosomes 6, 9, 10, 12 and 21 were repeatedly found (36) and predicted to harbor susceptibility genes for late-onset AD. To date, no gene has been reported to explain these linkage peaks with high confidence.

The design of hypothesis-driven association studies has gradually shifted from studies in which only a few SNPs per gene were investigated to studies employing a more extensive linkage disequilibrium (LD)-based approach covering the complete genetic variation in a gene or a gene region, including, for example, regulatory regions. Besides the amyloid hypothesis, numerous candidate genes fitting one or another AD-related hypothesis on neurodegenerative pathways were analyzed, such as APP cleavage and trafficking, cholesterol metabolism, calcium dysregulation and so on (Fig. 2). As such, an endless record of candidate-gene-based studies was publicized. Nevertheless, none of the associated candidate genes attained an effect size similar to *APOE* ϵ 4 and along with positive associations, negative replications for the same gene were described (their number will probably be higher since publication in the field is biased toward positive finding; <http://www.alzgene.org/>). Reasons for lack of reproducibility may include: insufficient study power to detect variants with minor contributions, biological, genetic and allelic heterogeneity, differences in study design and the presence of population substructure.

The comprehensive approach of genome-wide association (GWA) studies seemed very promising for complex disorders, as it permits the simultaneous assessment of thousands of genetic variants without prior assumption on biological pathways. Until now, 10 GWA studies have been published on AD, with some differences in terms of design (patient-control or family based), SNP selection method (LD-based or based upon biological function), ethnicity of population, number of AD patients and controls, number of SNPs and SNP genotyping platform. Apart from the fact that all GWA studies except one [which was conducted in two extended pedigrees (37)] substantiated *APOE* ϵ 4 as the most significant finding, other genes were proposed as risk factors for late-onset AD [for an overview (38)]. Although none met standard criteria for genome-wide significance, several loci were replicated in independent study populations (39,40), though it should be noted that few replication studies have been published so far.

The two most recent GWA studies differ largely from all previous ones (10,11). First and foremost, they independently

provided strong evidence for an association with the clusterin gene (*CLU* aka apolipoprotein J gene, *APOJ*), making *CLU* the first consistent risk gene in AD history since the identification of *APOE* ϵ 4. Secondly, major sample sizes were acquired by international collaboration (>13 000 individuals in the study of Lambert *et al.* and >16 000 individuals in the study of Harold *et al.*), surpassing the power hurdle to detect variants with a minor effect. Two other risk genes were detected, i.e. the receptor gene for the complement C3b protein, *CR1*, and *PICALM*, encoding the phosphatidylinositol-binding clathrin assembly protein. However, together the three risk genes explained only part of the genetic variance which at their discovery was even likely an overestimate of the true effect (41). Ongoing meta-analyses using raw data of GWA studies will undoubtedly reveal novel genes, given their increased power to detect alleles with a minor effect.

The three novel risk genes support the existing AD hypotheses. Several properties of *CLU*, an abundantly expressed apolipoprotein in brain, are directly connected to A β . Present in amyloid deposits (42), *CLU* has an increased expression in several AD brain regions (43). It acts as an A β chaperone blocking the aggregation of A β ₄₂ peptides (44,45). However, depending on the balance between A β and *CLU*, *CLU* can either enhance or prevent amyloid fibril formation and cytotoxicity (46), though it remains unknown whether the same holds true in AD patients. *CLU* mediates A β clearance at the blood–brain barrier (47) and by increased endocytosis into glial cells (48–50). *CR1* is linked to AD through fibrillar A β -induced activation of the C3 complement cascade (51). Circulating A β ₄₂ is cleared by C3b-mediated adherence to *CR1* at the erythrocyte surface, a process which is decreased in AD patients when compared with control individuals (52). Complement inhibition of C3 in transgenic mice leads to an increased A β deposition and neurodegeneration (53), suggesting a protective role of the complement system in AD mice. The precise role of *PICALM* in AD pathophysiology is unclear, but it could include a role in APP processing through the endocytotic pathway, synaptic fusion and memory formation during trafficking of vesicle-associated membrane protein 2 [VAMP2 (54,55), Fig. 2]. Previous associations were detected within the dynamin-binding protein gene (*DNMBP*) (55,56), underscoring that genetic variability in genes involved in synaptic functioning might contribute to AD risk. The primary genetic variants within the three novel genes *CLU*, *CR1* and *PICALM* underlying the associations with risk for AD remain to be elucidated.

LESSONS FROM GENETIC EVIDENCE

The fact that late-onset AD has a complex genetic heterogeneity is reflected by the dissimilar results obtained in the hypothesis-free genome scans and GWA studies. This casts some doubt on the appropriate detection methods for AD susceptibility factors. Late-onset AD might be caused by multiple common or rare alleles, as well as by a wide spectrum of alleles of different frequencies and phenotypic effect sizes. In the common disease-common variant hypothesis, complex diseases are caused by a limited number of common variants with small predisposing effects (57–59). GWA studies

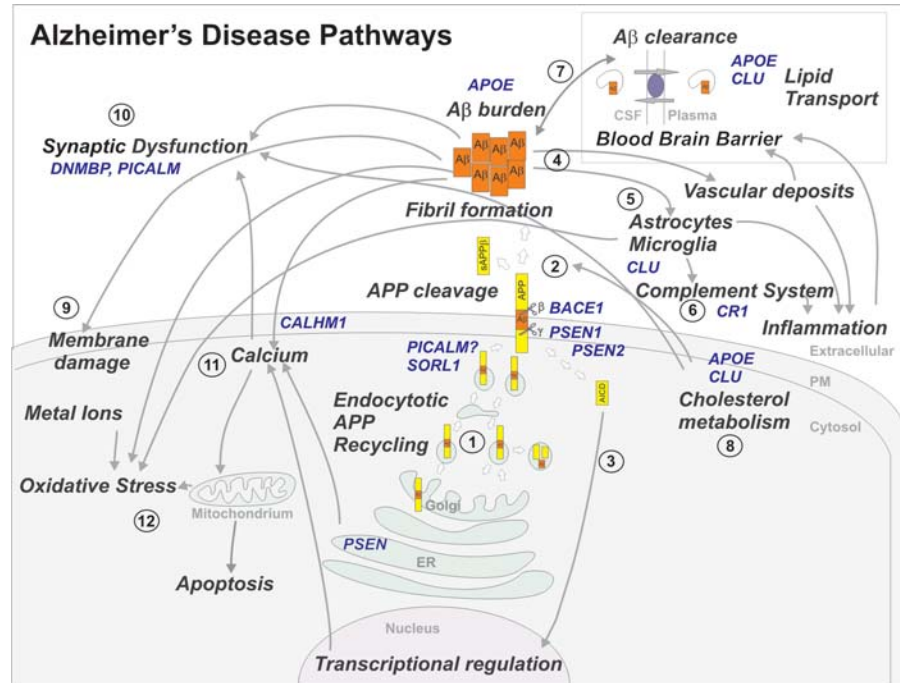


Figure 2. Overview of several disease pathways involved in AD pathogenesis. Causal AD genes and AD risk factors are marked in blue. APP is synthesized by the endoplasmic reticulum (ER) and the Golgi apparatus (1). Following the amyloidogenic pathway in neurons, APP is cleaved by β -secretase (BACE1) and γ -secretase (PSEN) to generate A β peptides and the amyloid intracellular domain [AICD] (2), which influences the transcription of several genes (3). In the APP retromer recycling pathway (1), APP is redirected to endosomes by SORL1. PICALM has a presumed role in APP endocytotic recycling (1). A β monomers aggregate into A β fibrils, causing amyloid plaques in brain parenchyma and vasculature (4). A β activates microglia and astrocytes, inducing the complement system, local inflammatory responses and oxidative stress (5). CR1 is the receptor of the complement C3b protein and participates in the clearance of A β from circulation (6). Besides causing increased A β endocytosis into glial cells, CLU is involved in A β clearance at the blood–brain barrier (7). APOE enhances amyloid plaque formation by conformational changes of A β . Clusterin (APOJ) and APOE are the main escorting proteins of A β in brain (7). Both are also important in cholesterol metabolism at the neuronal membrane (8) and high intracellular cholesterol may enhance APP amyloidogenic processing (2), which in turn can lead to membrane damage (9). Moreover, impaired cholesterol metabolism may influence synaptic dysfunction (10). Both PICALM and DNMBP are related at the synapse (10). Interaction of A β oligomers at the membrane is further connected to the calcium hypothesis in AD (11). Polymorphisms in the Ca²⁺ channel CALHM1 impair Ca²⁺ permeability at the plasma membrane (11). In addition, PSENs function as ER Ca²⁺-leak channels and several early-onset mutations impair Ca²⁺-leak-channel function, resulting in an excessive Ca²⁺ accumulation in the cytosol. An excessive Ca²⁺ is taken up by mitochondria, further leading to oxidative stress and apoptosis (12).

restricting the use of tagSNPs to common SNPs (minor allele frequency, MAF \geq 5%) have the potential to detect common variants since LD mapping depends on allele frequency of disease and marker locus (60–62). Even common variants with small effects (odds ratios in the range of 1.1–1.2) (63) will be detected when using very large sample sizes acquired by combining samples from many different settings.

On the contrary, rare variants will likely go undetected by GWA studies, no matter how large the sample size and the number of common SNPs being genotyped (64). Yet, rare variants might be implicated in complex pathways as previous studies suggested their contribution to common disease (65) and their importance in several disorders (66–68). Evidence suggested that rare variants in regulatory regions of causal genes contribute to AD susceptibility. For example, mutations detected within the *APP* 5' regulatory region in early- and late-onset AD patients might increase *APP* transcriptional activity (69,70), although this was not corroborated by a subsequent study (71). Whether 3'-UTR variants exert a functional effect on APP translation by miRNAs still remains to be assessed, but a recent re-sequencing effort detected rare mutations at highly conserved predicted miRNA target sites

in late-onset AD patients (72). In addition, regulatory *PSEN1* promoter variants were associated with AD in multiple early-onset populations (73–75) and for some a decreasing effect on transcriptional activity was shown (75,76). A deletion polymorphism inside the *PSEN2* promoter region was shown to increase *PSEN2* transcription by deprivation of transcription factor repression in a Russian early-onset AD population (77), although this could not be demonstrated in early- and late-onset AD populations of other ethnicities (78–80).

Besides the known AD genes, common or rare variants in genes associated with other neurodegenerative dementias might also confer risk to AD. For instance, missense mutations in progranulin (*PGRN*), in which null mutations result in FTL, are possible susceptibility factors for AD by influencing *PGRN* levels (81). Association findings with interesting susceptibility genes warrant further investigation of rare variants, since they might be functionally involved. Sequencing of the β -site APP-cleaving enzyme 1 (*BACE1*) 3'-UTR region identified rare patient-specific variants at predicted miRNA-binding sites (72). Besides the involvement of multiple genes in AD, several variants within one gene might confer risk to AD. For example, allelic heterogeneity was

supported for the sortilin-related receptor (*SORL1*), where the association with AD was found in two distinct gene regions (82,83).

Another type of genetic variation that has been underrepresented in genetic studies of AD but gained attention over the last years given its contribution to phenotypic diversity and complex diseases (84) is copy-number variations (CNVs). CNVs are implicated in a number of neurodegenerative disorders [for review, see (85)], including AD where *APP* duplications result in early-onset autosomal-dominant AD (18–21). In the future, genome-wide microarray data might shed light on the contribution of CNVs to complex AD.

SUMMARY AND FUTURE PROSPECTS: WHERE TO GO?

A marked evolution in study design has been seen in the field of AD genetics. Whereas classical, family-based linkage studies identified three causal AD genes and the *APOE* susceptibility factor, gene- and genome-based association studies have produced an intractable amount of association signals for late-onset AD, though the latter studies have been dominated by inconsistent findings (AlzGene database). Identification of *CLU*, *CR1* and *PICALM* as novel risk genes for late-onset AD was a major breakthrough and boost for the genetic field of late-onset AD. Besides these three novel risk genes, interesting genetic signals attaining sub-threshold significance should be examined in more detail. Conducting meta-analyses on GWA data will likely reveal new findings given the increased power to detect risk genes with smaller effect sizes. Conversely, this might bring about problems inherent to combining samples of different sources to obtain larger and powerful sample sizes, i.e. population-specific signals are diluted using mixed samples, especially taken the extensive genetic and allelic heterogeneity between populations. Furthermore, association outcomes might be different depending on the LD pattern between populations. Alternatively, studying isolated populations with a few founders might reduce the genetic heterogeneity and augment the chance to detect these otherwise ‘undetected’ signals. Another approach could be the use of intermediate phenotypes that resemble more closely the underlying disease pathogenesis and more directly interact with the genetic risk variance.

Since the failure to detect rare variants is another limitation of GWA studies, other detection methods are warranted to interrogate the complete allelic spectrum of AD. With the reducing costs of novel technologies, extensive re-sequencing efforts capturing all rare variants will eventually become possible in large AD populations. Whole exome-sequencing of probands of early-onset AD families has already started to take off, identifying a number of potential rare AD mutations [presented at ASHG2009, (86)]. Furthermore, whole-genome sequencing of AD patients will detect variations in non-coding regulatory sequences of RNA transcripts (such as miRNAs), and at an even higher level, variations in sequences mediating the expression of these regulatory sequences. Using the data of the 1000 Genomes Project (<http://www.1000genomes.org>), future GWA studies might include rare variants to ensure that a high proportion of rare SNPs are captured (61). The

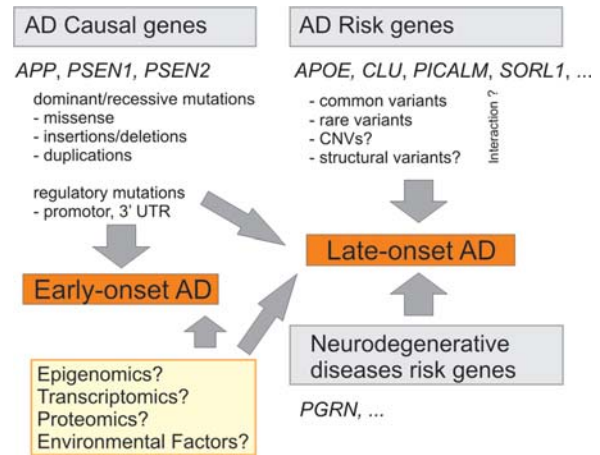


Figure 3. Schematic summary of the current knowledge of AD molecular genetics. Mutations in causal AD genes *APP*, *PSEN1* and *PSEN2* are associated with early-onset AD. Other mutations such as regulatory variants in promoter regions or 3' untranslated regions (3'-UTR) of causal AD genes may confer susceptibility to late-onset AD. Multiple common and/or rare variants in AD susceptibility genes confer risk to late-onset AD. The top four risk genes (Alzforum, status on 2 April 2010) are listed. The role of CNVs and other structural variants in late-onset AD remains to be clarified, likewise for the interaction between the different risk factors (indicated by question marks). Genes associated with other neurodegenerative dementias like *PGRN* are involved in AD genetic etiology as well. Several different approaches will likely reveal new genes involved in early and late-onset AD (yellow box).

role of structural variants, such as CNVs in relation to AD, should be interrogated across the complete genome. This is facilitated by the development of new generation SNP arrays and technology tools that combine SNP level and CNV dosage associations. In follow-up studies, all statistically significant associations should be functionally assessed, although this remains a hazardous task since many genetic variants play an unknown role and the functional effect may well be very subtle (87).

As no single method will fully elucidate the genetic spectrum of AD, more comprehensive approaches are recommended. The main challenge over the next years will be to devise methods allowing the integration and joint analysis of several types of variants and data, i.e. genetic data from GWA studies and re-sequencing experiments as well as transcriptomic, proteomic, epigenomic and metabolomic data. This genomic convergence approach has already been successful in prioritization of risk genes for AD on chromosome 10 (88). Once functionally established, epistasis should be evaluated by interaction studies between different susceptibility genes. One further challenge will be the study of the contribution of environmental risk factors in susceptibility to AD, since so far not much is known about the interplay of genetic and environmental factors.

The genetic architecture of AD is far from being completely unraveled (Fig. 3). Identifying additional genetic factors will remain challenging as genetic searches are complicated at several levels. However, promising strategies and tools are being developed to optimize comprehensive approaches. Detecting new susceptibility factors with a functional impact on AD will bring about major insights into the disease pathways, and initiate new lines of research toward improved

medical treatment. Though, it is still a long way to go, genetic risk profiles will eventually be translated into improved medical healthcare, including better and earlier diagnoses of AD as well as individualized care and treatments of AD patients.

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