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Review Article

Molecular genetics of early-onset Alzheimer's disease revisited

Rita Cacace[a,b], Kristel Sleegers[a,b], Christine Van Broeckhoven[a,b,*]

[a]Neurodegenerative Brain Diseases group, Department of Molecular Genetics, VIB, Antwerp, Belgium
[b]Laboratory of Neurogenetics, Institute Born-Bunge, University of Antwerp, Antwerp, Belgium

Abstract As the discovery of the Alzheimer's disease (AD) genes, *APP*, *PSEN1*, and *PSEN2*, in families with autosomal dominant early-onset AD (EOAD), gene discovery in familial EOAD came more or less to a standstill. Only 5% of EOAD patients are carrying a pathogenic mutation in one of the AD genes or a apolipoprotein E (*APOE*) risk allele ε4, most of EOAD patients remain unexplained. Here, we aimed at summarizing the current knowledge of EOAD genetics and its role in ongoing approaches to understand the biology of AD and disease symptomatology as well as developing new therapeutics. Next, we explored the possible molecular mechanisms that might underlie the missing genetic etiology of EOAD and discussed how the use of massive parallel sequencing technologies triggered novel gene discoveries. To conclude, we commented on the relevance of reinvestigating EOAD patients as a means to explore potential new avenues for translational research and therapeutic discoveries.

Keywords: Dementia; Early-onset Alzheimer's disease; Genetics; Genome and exome sequencing; Missing genetic etiology; Molecular pathways

1. Dementia and Alzheimer's disease

The term dementia is used to define a heterogeneous group of progressive and degenerative brain pathologies, clinically characterized by deterioration in memory, learning, orientation, language, comprehension, and judgment. AD (OMIM# 104300), in its typical clinical presentation with progressive loss of memory and disturbance of additional cognitive functions namely word-finding, spatial cognition, reasoning, judgment, and problem solving [1], is the leading cause of dementia in the elderly. Of all dementia patients, 50% to 75% present with AD, which affects between 23 and 35 million people worldwide [2]. Age is the most prominent biological risk factor [3], and the age of 65 years is often used to classify AD patients in early-onset (EOAD) and late-onset (LOAD) groups. Of all AD patients, around 10% are diagnosed with EOAD [4], and they present with their first symptoms between 30 and 65 years with most of the EOAD patients being diagnosed between 45 and 60 years. Besides the typical clinical presentation with memory impairment, atypical clinical presentation with focal cortical symptoms, for example, visual dysfunction, apraxia, dyscalculia, fluent and non-fluent aphasia, executive dysfunction, has also been reported. This atypical presentation is more frequently reported in EOAD patients compared to LOAD, who mostly present with typical memory phenotype [5]. Additionally, a nonmemory phenotype is seen in roughly 25% of EOAD patients in whom visual or apraxic and language phenotypes are more frequent [5].

The neuropathologic hallmarks of AD brains are extracellular accumulation of diffuse and neuritic amyloid plaques, composed of amyloid-β (Aβ) peptide, and frequently surrounded by dystrophic neurites and the intraneuronal accumulation of neurofibrillary tangles (NFTs) composed of hyperphosphorylated protein tau (p-tau) [6,7]. These pathologic features are accompanied by gliosis and the loss of neurons and synapses [7]. Although, some studies reported a larger neuropathologic burden [8] or a more widespread pathology extending outside the medial temporal lobe in younger patients [5], overall the

*Corresponding author. Tel.: +32 3 265 1101; Fax +32 3 265 1113.
E-mail address: christine.vanbroeckhoven@molgen.vib-ua.be

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pathologic features of EOAD and LOAD patients are largely similar, indicating that at the end-stage of disease, it is difficult to distinguish the two AD age groups by any other criterion than onset age.

The main incentive for this review was the recent renewed interest in EOAD genetic studies due to the availability of high-throughput, genome-sequencing and exome-sequencing technologies, and bioinformatic tools, permitting new attempts to unravel the missing genetic etiology of EOAD. The expectations are that these new genetic approaches will uncover new molecular pathways or new molecular components of already known pathways. Furthermore, the availability of new genetic markers will help refining the different genetic signatures of clinical AD, allowing a more accurate stratification of patient cohorts, preclinical and clinical, for medical research, and for clinical trials. In the long term, the ability to identify different underlying molecular pathologies of clinical AD patients or at risk individuals will pave the way for personalized medicine and health care.

2. The genetic etiology of EOAD

In contrast to LOAD which is a complex disorder with a heterogeneous etiology and an heritability of 70 to 80% [9,10], EOAD is an almost entirely genetically determined disease with a heritability ranging between 92% to 100% [9]. Between 35 to 60% of EOAD patients have at least one affected first-degree relative [11–13], and in 10% to 15% of those familial EOAD patients, the mode of inheritance is autosomal dominant transmission [11,13]. Genetic analysis of exceptionally large and informative monogenic pedigrees was the basis for the identification of high-penetrant mutations in the three EOAD genes, coding for the amyloid precursor protein (*APP*) and the presenilins 1 and 2 (*PSEN1* and *PSEN2*).

2.1. Identification of causal EOAD genes in extended pedigrees

Down syndrome (DS), caused by chromosome 21 (partial) trisomy, played a pivotal role in the early attempts to identify genes for inherited EOAD. DS patients were shown to present with a comparable brain pathology of amyloid plaques and tau tangles as AD patients [14]. The strong homology between the amyloid β (Aβ) protein peptides, isolated from vessels [15] and from plaques [16] from DS and AD brains, was a first indication that both diseases shared a common genetic mechanism associated with chromosome 21 [15,17]. Whole-genome-linkage (WGL) studies in AD families provided supporting evidence for a genetic defect located on chromosome 21q [18–20]. Cloning of the gene coding for the amyloid β precursor protein (APP) [21], from which the amyloid β peptides are produced, and its mapping to chromosome 21q21.2–21q21.3 [22,23], encouraged a series of genetic studies aiming at identifying mutations in AD patients and families. Initial genetic studies in large families with autosomal dominant AD were negative [24,25] but could be explained by the observation of a high degree of genetic heterogeneity in familial AD indicating that genes other than *APP* had to be involved [26].

A segregation study in extended multigenerational families with autosomal dominant cerebral hemorrhage with amyloidosis Dutch type (HCHWA-D), conclusively linked *APP* to the disease [27]. HCHWA-D brain pathology consists mainly of vascular amyloid depositions of the same Aβ observed in AD brains [28]. Sequencing identified a mutation affecting the Aβ sequence in patients with HCHWA-D [29] and segregated with disease [30]. The discovery of a *APP* mutation linked to vascular Aβ pathology in HCHWA-D encouraged new mutation studies of *APP* in AD families. A first mutation was identified confirming a direct role for *APP* in AD pathogenesis in some AD families [31].

Segregation studies in EOAD pedigrees, negative for *APP* mutations, led to the identification of a new locus for EOAD on chromosome 14q24.3 [32–35]. Genetic mapping and gene cloning followed by mutation screening of candidate genes [36–38] identified presenilin 1 (*PSEN1*) as an EOAD gene with at that time unknown functions [39]. Based on protein homology, a second presenilin protein was identified and mapped to chromosome 1q31–q42 [40,41], in the region that was linked to AD in a series of families known as descendants of Volga-Germans [42,43] and was named presenilin 2 (*PSEN2*).

2.2. Genetic and phenotypic heterogeneity in EOAD

To date, 52 pathogenic mutations in *APP* have been reported in 119 probands of autosomal dominant families (http://www.molgen.vib-ua.be/ADMutations) [44]. Most of the *APP* mutations are nonsynonymous within or flanking the Aβ sequence (Fig. 1A). However, 25 genomic duplications of variable size containing *APP* have been identified co-segregating with AD in as many autosomal dominant families, as reported in http://www.molgen.vib-ua.be/ADMutations [44] and reviewed in [45], mimicking partial trisomy 21. Furthermore, a recessive one amino acid deletion (p.E693Δ) [46] and a recessive missense mutation with dominant negative effect on amyloidogenesis (p.A673V) [47,48] were described (Table 1). Missense mutations are identified at least 4-folds more frequently than *APP* genomic duplications in AD patients. Disease onset of *APP* mutation carriers ranged between 45 and 60 years [49,50]. In contrast to the missense mutations, showing a near-complete disease penetrance, *APP* genomic duplications display reduced penetrance and higher variability in onset age [45]. Besides the pathogenic mutations, a rare protective variant p.A673T in *APP* was reported that was enriched in the Icelandic population [51]. At the same amino acid position, the p.A673V variant showed

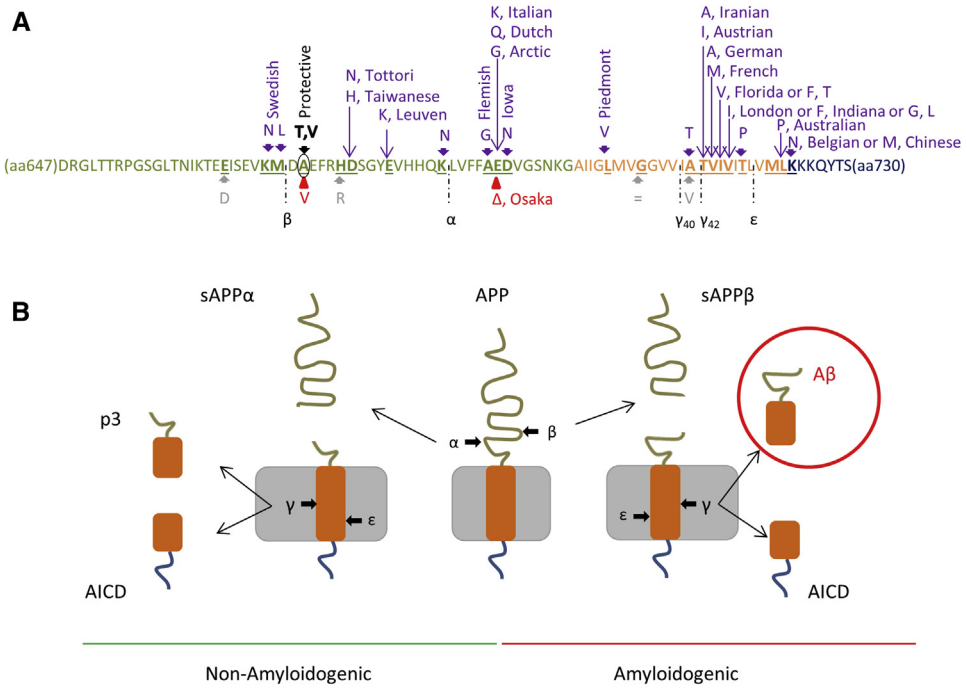

Fig. 1. APP protein mutations and structure. (A) APP protein sequence from amino acid residue 647 to 730 is presented, sequence in green depicts the extracellular domain, in orange the transmembrane domain and in dark blue the intracellular domain. Known pathogenic mutations are reported in purple and, if available, the mutation alias is shown, genomic duplications are not represented in the figure. In red are the two recessive pathogenic mutations, in gray the nonpathogenic mutations. A circle encloses the residue p.A673 because the change to T is described as protective against AD as well the change to V in the heterozygous state. An equal (=) marks a nonpathogenic silent mutation at residue p.G708. A delta (Δ) indicates a deletion. The cleavage sites of α, β, and γ secretases are marked with black dotted lines. (B) Schematic presentation of the APP proteolytic processes. The nonamyloidogenic and the amyloidogenic pathways are illustrated. For clarity, a general γ-secretase cleavage site is reported.

antiamyloidogenic properties when identified in the heterozygous state [47], suggestive of a protective effect (Fig. 1A).

Of all three EOAD genes, *PSEN1* is most frequently mutated with 215 mutations (Fig. 2A) in 475 probands (http://www.molgen.vib-ua.be/ADMutations) [44]. In *PSEN2*, 31 mutations have been identified, 15 pathogenic in 24 probands and 16 with pathogenicity nature unclear (Fig. 2B). The mutation spectrum of the *PSEN* genes includes missense mutations, small insertions, and deletions (indels) as well as genomic deletions specifically in *PSEN1*, which lead to in-frame exon 9 skipping (http://www.molgen.vib-ua.be/ADMutations) [44] (Table 1). The onset age of carriers of a *PSEN1* mutation ranged from 30 to 50 years. *PSEN2* mutation carriers have generally a wider onset age range from 40 to 70 years [49,50]. With a few exceptions, disease penetrance is complete for *PSEN1* mutations [50]. In case of *PSEN2* mutations, disease penetrance is more difficult to establish because far less families have been reported and the onset age range is much wider [50]. *PSEN* mutations are commonly inherited in an autosomal dominant manner, but *de novo* mutations in *PSEN1* have been described in EOAD patients with disease onset as early as 28 years [52,53].

Two families have been described that segregate dominant EOAD mutations in a homozygous state, a Italian family with the *APP* mutation p.A713T [54] and a Colombian family with the *PSEN1* mutation p.E280A [55]. In both families, the homozygous carriers did not seem to be more

Table 1
Genetic heterogeneity in Alzheimer's disease (AD): Known causal early-onset AD gene

Gene	Chromosome	Inheritance	Gene identification	Mutation spectrum	Mutations (N)
APP	21q21.1–21q21.3	Autosomal dominant Autosomal recessive Protective	Linkage analysis	Missense Gene Duplication Amino acid deletion	54*
PSEN1	14q24.3	Autosomal dominant *de novo*	Linkage analysis	Missense Small indels Genomic deletions	215
PSEN2	1q31–q42	Autosomal dominant	Linkage and homology mapping	Missense	31

*The total number of *APP* mutations includes two causal recessive mutations.

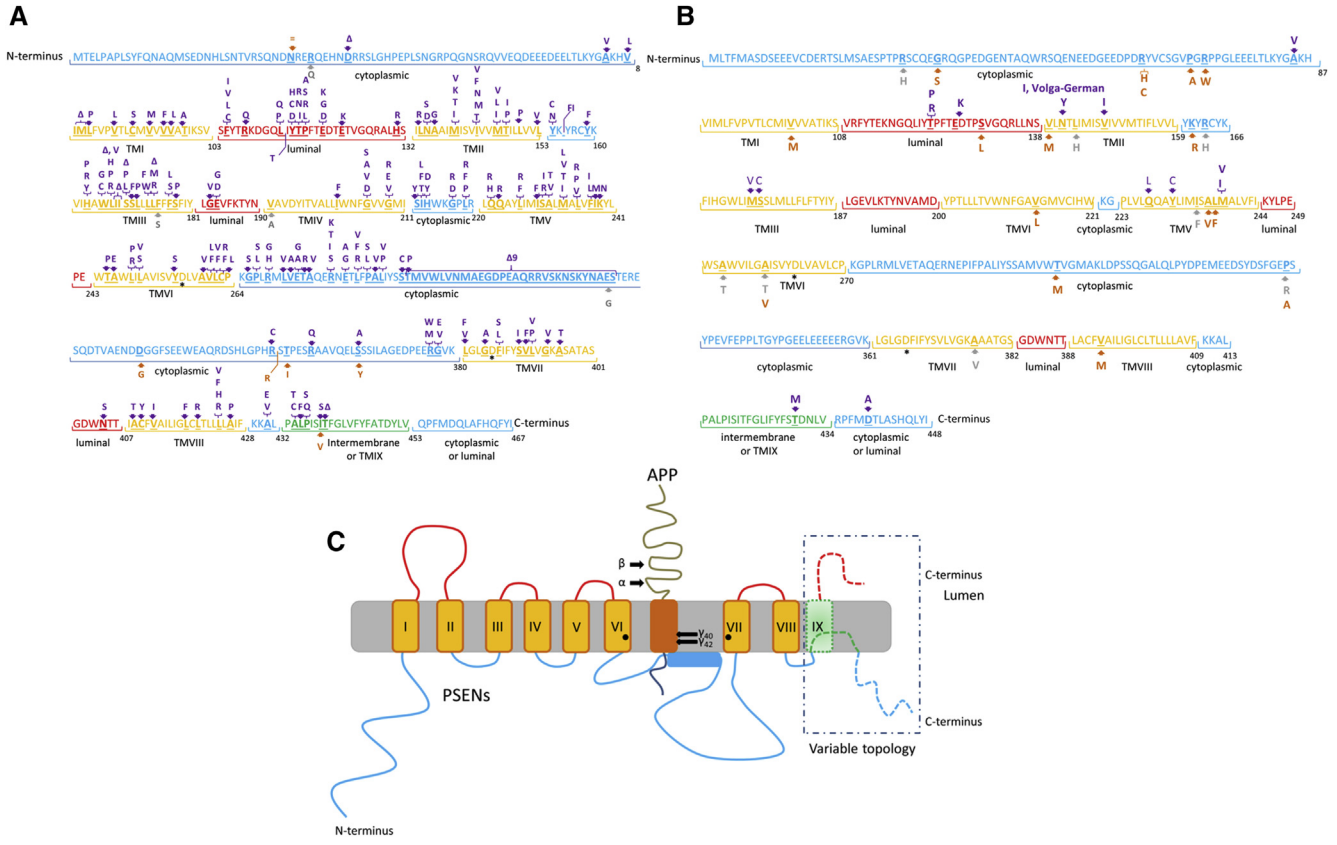

Fig. 2. PSENs protein mutations and structure. (A) PSEN1 and (B) PSEN2 protein sequences. In blue are marked the cytoplasmic domains, in yellow the transmembrane domains, in red the luminal domains, and in green the one intermembrane/IX transmembrane domain. Pathogenic or predicted pathogenic mutations are in purple. In orange are mutations with unclear pathogenicity and in gray are reported nonpathogenic mutations. Different nucleotide variants which lead to the same amino acid change, are reported only once. A delta (Δ) indicates deletions. An asterisk (*) marks two aspartates (D), which are the catalytic amino acids located in transmembrane domain VI (PSEN1 is D257 and PSEN2 is D263) and in transmembrane domain VII (PSEN1 is D385 and PSEN2 is D366). Arabic numbers indicate the last amino acid residue of every topological domain based on Uniprot database (PSEN1 P49768 and PSEN2 P49810). (C) Schematic presentation of APP and PSEN complexes. For PSENs, alternative predicted protein conformations are shown. The region with variable topology is framed in dotted line and includes either an intermembrane domain with intracellular C-terminus or a ninth transmembrane domain with extracellular C-terminus. The black dots on the transmembrane domains indicate the catalytic aspartates location. APP is inserted between PSENs transmembrane domains VI and VII. The α, β, and γ secretases cleavage sites are schematically indicated.

severely affected [54,55]. The major implication of these findings is that the homozygous state of autosomal dominant mutations in AD is not lethal as initially hypothesized. This may also suggest the possible presence of additional protective genetic factors, which may modify the disease presentation.

In an EOAD patient cohort, the estimated mutation frequencies for the three genes were <1% for *APP*, 6% for *PSEN1*, and 1% for *PSEN2* [56]. Together, they explain only 5%–10% of EOAD patients [9,56], whereas depending on the study, 23% to 88.2% of autosomal dominant patients remain genetically unexplained [9,11,57–59] (Fig. 3). The wide divergences in unexplained EOAD patients and families might result from differences in study design or sampling biases. Besides the genetic heterogeneity, phenotypic heterogeneity is also reported in EOAD. This complicates the clinical diagnosis in younger patients. Atypical presentation with language impairment was reported for specific *PSEN1* mutations [49,50]. Prominent behavioral symptoms (also presenting symptoms) like delusion, hallucinations, and apathy, are described for a subset of *PSEN1* and *PSEN2* mutations [49,50]. In some cases, the clinical symptoms fulfilled the criteria for a diagnosis of frontotemporal dementia, for example, in the case of the *PSEN1* mutation p.G183V. In this specific example, the autoptic pathologic and immunohistochemistry analysis of the brain showed pick-type tauopathy, normally caused by *MAPT* mutations, in absence of extracellular Aβ deposits [60]. The neurologic symptom of myoclonus is present in most of monogenic EOAD patients and increases with the disease duration; seizures were also reported as presenting symptom for some *PSEN1* mutations and are very common in *APP* duplication carriers (including Down syndrome patients) [49,50]. Extrapyramidal symptoms are not uncommon in *PSEN1* mutations carriers but very rare for *PSEN2* and *APP* and tend to appear after several years into the disease course [49,50]. Spastic paraparesis has been associated with specific *PSEN1*

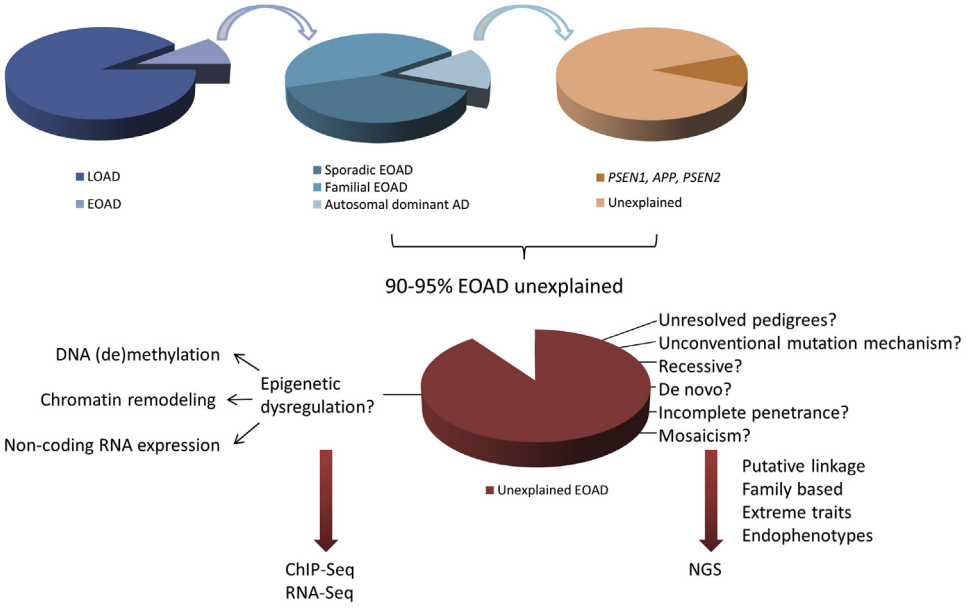

Fig. 3. Missing genetic etiology of early-onset Alzheimer's disease (EOAD). The pie charts indicate the distribution of EOAD and late-onset Alzheimer's disease (LOAD) patients (dark blue), the fraction of sporadic and familial EOAD patients with the sub-fraction of autosomal dominant patients (light emerald). The orange pie chart depicts the fraction of unexplained autosomal dominant families. The possible mechanisms that may explain the missing genetic etiology of EOAD are divided in two groups arising from the red pie chart: (1) possible undetected genetic alterations due to different causes, listed arising from the red pie chart (right side) and (2) possible undetected epigenetic dysregulation (left side). For both scenarios, some examples of study designs (e.g., family based, extreme trait design and so forth or investigation of DNA(de) methylation and so forth) and technological approaches such as next-generation sequencing (NGS), chromatin immunoprecipitation assay combined with sequencing (ChIP-Seq) and RNA sequencing (RNA-Seq) are schematically suggested.

mutations; in these cases, a peculiar pathologic finding of Aβ plaques without a dense core or neuritic dystrophy ("cotton wool plaques") is reported in the frontal cortex of the patients presenting the phenotype [61]. Cerebellar ataxia is a rare event but is present for specific *PSEN1* mutations, like in the case of the p.S170F mutation co-segregating with a *Cathepsin D* variant, suggesting a deleterious epistatic effect on the disease course [62]. Taken together, this indicates that it is reasonable to continue efforts to both unravel the genetic etiology of EOAD and to search for modifier of the phenotype in presence of known pathogenic mutations.

2.3. APOE *in EOAD*

A genome-wide linkage study in families with LOAD and subsequent association studies in patient/control cohorts identified the ε4 allele of the apolipoprotein E gene (*APOE*) as a major genetic risk factor for LOAD [63–65]. The presence of one or two copies of the *APOE* ε4 allele increased the risk to develop LOAD by a factor 3 up to 15-fold in a dose-dependent manner [66,67].

The *APOE* ε4 allele also increased risk for EOAD in carriers of at least one ε4 allele and was highest in those with a positive family history (Table 2) [12]. In carriers, the homozygosity for the *APOE* ε4 allele was sufficient to significantly increase risk for EOAD independent of other genetic factors. In contrast, in carriers, heterozygous for the *APOE* ε4 allele risk was only significantly increased in the presence of a positive family history of disease, indicating that the presence of one ε4 allele was insufficient to increase risk for AD before the age of 65 years. It also suggested that the *APOE* ε4 allele may modify the expression of other genetic factors contributing to disease. However,

Table 2
Risk variants and genetic modifiers in EOAD

Gene	Variant	Effect on EOAD	References
PSEN1	Promoter SNVs	Increased risk	[68,69]
APOE	ε4 allele	Increase risk–highest in the presence of positive family history–and earlier AAO	[12,70,71]
	ε2 allele	Delays AAO	[70–73]
CCL11	p.A23T	Delays AAO	[74]
PRNP	p.M129V	Increased risk–VV homozygosity and highest in presence of positive family history	[75]
	Octapeptide repeat insertions	Earlier AAO	[76]
SORL1	Nonsynonymous rare variants	Increased risk–signal driven by positive family history	[77]

Abbreviations: EOAD, early-onset Alzheimer's disease; SNVs, single nucleotide variants; AAO, age-at-onset.

familial aggregation of EOAD cannot fully be explained by *APOE* as both carriers and noncarriers of a ε4 allele have an increased risk in the presence of a positive family history. Contrary to the risk-increasing effect of the *APOE* ε4 allele, it was demonstrated that the ε2 allele exerted a protective effect [72,73]. Different from the mutations in *APP*, *PSEN1*, and *PSEN2*, the *APOE* ε4 allele was considered neither necessary nor sufficient to cause AD. However, this concept might need to be reconsidered based on a more recent study [78], which showed that the effect of *APOE* on AD risk is comparable to the effect of a major genetic factor with semidominant inheritance [78].

Analysis of the *APOE* genotypes in families segregating dominant *APP* (p.V717I) [79], *PSEN1* (p.E280A) [70], or *PSEN2* (p.N141I) mutations, showed that onset age decreased in presence of the ε4 allele in mutation carriers, whereas those with the ε2 allele had later onset ages [70,71]. A larger study of samples of the *PSEN1* (p.E280A) family [73] confirmed the *APOE* ε4 allele as an age-at-onset (AAO) modifier (∼12 years of delay) compared to the ε4 allele (Table 2) [73]. Nevertheless, these *APOE* genotype effects were not observed in all families with a pathogenic mutation [90]. Genome-wide searches in the Volga German families (P*SEN2* p.N141I) identified at least three additional genetic loci (1q23.3, 17p13.2, and 7q33) [91], which could harbor modifier genes. Furthermore, a whole genome-sequencing approach of a large Colombian kindred (*PSEN1* p.E280A), detected a protective variant (p.A23T, rs1129844) in the gene *CCL11* encoding eotaxin-1 that delayed onset age of AD by a decade [74]. Autosomal dominant families, segregating mutations in one of the 3 causal EOAD genes, show often variability in clinical presentation, even in the presence of the same mutation. A meta-analysis study showed that the phenotypic heterogeneity could be partially explained by the mutated gene, the type of mutation, and the family history. But, this is not always the case, indicating that in certain families, additional modifier factors (i.e., gene-gene and gene-environment interactions) may be involved [90].

3. The amyloid cascade

APP and its processed peptides are the cornerstone of the "amyloid cascade hypothesis" [92]. This hypothesis states that the accumulation of Aβ is the causative agent of AD by destroying the synapses, inducing the formation of NFTs, and ultimately inducing neuronal loss [92]. Two distinct and mutually exclusive pathways cleave APP (Fig. 1B). In one pathway, the cleavage by α-secretase and γ-secretase produces three fragments: a secreted C-terminal fragment (sAPPα), p3, and the APP intracellular domain (AICD; Fig. 1B). In the second, and thus amyloidogenic pathway, APP is cleaved by the β-secretase (beta-site APP cleaving enzyme 1, BACE1), followed by γ-secretase cleavage. The cleavage by β-secretase generates a large soluble extracellular secreted domain (sAPPβ). The remaining membrane bound APP fragment, C99, is processed by multiple γ-secretase cleavages. The first occurs at the cleavage site epsilon (ε-site, Fig. 1B) to produce the AICD and then, after γ-secretase cleavages trim the membrane bound producing Aβ species that differ in protein length including Aβ38, Aβ40 (most common fragment), Aβ42 (self-aggregative fragment), and Aβ43 [93]. Genetic mutations in *APP* are located in or near the Aβ sequence and in the proximity of the cleavage sites of the secretases (Fig. 1A), whereas *PSEN1* and *PSEN2* mutations are scattered all over the protein (Fig. 2A and 2B). The mutations are predicted to cause AD through aberrant APP processing determining either increased Aβ levels or increased production of Aβ42 (and Aβ43) peptide over Aβ40, triggering Aβ aggregation [93]. From its postulation, the "amyloid cascade hypothesis" has been supported and questioned (as reviewed by Morris et al. [94]) acknowledging that Aβ has an important role in AD pathogenesis although its role in the disease process is most likely much more multifaceted. This was corroborated by the observation of a subgroup of clinically diagnosed AD patients with neurodegeneration and cognitive deficits but near absence of Aβ deposits, defined as "suspected non-AD pathology" (SNAP) [95]. In a clinical trial of anti-Aβ passive immunotherapy, 16.3% of the clinically diagnosed AD patients had a negative-baseline amyloid positron emission tomography scan [96]. Studies on mild cognitive impairment cohorts are ongoing to phenotypically classify the SNAP patients [97,98], to better understand, if they represent a subgroup of AD with pathologic features independent from the amyloid cascade [99].

4. The role of other neurodegenerative brain diseases genes in EOAD expression

Possibly, some of the SNAP patients might be associated with mutations in genes involved in other neurodegenerative brain diseases (NBD). Mutations have been identified, at low frequencies (less than 1%–2%), in genes causing frontotemporal lobar degeneration (FTLD), that is, mutations in *MAPT* [83,84] and in granulin (*GRN*) [80–82], and the repeat expansion in *C9orf72* [85–87] (Table 3). The genetic heterogeneity of the phenotypic presentation of AD supports that both diseases form part of an AD-FTLD disease continuum [100,101]. An AD-like phenotype was also described in the presence of a nonsense mutation in the prion protein

Table 3
Genetic heterogeneity in AD: Neurodegenerative brain diseases genes presenting with AD-like phenotype

Gene	Chromosome	Typical phenotype	Mutation frequency in AD (%)	References
GRN	17q21.31	FTLD	<2%	[80–82]
MAPT	17q21.31	FTLD	<1%	[83,84]
C9orf72	9p21.2	FTLD	<1%	[85–87]
PRNP	20p13	Prion disease	<1%	[88,89]

(*PRNP* p.Q160*) [88,89], gene responsible for inherited neurodegenerative spongiform encephalopathies. Histopathologic analysis of nonsense mutation carrier showed neuritic plaque-like pathology and severe NTFs, the plaques were immunonegative for Aβ but immunopositive to PrP leading to a pathological diagnosis of prion disease [88] (Table 3). Moreover, a common coding polymorphism, Methionine (M) to Valine (V) at position 129 of *PRNP* has been associated with EOAD when identified in homozygous state (MM and VV; Table 2) [75]. The risk was higher for the VV and increased in patients with positive family history [75]. In addition, increasing number of octapeptide repeat insertions in *PRNP* have also been associated with younger onset age in AD patients (Table 2) [76]. The genetic heterogeneity in some dementia patients leads to a later clinical diagnosis and to flawed genetic testing. With the current availability of massive parallel sequencing (MPS), causal genes across NBD clinical diagnostic dementia subgroups can be screened simultaneously, increasing the chances to identify pathologic mutations independent of the clinical diagnosis. It can be expected that the breadth and depth of this etiological disease heterogeneity will be clarified more fully in the coming years, as discussed in section 5 and 6.

5. The role of EOAD genes in AD susceptibility

There is substantial evidence that apart from pathogenic mutations, the EOAD genes also harbor genetic variations that contribute to increased susceptibility for AD. For example, single nucleotide variants (SNVs) in the promoter region of *APP* or *PSEN1* have been associated with increased risk of LOAD [68,69,102]. Some *APP* promoter SNVs, increase neuron-specific *APP* transcriptional activity by near two-fold [103,104], and as such increase risk to develop LOAD. *PSEN1* promoter SNVs [68,69,102] could alter protein expression in neurons [69,102] leading to increased risk of both EOAD (Table 2) [68,69] and LOAD [69]. Likewise, coding variants in the known causal genes might influence AD risk; however, to date, there is little information available because causal EOAD genes are rarely screened in LOAD patients [56,105]. Moreover, in EOAD patients, the routine genetic testing paradigm was based on mutation frequency (*PSEN1* > *APP* > *PSEN2*) and location of known mutations (exons 16 and 17 in *APP* covering the Aβ peptide cleavages sites) and was stopped once a pathogenic mutation was identified.

Today, the availability of extended gene panels and MPS technology, allows near unlimited screening of full coding sequences of multiple disease genes in parallel across clinical phenotypes ([105,106] and unpublished data). Systematic screening of EOAD and LOAD patient cohorts showed rare or novel mutations in causal AD genes in LOAD patients ([105] and unpublished data) mutations in *APP* outside the Aβ coding exons and the occurrence in one individual of mutations in more than one NBD genes (unpublished data). This suggests that mutations in known EOAD genes might act differently on disease expression, which ranges from high penetrance (= causal allele) and early-onset age to low penetrance (= risk allele) and late-onset age, depending on the effect of the mutant allele on protein function ([105] and unpublished data). The new challenge that we are facing when using these gene panels is the high potential of identifying variants of uncertain significance (VUSs). These VUSs lack supportive functional data and can only be classified based on their likelihood of pathogenicity using *in silico* information obtained using bioinformatics prediction tools (e.g., predicting the impact of the mutated amino acid residue on protein stability/function) [107], data which are insufficient in a clinical diagnostic setting.

6. The missing genetics of EOAD

Rare high-penetrant mutations in *APP*, *PSEN1*, and *PSEN2* could explain only a small fraction of EOAD families, leaving a large group of autosomal dominant pedigrees genetically unexplained [9,11,57–59] (Fig. 3). Additionally, it was estimated that only between 5%–10% of EOAD [9,56] can be explained by mutations in the three known EOAD genes (Fig. 3). The limited number of resolved pedigrees and large number of genetically unexplained EOAD patients, indicated that additional causal genes remain to be identified. Next generation sequencing technologies (NGS), like whole-genome-sequencing (WGS) and whole-exome-sequencing (WES), offered a next step to gain new insights into the molecular genetic etiology of AD. Particularly, these NGS technologies made it conceivable to reconsider those small families that had been excluded from WGL studies because of their limited genetic information content and thus lack statistical power. Additionally, it became possible to examine, in an unbiased manner, groups of unrelated AD patients selected for their extreme phenotypic characteristics, for example, very early-onset age, familial history of disease, or atypical clinical and/or neuropathological phenotypes.

An early NGS study produced WES data of a clinically diagnosed EOAD patient and identified in *NOTCH3*, a known mutation that had been associated with cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy [108], a dementia disorder that shares clinical similarities with EOAD. The index patient belonged to a consanguineous Turkish family with a complex clinical history of neurologic and immunologic disorders [108]. The study demonstrated that WES is a valid tool to unravel the genetic etiology of complex diseases [108]. A WES study in EOAD probands of autosomal dominant families, who were negative for mutations in the three EOAD genes, identified seven mutations on screening of 29 probands (five missense and two nonsense mutations) in the sorting protein-related receptor gene (*SORL1*) [109]. Previous studies have shown reduced expression of the SORL1 transcript in AD patients using

microarray screening [110] and the involvement of the protein in APP and Aβ trafficking [111]. One of the EOAD missense mutations in *SORL1* (p.G511R) that co-segregates with autosomal dominant AD in one family [109], is located within the VPS10P domain of SORL1. This specific mutation was shown to reduce the capability of the VPS10P domain to bind Aβ, resulting in accumulation of the peptide and lower turnover [112]. More, the investigation of an EOAD patient-control cohort confirmed the role of rare nonsynonymous coding variants in *SORL1* as risk factor for EOAD (Table 2), with the association signal driven by familial patients [77]. Also, both common and rare, noncoding and coding variants in *SORL1* have been associated with increased risk of LOAD [113–115]. Three *SORL1* coding mutations (p.E270K and p.T947M, p.A528T) identified in a family-based and cohort-based association study on LOAD have been shown to increase Aβ40 and/or Aβ42 secretion when expressed *in vitro* [115]. These studies reinforced the role of rare variants in *SORL1* in both EOAD and LOAD risk. The screening of *SORL1* in larger patient/control groups will help defining the contribution of rare genetic variants in *SORL1* to AD etiology.

6.1. Causal genes in unexplained EOAD families

Scarcity of sufficiently informative EOAD families, the small number of family members available for genetic analyses and the clinical and genetic heterogeneity, has hampered WGL studies in identifying the actual underlying gene defects [116]. An example is provided by the family-based WGL study in the Swedish series, which includes both EOAD and LOAD patients. The original WGL study in multiple autosomal dominant families detected a suggestive linkage at chromosome 5q35 [117]. The inclusion of additional families to the WGL analysis did not confirm the 5q35 locus [118], but the investigation of a selected number of families with autopsy confirmed AD revealed a suggestive locus on chromosome 8q24 [119]. An independent locus on chromosome 8q was identified in a Swedish family in another WGL study [120]. In the aforementioned examples, the underlying genetic defect has not yet been pinpointed. We also identified in one extended Dutch family, a significant WGL peak at chromosome 7q36 but candidate gene screening in this locus did not identify a mutation that could explain the linkage [121].

Today, NGS technologies are used to examine unexplained EOAD families [122]. In large extended families, the NGS approach can be preceded by a WGL analysis to prioritize chromosomal regions that are more likely to contain the disease gene. NGS data of two, distantly related, patients can be sufficient to identify shared, protein affecting, genetic variants in genes located in the linked loci. An advantage of this combined approach of WGL and NGS is a significant decrease in complexity of the NGS data under analysis. Furthermore, the success rate of this approach can be substantially increased by including additional family members both affected and/or unaffected. The main strength of NGS technologies, however, is their capability to identify putative disease genes by direct analysis of the data obtained of related family members belonging to small nuclear families. Because of the familial nature, these NGS studies are often limiting their first analysis to variants in coding sequences, particularly variants predicted to have a high impact on the protein functioning and thus identifying high-penetrant causal mutations. But silent, noncoding and genomic variations can also exert an effect on disease risk or causality. For example, the intron 4 variant (c.358–304 C > G) in *SOD1* defines the activation of a cryptic splice site with the inclusion of a pseudo-exon leading to amyotrophic lateral sclerosis [123]. Also, genomic duplications of the *APP* locus have been linked to EOAD (as reviewed in section 2.2), and an inversion polymorphism comprising *MAPT* has been associated to increased risk for neurodegenerative brain diseases like progressive supranuclear palsy [124,125]. In case, a candidate gene is identified in this NGS approach, the next major step is to generate confirmatory evidence of its pathogenic role in the disease. This can be obtained by co-segregation studies of the putative disease causing mutation in the family, by the screening of the candidate gene for mutations in a patient cohort and its absence in a control cohort. Limiting factors to the segregation analysis are the size of the pedigree as well as the number of available samples. Also, incomplete or age-dependent penetrance of the genetic variants can complicate the interpretation of both the segregation data as well as the presence of the variant in control individuals.

Alternatively, an extreme trait design can be used to sequence a small number of individuals at one or both extremes of the disease (endo) phenotype [126] as shown by Nho et al. [127]. This approach, though it identified frequent variants that may be associated with AD risk, provides an example of using NGS with quantifiable traits as endophenotype.

6.2. Other mechanisms

In most of the EOAD families, the disease inheritance is consistent with autosomal dominant transmission. Occasionally, other transmission patterns were reported like recessive mutations in *APP* [46,47] and *de novo* mutations in *PSEN1* [52,53] (described in section 2.2), but they have not been systematically explored. A potential study design to optimize their detection is the use of WGS in small nuclear families or trios with neurologically healthy parents and EOAD patients negative for the *APOE* ε4 allele. It is possible to approach the data analysis, based on the family history of disease, to identify either recessive (positive family history) or *de novo* (negative family history) mutations. In specific cases, that is, when the family history is unknown, both hypotheses can be

followed in parallel. The WGS data of the affected offspring can be used to define a genome-wide map of runs of homozygosity (ROH), and these ROH regions can be analyzed together with the WGS data of the parents to identify recessive mutations. Recessive inheritance of AD has already been examined before using either polymorphic simple-tandem-repeat markers or high-density single nucleotide polymorphism genotyping arrays, identifying homozygous regions harboring potential candidate genes [128–131]. Conversely, the WGS data can be analyzed for *de novo* mutations present in the offspring but absent in the parents. Limitations to these study designs are the unavailability of both parents and, the possibility that EOAD in the offspring resulted from a dominant mutation that was undetected in the parent due to incomplete penetrance or an AAO modifier. Alternative to recessive or *de novo* mutations, sporadic EOAD patients can also be explained by germline or somatic mosaicism [132,133] as shown for the *PSEN1* p.P436Q mutation [132], with the degree of mosaicism affecting onset age and clinical presentation. Also, epigenetic dysregulation can affect gene expression in AD. Deoxyribonucleic acid (DNA) (de)methylation, chromatin remodeling, and noncoding RNAs (ncRNAs) expression could play a more impacting role than initially thought in AD onset and expression (Fig. 3). Alteration in methylation in the promoter region of *APP*, *PSEN1*, and *APOE* have been described in AD patients as well as histone acetylation which was altered in both AD patients and in AD transgenic mouse models (as reviewed by Lardenoije et al., [134]). Imbalanced expression of noncoding RNAs, miRNA particularly, has been identified in relation to AD (as reviewed by van den Hove et al. [135]). These mechanisms affect gene expression without altering the DNA sequence and are currently underinvestigated in EOAD patients. Two epigenetic studies in LOAD [136,137], adopted novel designs to control for confounding factors like the different methylation pattern in different cells/tissues, interindividual variability within the same tissue [138]. Both studies independently detected changes in methylation in four CpG sites close to *ANK1*, *RPL13*, *CDH23*, and *RHBDF2* [136,137]. This confirmed the respective findings and supported the strength of the study designs. Additionally, the recent availability of the epigenome roadmap [139], a public data resource containing epigenetic information from different cell types and tissues, is paving the way to more comprehensive research. This effort resulted, among others, in the identification of conserved epigenomic signals at orthologous regions between mice and humans, which upregulate the immune response genes and downregulate the synaptic plasticity genes in the CK-p25 mouse model of AD [140]. The major challenge of this type of studies, in the years to come, will be to determine whether the epigenetic change is a cause or a consequence of the disease and how the disease risk is influenced by the alteration of the chromatin structure [141]. Moreover, it will be relevant to comprehend how the epigenome signature varies between populations and how the epigenetic findings will translate from mouse models (e.g., CK-p25) to humans. The availability of epigenome data will allow the integration of genetic results with epigenome regulation to better understand disease signatures which may represent a potential target for future diagnostic and treatment interventions.

7. Translational research in autosomal dominant families

On the one hand, the investigation of unexplained EOAD families and patients aims at identifying novel causal genes and pathways. On the other hand, an international effort is ongoing to better understand the temporal sequence and the earliest events, which lead to the endpoint disease clinical manifestation. Longitudinal studies on families segregating a monogenic form of AD and patients with a known causal mutation are currently performed to investigate the prodromal phase of the neurodegenerative processes which ultimately lead to AD [142]. The understanding of the post-genomic consequences of the genetic mutations aims at improving the AD diagnosis through the identification of novel sensitive biomarkers (Box 1), which correlate with earliest pathologic events and with disease conversion and progression. This will also help in the definition of targets for therapeutic screening and the selection of uniform patients groups for targeted pharmaceutical approaches. Longitudinal studies in asymptomatic mutation carriers have already demonstrated that at least 20 years before the clinical manifestation of cognitive deficits it is possible to detect the pathologic changes happening in the brain using both biochemical and instrumental biomarkers (Box 1) [142,144]. The longitudinal data investigation of cognitively unimpaired mutations carriers from the Colombian *PSEN1* kindred included in the Alzheimer's Prevention Initiative (API), for example, led to the identification of a composite cognitive test score that has improved power to detect preclinical AD decline [150]. A direct application of this score could be in identifying preclinical patients to include in prevention trials [150]. In the API cohort, the anti-Aβ monoclonal antibody crenezumab testing is ongoing [151]. Another example of translational research is provided by the dominantly inherited Alzheimer's network trials unit, which is currently testing in presymptomatic known mutation carriers two monoclonal anti-Aβ antibodies (gantenerumab and solanezumab) that target different Aβ species [151]. $A\beta_{42}$-reducing approaches may have more efficacy in familial patients, because the increased production of $A\beta_{42}$ is caused by genetic mutations, in this way, it is possible to attempt to increase the clearance of the toxic peptide before the accumulation is too extensive. Taken together, these examples demonstrate that families segregating a monogenic form of AD and patients with a known causal

Box 1
Genetic, biochemical, neuroanatomic, and metabolic markers in AD diagnosis

Genetic markers: *APP*, *PSEN1*, and *PSEN2*

APP (OMIM# 104760) contains 18 exons and spans a genomic region of ±290 kb at chromosome 21q21.1–21q21.3. *APP* has several isoforms generated by alternative splicing. The APP770 is used as canonical isoform for mutation nomenclature. Reference sequences are Gene NG_007376.1, cDNA NM_000484.2, and Protein NP_000475.1. The Aβ protein is encoded by exons 16 and 17. APP is a type I transmembrane protein (Fig. 1).

PSEN1 (OMIM# 104311) counts 13 exons and spans ± 84 kb at chromosome 14q24.3. The first 4 exons contain untranslated sequence, and exons 1 and 2 represent alternate transcription initiation sites. PSEN1 counts 467 amino acids residues. Reference sequences are Gene NG_007386.2, cDNA NM_000021.2, and protein NP_000012.1.

PSEN2 (OMIM# 600759) contains 12 exons and spans a region of ±25 kb at chromosome 1q42.13. The first two exons encode the 5-prime untranslated region. PSEN2 has 448 amino acids. Reference sequences are Gene NG_007381.1, cDNA NM_000447.1, and protein NP_000438.1. The PSENs share an overall amino acid homology of 67% and are multimeric protein predicted to cross the membrane 7–9 times (Fig. 2C).

Diagnostic molecular genetic screenings have been performed so far by sequencing exon 16 and 17 (Aβ coding exons) of *APP* and all coding exons of *PSEN1* and *PSEN2*, after a decision paradigm based on gene mutation frequency *PSEN1* > *APP* > *PSEN2*. NGS technologies are sequencing in parallel the entire coding sequence of all three causal genes either by gene-panels approach [106] or by whole-exome sequencing [143].

Specific (or core) biochemical markers are $A\beta_{42}$, total tau (t-tau), and phosphorylated tau (p-tau). These are dosed in cerebrospinal fluid (CSF) on lumbar puncture. These biomarkers are measured to provide a reflection of the cerebral metabolic process of the protein transport between the brain and the CSF. General consensus is that in AD patients, the CSF levels of $A\beta_{42}$ are reduced, because of amyloid plaque formation in the brain, and that t-tau and p-tau are increased because of neuronal cell loss [144,145]. Furthermore, t-tau CSF levels increase with AD progression [145]. The better understanding of the pathologic processes occurring in AD brains are triggering the validation of novel CSF biomarkers (as reviewed in [146]) as the APP-cleaving enzyme 1 (BACE1) levels, the dosage of truncated amyloid-β isoforms, both linked to amyloidogenesis and result increased in patients. APP cleavage products, which are produced during APP processing can be measured to assess drug efficacy. F2-isoprostane a marker of mithocandrial dysfunction as well as markers of synaptic degeneration (e.g., synaptotagmin, synapsin, synaptophysin and so forth) and markers of brain injury (i.e., visinin-like protein 1 (VILIP-1) [147]), are under investigation to assess their applicability in diagnostic.

Neuroanatomic markers such as computer-assisted tomography (CT) and volumetric magnetic resonance imaging (MRI) are used to measure structural brain atrophy. Alteration in brain metabolic processed are measured using metabolic markers, that is, positron-emission tomography and single photon-emission computed tomography (SPECT) with radiopharmaceutical agents, for example, fluorodeoxyglucose (FDG). It is also possible to image *in vivo* amyloid deposition, imaged by PET with the use of Pittsburgh compound B (PiB). A recent study shows that also tau tangles correlate well with AD diagnosis, showing the strongest predictive value for progression to AD [148]. Although tau is a biomarker of brain injury, synapse loss, and progression to neurodegeneration, selective tau tracers for PET are not yet available [149]. *In vivo* tau imaging will help in clarifying the role of tau in AD onset and progression and will support the clinical disease diagnosis [149].

mutation are still in the central core of preclinical and clinical research. The knowledge of the molecular and cellular biology of AD has already supported the development of tools for disease diagnosis (Box 1), but efforts are still ongoing to identify novel biomarkers for disease prediction and prognosis. These will allow the selection of homogeneous cohorts of participants for clinical trials with a close monitoring of the drug response.

8. Lesson learned from research in LOAD

Locus heterogeneity and limitations in both pedigree size and technologies were probably the major contributors to the lack of additional genetic breakthroughs in EOAD. Meanwhile, the investigation of LOAD, using genome-wide association studies (GWAS), led to the identification of common risk variants within >20 genetic loci [114,152–156]. These genetic loci could be grouped in three major biological pathways: immune system; lipid metabolism; and synaptic dysfunction/cell membrane processes (e.g., endocytosis) [116,157,158]. Additionally, NGS resequencing efforts are identifying rare variants (minor allele frequency <1% in the general population) with a strong effect on disease risk in additional genes or in genes previously associated with LOAD. Examples are provided by rare variants detected in *TREM2* [159,160] or *ABCA7* [161,162]. Also, in these cases, the main pathways involved are the immune response (*TREM2*, *ABCA7*) and lipid metabolism (*ABCA7*) [157]. As

for *APOE ε4* (discussed in section 2.3), a few rare variants in the risk genes (e.g., *TREM2* [163], *ABCA7* [162], *EPHA1* [164]) have been shown segregating in families, suggesting a possible effect on disease penetrance, although further studies are needed to strengthen this hypothesis. The role of rare variants in EOAD has been less investigated nonetheless a few examples are available like the rare protective variant detected in *APP* [51] and the variant in *CCL11* (eotaxin-1) [74]. This last example is of particular relevance, as it demonstrated an involvement of the immune pathway in modulating the disease onset in presence of a highly penetrant mutation in *PSEN1*. The molecular mechanism through which eotaxin-1 modulates AD onset needs further investigation but, if validated, may represent a first step to develop therapies able to delay the onset of AD. This finding also supports the hypothesis that rare variants can modulate the disease onset and most likely also the disease progression. Taken together, the findings suggest that Aβ processing in LOAD could have a lesser central role, whereas the clearance of the aggregates might be involved in the disease pathogenesis [165].

On the one hand, the identification in EOAD of AAO disease modifiers (*APOE*: lipid metabolism and *CCL11*: immune pathway) in genes belonging to two of the biological pathways detected by GWAS in LOAD, is indicative of multiple (common) pathways modulating the clinical expression of AD. On the other hand, the shared clinical features as well as the pathologic findings in brain tissue of both EOAD and LOAD suggest that discoveries obtained in EOAD will likely translate to LOAD.

9. Conclusions

The renewed interest in EOAD in the NGS era has the potential to implement the knowledge of the molecular and cellular mechanisms which ultimately lead to AD. The in-depth genetic characterization, by systematic screening of the dementia causal genes, will allow patient stratification in more homogenous groups, leading to the selection of unexplained EOAD, both familiar and sporadic patients to include in further research. This can be gene hunting projects or trials for biomarkers selection or compound testing, paving the way to personalized medicine.

In parallel to the deep genetic profiling of patients cohorts, the longitudinal follow-up of families with known mutations will provide additional insights in the known disease process that could have implications for diagnostics, for the development of predictive tests, and for clinical trial design. In a future prospective, the integration of NGS with data coming from other–omics analysis (i.e., epigenomics, proteomics, transcriptomics, and metabolomics), might lead to the identification of molecular disease signatures, identifying pattern(s) of altered protein expression that are better able to discriminate early disease stages and facilitate the identification of novel causal proteins and pathways or to prioritize genes and proteins as "druggable" molecular targets for novel therapeutic approaches. In conclusion, tremendous progresses have been made in the last years in disclosing the pathologic mechanisms of AD. Novel regulatory mechanism and biological pathways have been disclosed, but prevention of the disease is still a challenge ahead. In this review, we have summarized the main aspects of the research in EOAD and how these have influenced the current knowledge of the disease mechanisms. We suggest that continuation of the investigations of families segregating known mutations and the elucidation of the missing genetic etiology in unexplained EOAD patients still has a vast potential to deliver novel crucial pieces that will lead to a better understanding of the complex puzzle of AD at large.

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RESEARCH IN CONTEXT

1. Systematic review: PubMed search, meeting abstracts and presentations, were used to collect information concerning Alzheimer's disease (AD). The search focus was on the genetics of early-onset AD (EOAD), on the application of high-throughput sequencing technologies and on ongoing translational studies on EOAD.
2. Interpretation: We provide a comprehensive review on EOAD genetics. Despite the tremendous advance in the field, a large part of the EOAD patients is still unexplained. Thus, possible molecular mechanisms that might underlie the missing genetic etiology of EOAD are explored.
3. Future directions: The basis of the current knowledge of AD is derived from genetic studies on large EOAD pedigrees in the early 90s. Here, we suggest that the use of high-throughput sequencing technologies applied to EOAD patients has a vast potential to deliver crucial information to help in better understanding the molecular mechanisms of AD in general.

References

[1] McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR Jr, Kawas CH, et al. The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement 2011;7:263–9.

[2] Alzheimer's Disease International. World Alzheimer Report 2015: The global impact of dementia an analysis of prevalence, incidence, costs and trends. London: Alzheimer's Disease International (ADI); 2015. Available at: http://www.alz.co.uk/.

[3] Carr DB, Goate A, Phil D, Morris JC. Current concepts in the pathogenesis of Alzheimer's disease. Am J Med 1997;103:3S–10.

[4] Alzheimer's Disease International. World Alzheimer Report 2009: The Global Prevalence of Dementia. London: Alzheimer's Disease International (ADI); 2009. p. 1–96. Available at: http://www.alz.co.uk/.

[5] van der Flier WM, Pijnenburg YA, Fox NC, Scheltens P. Early-onset versus late-onset Alzheimer's disease: the case of the missing APOE varepsilon4 allele. Lancet Neurol 2011;10:280–8.

[6] Braak H, Braak E. Demonstration of amyloid deposits and neurofibrillary changes in whole brain sections. Brain Pathol 1991;1:213–6.

[7] Hyman BT, Phelps CH, Beach TG, Bigio EH, Cairns NJ, Carrillo MC, et al. National Institute on Aging-Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease. Alzheimers Dement 2012;8:1–13.

[8] Marshall GA, Fairbanks LA, Tekin S, Vinters HV, Cummings JL. Early-onset Alzheimer's disease is associated with greater pathologic burden. J Geriatr Psychiatry Neurol 2007;20:29–33.

[9] Wingo TS, Lah JJ, Levey AI, Cutler DJ. Autosomal recessive causes likely in early-onset Alzheimer disease. Arch Neurol 2012;69:59–64.

[10] Gatz M, Reynolds CA, Fratiglioni L, Johansson B, Mortimer JA, Berg S, et al. Role of genes and environments for explaining Alzheimer disease. Arch Gen Psychiatry 2006;63:168–74.

[11] Jarmolowicz AI, Chen HY, Panegyres PK. The Patterns of Inheritance in Early-Onset Dementia: Alzheimer's Disease and Frontotemporal Dementia. Am J Alzheimers Dis Other Demen 2014;30:299–306.

[12] van Duijn CM, de Knijff P, Cruts M, Wehnert A, Havekes LM, Hofman A, et al. Apolipoprotein E4 allele in a population-based study of early-onset Alzheimer's disease. Nat Genet 1994;7:74–8.

[13] Campion D, Dumanchin C, Hannequin D, Dubois B, Belliard S, Puel M, et al. Early-onset autosomal dominant Alzheimer disease: prevalence, genetic heterogeneity, and mutation spectrum. Am J Hum Genet 1999;65:664–70.

[14] Wisniewski KE, Dalton AJ, McLachlan C, Wen GY, Wisniewski HM. Alzheimer's disease in Down's syndrome: clinicopathologic studies. Neurology 1985;35:957–61.

[15] Glenner GG, Wong CW. Alzheimer's disease and Down's syndrome: sharing of a unique cerebrovascular amyloid fibril protein. Biochem Biophys Res Commun 1984;122:1131–5.

[16] Masters CL, Simms G, Weinman NA, Multhaup G, McDonald BL, Beyreuther K. Amyloid plaque core protein in Alzheimer disease and Down syndrome. Proc Natl Acad Sci U S A 1985;82:4245–9.

[17] Glenner GG, Wong CW. Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. Biochem Biophys Res Commun 1984;120:885–90.

[18] George-Hyslop PH, Tanzi RE, Polinsky RJ, Haines JL, Nee L, Watkins PC, et al. The genetic defect causing familial Alzheimer's disease maps on chromosome 21. Science 1987;235:885–90.

[19] Goate AM, Haynes AR, Owen MJ, Farrall M, James LA, Lai LY, et al. Predisposing locus for Alzheimer's disease on chromosome 21. Lancet 1989;1:352–5.

[20] Hardy J, Goate A, Owen M, Rossor M. Presenile dementia associated with mosaic trisomy 21 in a patient with a Down syndrome child. Lancet 1989;2:743.

[21] Kang J, Lemaire HG, Unterbeck A, Salbaum JM, Masters CL, Grzeschik KH, et al. The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. Nature 1987;325:733–6.

[22] Goldgaber D, Lerman MI, McBride OW, Saffiotti U, Gajdusek DC. Characterization and chromosomal localization of a cDNA encoding brain amyloid of Alzheimer's disease. Science 1987;235:877–80.

[23] Tanzi RE, Gusella JF, Watkins PC, Bruns GA, George-Hyslop P, Van Keuren ML, et al. Amyloid beta protein gene: cDNA, mRNA distribution, and genetic linkage near the Alzheimer locus. Science 1987;235:880–4.

[24] Van Broeckhoven C, Genthe AM, Vandenberghe A, Horsthemke B, Backhovens H, Raeymaekers P, et al. Failure of familial Alzheimer's disease to segregate with the A4- amyloid gene in several European families. Nature 1987;329:153–5.

[25] Tanzi RE, George-Hyslop PH, Haines JL, Polinsky RJ, Nee L, Foncin JF, et al. The genetic defect in familial Alzheimer's disease is not tightly linked to the amyloid beta-protein gene. Nature 1987;329:156–7.

[26] St George-Hyslop PH, Haines JL, Farrer LA, Polinsky R, Van Broeckhoven C, Goate A, et al. Genetic linkage studies suggest that Alzheimer's disease is not a single homogeneous disorder. Nature 1990;347:194–7.

[27] Van Broeckhoven C, Haan J, Bakker E, Hardy JA, Van Hul W, Wehnert A, et al. Amyloid beta protein precursor gene and hereditary cerebral hemorrhage with amyloidosis (Dutch). Science 1990;248:1120–2.

[28] Coria F, Prelli F, Castano EM, Larrondo-Lillo M, Fernandez-Gonzalez J, van Duinen SG, et al. Beta-protein deposition: a pathogenetic link between Alzheimer's disease and cerebral amyloid angiopathies. Brain Res 1988;463:187–91.

[29] Levy E, Carman MD, Fernandez-Madrid IJ, Power MD, Lieberburg I, van Duinen SG, et al. Mutation of the Alzheimer's disease amyloid gene in hereditary cerebral hemorrhage, Dutch type. Science 1990;248:1124–6.

[30] Bakker E, Van Broeckhoven C, Haan J, Voorhoeve E, Van Hul W, Levy E, et al. DNA diagnosis for hereditary cerebral hemorrhage with amyloidosis (Dutch type). Am J Hum Genet 1991;49:518–21.

[31] Goate A, Chartier-Harlin MC, Mullan M, Brown J, Crawford F, Fidani L, et al. Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. Nature 1991;349:704–6.

[32] Schellenberg GD, Bird TD, Wijsman EM, Orr HT, Anderson L, Nemens E, et al. Genetic linkage evidence for a familial Alzheimer's disease locus on chromosome 14. Science 1992;258:668–71.

[33] George-Hyslop PH, Haines J, Rogaev E, Mortilla M, Vaula G, Pericak-Vance M, et al. Genetic evidence for a novel familial Alzheimer's disease locus on chromosome 14. Nat Genet 1992;2:330–4.

[34] Van Broeckhoven C, Backhovens H, Cruts M, De Winter G, Bruyland M, Cras P, et al. Mapping of a gene predisposing to early-onset Alzheimer's disease to chromosome 14q24.3. Nat Genet 1992;2:335–9.

[35] Mullan M, Houlden H, Windelspecht M, Fidani L, Lombardi C, Diaz P, et al. A locus for familial early-onset Alzheimer's disease on the long arm of chromosome 14, proximal to the alpha 1-antichymotrypsin gene. Nat Genet 1992;2:340–2.

[36] Clark RF, Cruts M, Korenblat KM, He C, Talbot C, Van Broeckhoven C, et al. A yeast artificial chromosome contig from human chromosome 14q24 spanning the Alzheimer's disease locus AD3. Hum Mol Genet 1995;4:1347–54.

[37] Mitsunaga Y, Takahashi K, Tabira T, Tasaki H, Watanabe S. Assignment of a familial Alzheimer's disease locus between D14S289 and D14S53. Lancet 1994;344:1154–5.

[38] Cruts M, Backhovens H, Theuns J, Clark RF, Le Paslier D, Weissenbach J, et al. Genetic and physical characterization of the early-onset Alzheimer's disease AD3 locus on chromosome 14q24.3. Hum Mol Genet 1995;4:1355–64.

[39] Sherrington R, Rogaev EI, Liang Y, Rogaeva EA, Levesque G, Ikeda M, et al. Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. Nature 1995;375:754–60.
[40] Rogaev EI, Sherrington R, Rogaeva EA, Levesque G, Ikeda M, Liang Y, et al. Familial Alzheimer's disease in kindreds with missense mutations in a gene on chromosome 1 related to the Alzheimer's disease type 3 gene. Nature 1995;376:775–8.
[41] Levy-Lahad E, Wasco W, Poorkaj P, Romano DM, Oshima J, Pettingell WH, et al. Candidate gene for the chromosome 1 familial Alzheimer's disease locus. Science 1995;269:973–7.
[42] Bird TD, Lampe TH, Nemens EJ, Miner GW, Sumi SM, Schellenberg GD. Familial Alzheimer's disease in American descendants of the Volga Germans: probable genetic founder effect. Ann Neurol 1988;23:25–31.
[43] Levy-Lahad E, Wijsman EM, Nemens E, Anderson L, Goddard KA, Weber JL, et al. A familial Alzheimer's disease locus on chromosome 1. Science 1995;269:970–3.
[44] Cruts M, Theuns J, Van Broeckhoven C. Locus-specific mutation databases for neurodegenerative brain diseases. Hum Mutat 2012; 33:1340–4.
[45] Hooli BV, Mohapatra G, Mattheisen M, Parrado AR, Roehr JT, Shen Y, et al. Role of common and rare APP DNA sequence variants in Alzheimer disease. Neurology 2012;78:1250–7.
[46] Tomiyama T, Nagata T, Shimada H, Teraoka R, Fukushima A, Kanemitsu H, et al. A new amyloid beta variant favoring oligomerization in Alzheimer's-type dementia. Ann Neurol 2008;63:377–87.
[47] Di Fede G, Catania M, Morbin M, Rossi G, Suardi S, Mazzoleni G, et al. A recessive mutation in the APP gene with dominant-negative effect on amyloidogenesis. Science 2009;323:1473–7.
[48] Giaccone G, Morbin M, Moda F, Botta M, Mazzoleni G, Uggetti A, et al. Neuropathology of the recessive A673V APP mutation: Alzheimer disease with distinctive features. Acta Neuropathol 2010; 120:803–12.
[49] Ryan NS, Rossor MN. Correlating familial Alzheimer's disease gene mutations with clinical phenotype. Biomark Med 2010;4:99–112.
[50] Pilotto A, Padovani A, Borroni B. Clinical, biological, and imaging features of monogenic Alzheimer's Disease. Biomed Res Int 2013; 2013:689591.
[51] Jonsson T, Atwal JK, Steinberg S, Snaedal J, Jonsson PV, Bjornsson S, et al. A mutation in APP protects against Alzheimer's disease and age-related cognitive decline. Nature 2012;488:96–9.
[52] Portet F, Dauvilliers Y, Campion D, Raux G, Hauw JJ, Lyon-Caen O, et al. Very early onset AD with a de novo mutation in the presenilin 1 gene (Met 233 Leu). Neurology 2003;61:1136–7.
[53] Golan MP, Styczynska M, Jozwiak K, Walecki J, Maruszak A, Pniewski J, et al. Early-onset Alzheimer's disease with a de novo mutation in the presenilin 1 gene. Exp Neurol 2007;208:264–8.
[54] Conidi ME, Bernardi L, Puccio G, Smirne N, Muraca MG, Curcio SA, et al. Homozygous carriers of APP A713T mutation in an autosomal dominant Alzheimer disease family. Neurology 2015; 84:2266–73.
[55] Kosik KS, Munoz C, Lopez L, Arcila ML, Garcia G, Madrigal L, et al. Homozygosity of the autosomal dominant Alzheimer disease presenilin 1 E280A mutation. Neurology 2015;84:206–8.
[56] Brouwers N, Sleegers K, Van Broeckhoven C. Molecular genetics of Alzheimer's disease: an update. Ann Med 2008;40:562–83.
[57] Wallon D, Rousseau S, Rovelet-Lecrux A, Quillard-Muraine M, Guyant-Marechal L, Martinaud O, et al. The French series of autosomal dominant early onset Alzheimer's disease cases: mutation spectrum and cerebrospinal fluid biomarkers. J Alzheimers Dis 2012;30:847–56.
[58] Brickell KL, Steinbart EJ, Rumbaugh M, Payami H, Schellenberg GD, Van Deerlin V, et al. Early-onset Alzheimer disease in families with late-onset Alzheimer disease: a potential important subtype of familial Alzheimer disease. Arch Neurol 2006; 63:1307–11.
[59] Janssen JC, Beck JA, Campbell TA, Dickinson A, Fox NC, Harvey RJ, et al. Early onset familial Alzheimer's disease: Mutation frequency in 31 families. Neurology 2003;60:235–9.
[60] Dermaut B, Kumar-Singh S, Engelborghs S, Theuns J, Rademakers R, Saerens J, et al. A novel presenilin 1 mutation associated with Pick's disease but not beta-amyloid plaques. Ann Neurol 2004;55:617–26.
[61] Karlstrom H, Brooks WS, Kwok JB, Broe GA, Kril JJ, McCann H, et al. Variable phenotype of Alzheimer's disease with spastic paraparesis. J Neurochem 2008;104:573–83.
[62] Ehling R, Noskova L, Stranecky V, Hartmannova H, Pristoupilova A, Hodanova K, et al. Cerebellar dysfunction in a family harboring the PSEN1 mutation co-segregating with a cathepsin D variant p.A58V. J Neurol Sci 2013;326:75–82.
[63] Pericak-Vance MA, Bebout JL, Gaskell PC Jr, Yamaoka LH, Hung WY, Alberts MJ, et al. Linkage studies in familial Alzheimer disease: evidence for chromosome 19 linkage. Am J Hum Genet 1991;48:1034–50.
[64] Saunders AM, Strittmatter WJ, Schmechel D, George-Hyslop PH, Pericak-Vance MA, Joo SH, et al. Association of apolipoprotein E allele epsilon 4 with late-onset familial and sporadic Alzheimer's disease. Neurology 1993;43:1467–72.
[65] Strittmatter WJ, Saunders AM, Schmechel D, Pericak-Vance M, Enghild J, Salvesen GS, et al. Apolipoprotein E: high-avidity binding to beta-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. Proc Natl Acad Sci U S A 1993; 90:1977–81.
[66] Corder EH, Saunders AM, Strittmatter WJ, Schmechel DE, Gaskell PC, Small GW, et al. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. Science 1993;261:921–3.
[67] Farrer LA, Cupples LA, Haines JL, Hyman B, Kukull WA, Mayeux R, et al. Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. APOE and Alzheimer Disease Meta Analysis Consortium. JAMA 1997;278:1349–56.
[68] van Duijn CM, Cruts M, Theuns J, Van Gassen G, Backhovens H, Van den Broeck M, et al. Genetic association of the presenilin-1 regulatory region with early- onset Alzheimer's disease in a population-based sample. Eur J Hum Genet 1999;7:801–6.
[69] Lambert JC, Mann DM, Harris JM, Chartier-Harlin MC, Cumming A, Coates J, et al. The -48 C/T polymorphism in the presenilin 1 promoter is associated with an increased risk of developing Alzheimer's disease and an increased Abeta load in brain. J Med Genet 2001;38:353–5.
[70] Pastor P, Roe CM, Villegas A, Bedoya G, Chakraverty S, Garcia G, et al. Apolipoprotein Eepsilon4 modifies Alzheimer's disease onset in an E280A PS1 kindred. Ann Neurol 2003;54:163–9.
[71] Wijsman EM, Daw EW, Yu X, Steinbart EJ, Nochlin D, Bird TD, et al. APOE and other loci affect age-at-onset in Alzheimer's disease families with PS2 mutation. Am J Med Genet B Neuropsychiatr Genet 2005;132B:14–20.
[72] Corder EH, Saunders AM, Risch NJ, Strittmatter WJ, Schmechel DE, Gaskell PC Jr, et al. Protective effect of apolipoprotein E type 2 allele for late onset Alzheimer disease. Nat Genet 1994;7:180–4.
[73] Velez JI, Lopera F, Sepulveda-Falla D, Patel HR, Johar AS, Chuah A, et al. APOE*E2 allele delays age of onset in PSEN1 E280A Alzheimer's disease. Mol Psychiatry 2015; http://dx.doi.org/10.1038/mp.2015.177.
[74] Lalli MA, Bettcher BM, Arcila ML, Garcia G, Guzman C, Madrigal L, et al. Whole-genome sequencing suggests a chemokine gene cluster that modifies age at onset in familial Alzheimer's disease. Mol Psychiatry 2015;20:1294–300.
[75] Dermaut B, Croes EA, Rademakers R, Van den Broeck M, Cruts M, Hofman A, et al. PRNP Val129 homozygosity increases risk for early-onset Alzheimer's disease. Ann Neurol 2003;53:409–12.

[76] Croes EA, Theuns J, Houwing-Duistermaat JJ, Dermaut B, Sleegers K, Roks G, et al. Octapeptide repeat insertions in the prion protein gene and early onset dementia. J Neurol Neurosurg Psychiatry 2004;75:1166–70.
[77] Nicolas G, Charbonnier C, Wallon D, Quenez O, Bellenguez C, Grenier-Boley B, et al. SORL1 rare variants: a major risk factor for familial early-onset Alzheimer's disease. Mol Psychiatry 2015; http://dx.doi.org/10.1038/mp.2015.121.
[78] Genin E, Hannequin D, Wallon D, Sleegers K, Hiltunen M, Combarros O, et al. APOE and Alzheimer disease: a major gene with semi-dominant inheritance. Mol Psychiatry 2011;16:903–7.
[79] Sorbi S, Nacmias B, Forleo P, Piacentini S, Latorraca S, Amaducci L. Epistatic effect of APP717 mutation and apolipoprotein E genotype in familial Alzheimer's disease. Ann Neurol 1995;38:124–7.
[80] Brouwers N, Sleegers K, Engelborghs S, Maurer-Stroh S, Gijselinck I, van der Zee J, et al. Genetic variability in progranulin contributes to risk for clinically diagnosed Alzheimer disease. Neurology 2008;71:656–64.
[81] Kelley BJ, Haidar W, Boeve BF, Baker M, Shiung M, Knopman DS, et al. Alzheimer disease-like phenotype associated with the c.154delA mutation in progranulin. Arch Neurol 2010;67:171–7.
[82] Cortini F, Fenoglio C, Guidi I, Venturelli E, Pomati S, Marcone A, et al. Novel exon 1 progranulin gene variant in Alzheimer's disease. Eur J Neurol 2008;15:1111–7.
[83] Rademakers R, Dermaut B, Peeters K, Cruts M, Heutink P, Goate A, et al. Tau (MAPT) mutation Arg406Trp presenting clinically with Alzheimer disease does not share a common founder in Western Europe. Hum Mutat 2003;22:409–11.
[84] Lindquist SG, Holm IE, Schwartz M, Law I, Stokholm J, Batbayli M, et al. Alzheimer disease-like clinical phenotype in a family with FTDP-17 caused by a MAPT R406W mutation. Eur J Neurol 2008;15:377–85.
[85] Cacace R, Van Cauwenberghe C, Bettens K, Gijselinck I, van der Zee J, Engelborghs S, et al. C9orf72 G4C2 repeat expansions in Alzheimer's disease and mild cognitive impairment. Neurobiol Aging 2013;34:1712–7.
[86] Kohli MA, John-Williams K, Rajbhandary R, Naj A, Whitehead P, Hamilton K, et al. Repeat expansions in the C9ORF72 gene contribute to Alzheimer's disease in Caucasians. Neurobiol Aging 2012;34:1519.e5–151912.
[87] Harms M, Benitez BA, Cairns N, Cooper B, Cooper P, Mayo K, et al. C9orf72 hexanucleotide repeat expansions in clinical Alzheimer disease. JAMA Neurol 2013;70:736–41.
[88] Jayadev S, Nochlin D, Poorkaj P, Steinbart EJ, Mastrianni JA, Montine TJ, et al. Familial prion disease with Alzheimer disease-like tau pathology and clinical phenotype. Ann Neurol 2011;69:712–20.
[89] Guerreiro R, Bras J, Wojtas A, Rademakers R, Hardy J, Graff-Radford N. A nonsense mutation in PRNP associated with clinical Alzheimer's disease. Neurobiol Aging 2014;35:2656.e13–6.
[90] Ryman DC, Acosta-Baena N, Aisen PS, Bird T, Danek A, Fox NC, et al. Symptom onset in autosomal dominant Alzheimer disease: a systematic review and meta-analysis. Neurology 2014;83:253–60.
[91] Marchani EE, Bird TD, Steinbart EJ, Rosenthal E, Yu CE, Schellenberg GD, et al. Evidence for three loci modifying age-at-onset of Alzheimer's disease in early-onset PSEN2 families. Am J Med Genet B Neuropsychiatr Genet 2010;153B:1031–41.
[92] Hardy JA, Higgins GA. Alzheimer's disease: the amyloid cascade hypothesis. Science 1992;256:184–5.
[93] Dimitrov M, Alattia JR, Lemmin T, Lehal R, Fligier A, Houacine J, et al. Alzheimer's disease mutations in APP but not gamma-secretase modulators affect epsilon-cleavage-dependent AICD production. Nat Commun 2013;4:2246.
[94] Morris GP, Clark IA, Vissel B. Inconsistencies and controversies surrounding the amyloid hypothesis of Alzheimer's disease. Acta Neuropathol Commun 2014;2:135.
[95] Chetelat G, La JR, Villain N, Perrotin A, de La Sayette V, Eustache F, et al. Amyloid imaging in cognitively normal individuals, at-risk populations and preclinical Alzheimer's disease. Neuroimage Clin 2013;2:356–65.
[96] Serrano-Pozo A, Qian J, Monsell SE, Blacker D, Gomez-Isla T, Betensky RA, et al. Mild to moderate Alzheimer dementia with insufficient neuropathological changes. Ann Neurol 2014;75:597–601.
[97] Jack CR Jr, Wiste HJ, Weigand SD, Knopman DS, Lowe V, Vemuri P, et al. Amyloid-first and neurodegeneration-first profiles characterize incident amyloid PET positivity. Neurology 2013;81:1732–40.
[98] Caroli A, Prestia A, Galluzzi S, Ferrari C, van der Flier WM, Ossenkoppele R, et al. Mild cognitive impairment with suspected nonamyloid pathology (SNAP): Prediction of progression. Neurology 2015;84:508–15.
[99] Chetelat G. Alzheimer disease: Abeta-independent processes-rethinking preclinical AD. Nat Rev Neurol 2013;9:123–4.
[100] Dermaut B, Kumar-Singh S, Rademakers R, Theuns J, Cruts M, Van Broeckhoven C. Tau is central in the genetic Alzheimer-frontotemporal dementia spectrum. Trends Genet 2005;21:664–72.
[101] van der Zee J, Sleegers K, Van Broeckhoven C. Invited article: the Alzheimer disease-frontotemporal lobar degeneration spectrum. Neurology 2008;71:1191–7.
[102] Theuns J, Remacle J, Killick R, Corsmit E, Vennekens K, Huylebroeck D, et al. Alzheimer-associated C allele of the promoter polymorphism -22C>T causes a critical neuron-specific decrease of presenilin 1 expression. Hum Mol Genet 2003;12:869–77.
[103] Theuns J, Brouwers N, Engelborghs S, Sleegers K, Bogaerts V, Corsmit E, et al. Promoter mutations that increase amyloid precursor-protein expression are associated with Alzheimer disease. Am J Hum Genet 2006;78:936–46.
[104] Brouwers N, Sleegers K, Engelborghs S, Bogaerts V, Serneels S, Kamali K, et al. Genetic risk and transcriptional variability of amyloid precursor protein in Alzheimer's disease. Brain 2006;129:2984–91.
[105] Cruchaga C, Haller G, Chakraverty S, Mayo K, Vallania FL, Mitra RD, et al. Rare variants in APP, PSEN1 and PSEN2 increase risk for AD in late-onset Alzheimer's disease families. PLoS One 2012;7:e31039.
[106] Beck J, Pittman A, Adamson G, Campbell T, Kenny J, Houlden H, et al. Validation of next-generation sequencing technologies in genetic diagnosis of dementia. Neurobiol Aging 2014;35:261–5.
[107] Clarke AJ. Managing the ethical challenges of next-generation sequencing in genomic medicine. Br Med Bull 2014;111:17–30.
[108] Guerreiro RJ, Lohmann E, Kinsella E, Bras JM, Luu N, Gurunlian N, et al. Exome sequencing reveals an unexpected genetic cause of disease: NOTCH3 mutation in a Turkish family with Alzheimer's disease. Neurobiol Aging 2012;33:1008–23.
[109] Pottier C, Hannequin D, Coutant S, Rovelet-Lecrux A, Wallon D, Rousseau S, et al. High frequency of potentially pathogenic SORL1 mutations in autosomal dominant early-onset Alzheimer disease. Mol Psychiatry 2012;17:875–9.
[110] Scherzer CR, Offe K, Gearing M, Rees HD, Fang G, Heilman CJ, et al. Loss of apolipoprotein E receptor LR11 in Alzheimer disease. Arch Neurol 2004;61:1200–5.
[111] Andersen OM, Reiche J, Schmidt V, Gotthardt M, Spoelgen R, Behlke J, et al. Neuronal sorting protein-related receptor sorLA/LR11 regulates processing of the amyloid precursor protein. Proc Natl Acad Sci U S A 2005;102:13461–6.
[112] Caglayan S, Takagi-Niidome S, Liao F, Carlo AS, Schmidt V, Burgert T, et al. Lysosomal sorting of amyloid-beta by the SORLA receptor is impaired by a familial Alzheimer's disease mutation. Sci Transl Med 2014;6:223ra20.
[113] Rogaeva E, Meng Y, Lee JH, Gu Y, Kawarai T, Zou F, et al. The neuronal sortilin-related receptor SORL1 is genetically associated with Alzheimer disease. Nat Genet 2007;39:168–77.

[114] Lambert JC, Ibrahim-Verbaas CA, Harold D, Naj AC, Sims R, Bellenguez C, et al. Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease. Nat Genet 2013; 45:1452–8.
[115] Vardarajan BN, Zhang Y, Lee JH, Cheng R, Bohm C, Ghani M, et al. Coding mutations in SORL1 and Alzheimer disease. Ann Neurol 2015;77:215–27.
[116] Bettens K, Sleegers K, Van Broeckhoven C. Genetic insights in Alzheimer's disease. Lancet Neurol 2013;12:92–104.
[117] Sillen A, Forsell C, Lilius L, Axelman K, Bjork BF, Onkamo P, et al. Genome scan on Swedish Alzheimer's disease families. Mol Psychiatry 2006;11:182–6.
[118] Sillen A, Andrade J, Lilius L, Forsell C, Axelman K, Odeberg J, et al. Expanded high-resolution genetic study of 109 Swedish families with Alzheimer's disease. Eur J Hum Genet 2008;16:202–8.
[119] Sillen A, Brohede J, Forsell C, Lilius L, Andrade J, Odeberg J, et al. Linkage analysis of autopsy-confirmed familial Alzheimer disease supports an Alzheimer disease locus in 8q24. Dement Geriatr Cogn Disord 2011;31:109–18.
[120] Giedraitis V, Hedlund M, Skoglund L, Blom E, Ingvast S, Brundin R, et al. New Alzheimer's disease locus on chromosome 8. J Med Genet 2006;43:931–5.
[121] Rademakers R, Cruts M, Sleegers K, Dermaut B, Theuns J, Aulchenko Y, et al. Linkage and association studies identify a novel locus for Alzheimer disease at 7q36 in a Dutch population-based sample. Am J Hum Genet 2005;77:643–52.
[122] Van Broeckhoven C. The future of genetic research on neurodegeneration. Nat Med 2010;16:1215–7.
[123] Valdmanis PN, Belzil VV, Lee J, Dion PA, St-Onge J, Hince P, et al. A mutation that creates a pseudoexon in SOD1 causes familial ALS. Ann Hum Genet 2009;73:652–7.
[124] Cruts M, Rademakers R, Gijselinck I, van der Zee J, Dermaut B, De Pooter T, et al. Genomic architecture of human 17q21 linked to frontotemporal dementia uncovers a highly homologous family of low-copy repeats in the tau region. Hum Mol Genet 2005; 14:1753–62.
[125] Rademakers R, Melquist S, Cruts M, Theuns J, Del-Favero J, Poorkaj P, et al. High-density SNP haplotyping suggests altered regulation of tau gene expression in progressive supranuclear palsy. Hum Mol Genet 2005;14:3281–92.
[126] Cirulli ET, Goldstein DB. Uncovering the roles of rare variants in common disease through whole-genome sequencing. Nat Rev Genet 2010;11:415–25.
[127] Nho K, Corneveaux JJ, Kim S, Lin H, Risacher SL, Shen L, et al. Whole-exome sequencing and imaging genetics identify functional variants for rate of change in hippocampal volume in mild cognitive impairment. Mol Psychiatry 2013;18:781–7.
[128] Clarimon J, Djaldetti R, Lleo A, Guerreiro RJ, Molinuevo JL, Paisan-Ruiz C, et al. Whole genome analysis in a consanguineous family with early onset Alzheimer's disease. Neurobiol Aging 2009; 30:1986–91.
[129] Ghani M, Sato C, Lee JH, Reitz C, Moreno D, Mayeux R, et al. Evidence of recessive Alzheimer disease loci in a Caribbean Hispanic data set: genome-wide survey of runs of homozygosity. JAMA Neurol 2013;70:1261–7.
[130] Nalls MA, Guerreiro RJ, Simon-Sanchez J, Bras JT, Traynor BJ, Gibbs JR, et al. Extended tracts of homozygosity identify novel candidate genes associated with late-onset Alzheimer's disease. Neurogenetics 2009;10:183–90.
[131] Farrer LA, Bowirrat A, Friedland RP, Waraska K, Korczyn AD, Baldwin CT. Identification of multiple loci for Alzheimer disease in a consanguineous Israeli-Arab community. Hum Mol Genet 2003;12:415–22.
[132] Beck JA, Poulter M, Campbell TA, Uphill JB, Adamson G, Geddes JF, et al. Somatic and germline mosaicism in sporadic early-onset Alzheimer's disease. Hum Mol Genet 2004;13:1219–24.
[133] Frigerio CS, Lau P, Troakes C, Deramecourt V, Gele P, Van Loo P, et al. On the identification of low allele frequency mosaic mutations in the brains of Alzheimer disease patients. Alzheimers Dement 2015;11:1265–76.
[134] Lardenoije R, Iatrou A, Kenis G, Kompotis K, Steinbusch HW, Mastroeni D, et al. The epigenetics of aging and neurodegeneration. Prog Neurobiol 2015;131:21–64.
[135] van den Hove DL, Kompotis K, Lardenoije R, Kenis G, Mill J, Steinbusch HW, et al. Epigenetically regulated microRNAs in Alzheimer's disease. Neurobiol Aging 2014;35:731–45.
[136] De Jager PL, Srivastava G, Lunnon K, Burgess J, Schalkwyk LC, Yu L, et al. Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci. Nat Neurosci 2014;17:1156–63.
[137] Lunnon K, Smith R, Hannon E, De Jager PL, Srivastava G, Volta M, et al. Methylomic profiling implicates cortical deregulation of ANK1 in Alzheimer's disease. Nat Neurosci 2014;17:1164–70.
[138] Davies MN, Volta M, Pidsley R, Lunnon K, Dixit A, Lovestone S, et al. Functional annotation of the human brain methylome identifies tissue-specific epigenetic variation across brain and blood. Genome Biol 2012;13:R43.
[139] Skipper M, Eccleston A, Gray N, Heemels T, Le Bot N, Marte B, et al. Presenting the epigenome roadmap. Nature 2015;518:313.
[140] Gjoneska E, Pfenning AR, Mathys H, Quon G, Kundaje A, Tsai LH, et al. Conserved epigenomic signals in mice and humans reveal immune basis of Alzheimer's disease. Nature 2015; 518:365–9.
[141] Bennett DA, Yu L, Yang J, Srivastava GP, Aubin C, De Jager PL. Epigenomics of Alzheimer's disease. Transl Res 2015;165:200–20.
[142] Bateman RJ, Xiong C, Benzinger TL, Fagan AM, Goate A, Fox NC, et al. Clinical and biomarker changes in dominantly inherited Alzheimer's disease. N Engl J Med 2012;367:795–804.
[143] Yang Y, Muzny DM, Reid JG, Bainbridge MN, Willis A, Ward PA, et al. Clinical whole-exome sequencing for the diagnosis of mendelian disorders. N Engl J Med 2013;369:1502–11.
[144] Fagan AM, Xiong C, Jasielec MS, Bateman RJ, Goate AM, Benzinger TL, et al. Longitudinal change in CSF biomarkers in autosomal-dominant Alzheimer's disease. Sci Transl Med 2014; 6:226ra30.
[145] Reitz C, Brayne C, Mayeux R. Epidemiology of Alzheimer disease. Nat Rev Neurol 2011;7:137–52.
[146] Wattamwar PR, Mathuranath PS. An overview of biomarkers in Alzheimer's disease. Ann Indian Acad Neurol 2010;13:S116–23.
[147] Sutphen CL, Jasielec MS, Shah AR, Macy EM, Xiong C, Vlassenko AG, et al. Longitudinal Cerebrospinal Fluid Biomarker Changes in Preclinical Alzheimer Disease During Middle Age. JAMA Neurol 2015;72:1029–42.
[148] Toledo JB, Weiner MW, Wolk DA, Da X, Chen K, Arnold SE, et al. Neuronal injury biomarkers and prognosis in ADNI subjects with normal cognition. Acta Neuropathol Commun 2014;2:26.
[149] Villemagne VL, Fodero-Tavoletti MT, Masters CL, Rowe CC. Tau imaging: early progress and future directions. Lancet Neurol 2015; 14:114–24.
[150] Ayutyanont N, Langbaum JB, Hendrix SB, Chen K, Fleisher AS, Friesenhahn M, et al. The Alzheimer's prevention initiative composite cognitive test score: sample size estimates for the evaluation of preclinical Alzheimer's disease treatments in presenilin 1 E280A mutation carriers. J Clin Psychiatry 2014;75:652–60.
[151] Schneider LS, Mangialasche F, Andreasen N, Feldman H, Giacobini E, Jones R, et al. Clinical trials and late-stage drug development for Alzheimer's disease: an appraisal from 1984 to 2014. J Intern Med 2014;275:251–83.
[152] Harold D, Abraham R, Hollingworth P, Sims R, Gerrish A, Hamshere ML, et al. Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease. Nat Genet 2009;41:1088–93.

[153] Hollingworth P, Harold D, Sims R, Gerrish A, Lambert JC, Carrasquillo MM, et al. Common variants at ABCA7, MS4A6A/MS4A4E, EPHA1, CD33 and CD2AP are associated with Alzheimer's disease. Nat Genet 2011;43:429–35.
[154] Lambert JC, Heath S, Even G, Campion D, Sleegers K, Hiltunen M, et al. Genome-wide association study identifies variants at CLU and CR1 associated with Alzheimer's disease. Nat Genet 2009;41:1094–9.
[155] Naj AC, Jun G, Beecham GW, Wang LS, Vardarajan BN, Buros J, et al. Common variants at MS4A4/MS4A6E, CD2AP, CD33 and EPHA1 are associated with late-onset Alzheimer's disease. Nat Genet 2011;43:436–41.
[156] Seshadri S, Fitzpatrick AL, Ikram MA, DeStefano AL, Gudnason V, Boada M, et al. Genome-wide analysis of genetic loci associated with Alzheimer disease. JAMA 2010;303:1832–40.
[157] Karch CM, Goate AM. Alzheimer's disease risk genes and mechanisms of disease pathogenesis. Biol Psychiatry 2015;77:43–51.
[158] Morgan K. The three new pathways leading to Alzheimer's disease. Neuropathol Appl Neurobiol 2011;37:353–7.
[159] Guerreiro R, Wojtas A, Bras J, Carrasquillo M, Rogaeva E, Majounie E, et al. TREM2 variants in Alzheimer's disease. N Engl J Med 2013;368:117–27.
[160] Jonsson T, Stefansson H, Steinberg S, Jonsdottir I, Jonsson PV, Snaedal J, et al. Variant of TREM2 associated with the risk of Alzheimer's disease. N Engl J Med 2013;368:107–16.
[161] Steinberg S, Stefansson H, Jonsson T, Johannsdottir H, Ingason A, Helgason H, et al. Loss-of-function variants in ABCA7 confer risk of Alzheimer's disease. Nat Genet 2015;47:445–7.
[162] Cuyvers E, De Roeck A, Van den Bossche T, Van Cauwenberghe C, Bettens K, Vermeulen S, et al. Mutations in ABCA7 in a Belgian cohort of Alzheimer's disease patients: a targeted resequencing study. Lancet Neurol 2015;14:814–22.
[163] Korvatska O, Leverenz JB, Jayadev S, McMillan P, Kurtz I, Guo X, et al. R47H Variant of TREM2 Associated With Alzheimer Disease in a Large Late-Onset Family: Clinical, Genetic, and Neuropathological Study. JAMA Neurol 2015;72:920–7.
[164] Vardarajan BN, Ghani M, Kahn A, Sheikh S, Sato C, Barral S, et al. Rare coding mutations identified by sequencing of Alzheimer disease genome-wide association studies loci. Ann Neurol 2015;78:487–98.
[165] Guerreiro RJ, Hardy J. Alzheimer's disease genetics: lessons to improve disease modelling. Biochem Soc Trans 2011;39:910–6.