

Review Article

Molecular genetics of early-onset Alzheimer's disease revisited

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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 $\epsilon 4$, 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.

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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 ($A\beta$) 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

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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, pre-clinical 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 ($A\beta$) 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 $A\beta$ observed in AD brains [28]. Sequencing identified a mutation affecting the $A\beta$ sequence in patients with HCHWA-D [29] and segregated with disease [30]. The discovery of a *APP* mutation linked to vascular $A\beta$ 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 $A\beta$ 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.E693 Δ) [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

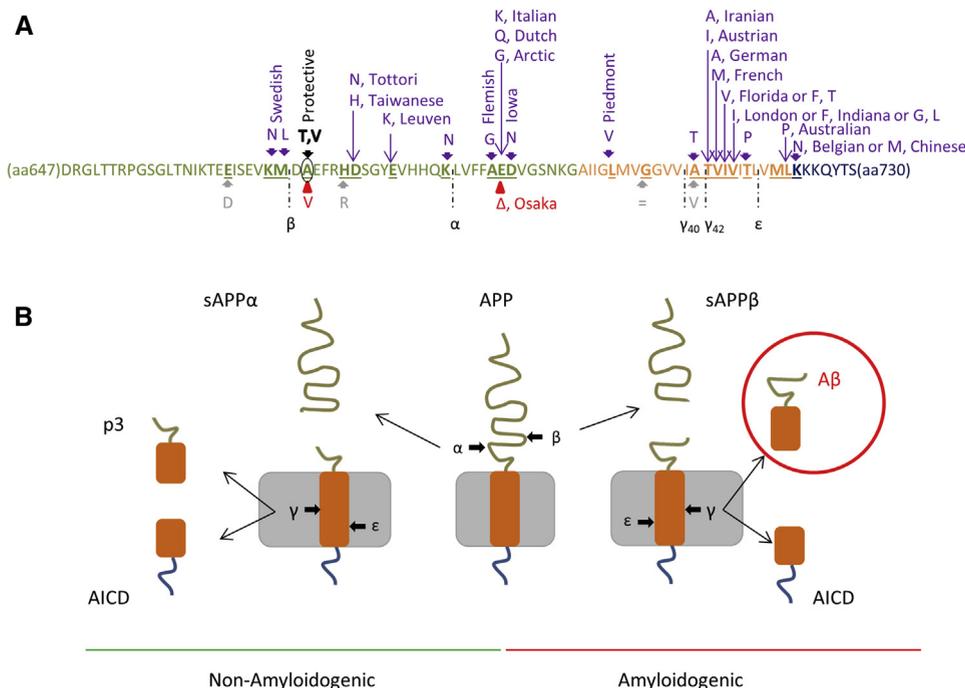


Fig. 1. APP protein mutations and structure. (A) APP protein sequence from amino acid residue 647 to 730 is presented, sequence in green depicts the extracellular domain, in orange the transmembrane domain and in dark blue the intracellular domain. Known pathogenic mutations are reported in purple and, 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)
<i>APP</i>	21q21.1–21q21.3	Autosomal dominant Autosomal recessive Protective	Linkage analysis	Missense Gene Duplication Amino acid deletion	54*
<i>PSEN1</i>	14q24.3	Autosomal dominant <i>de novo</i>	Linkage analysis	Missense Small indels Genomic deletions	215
<i>PSEN2</i>	1q31–q42	Autosomal dominant	Linkage and homology mapping	Missense	31

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

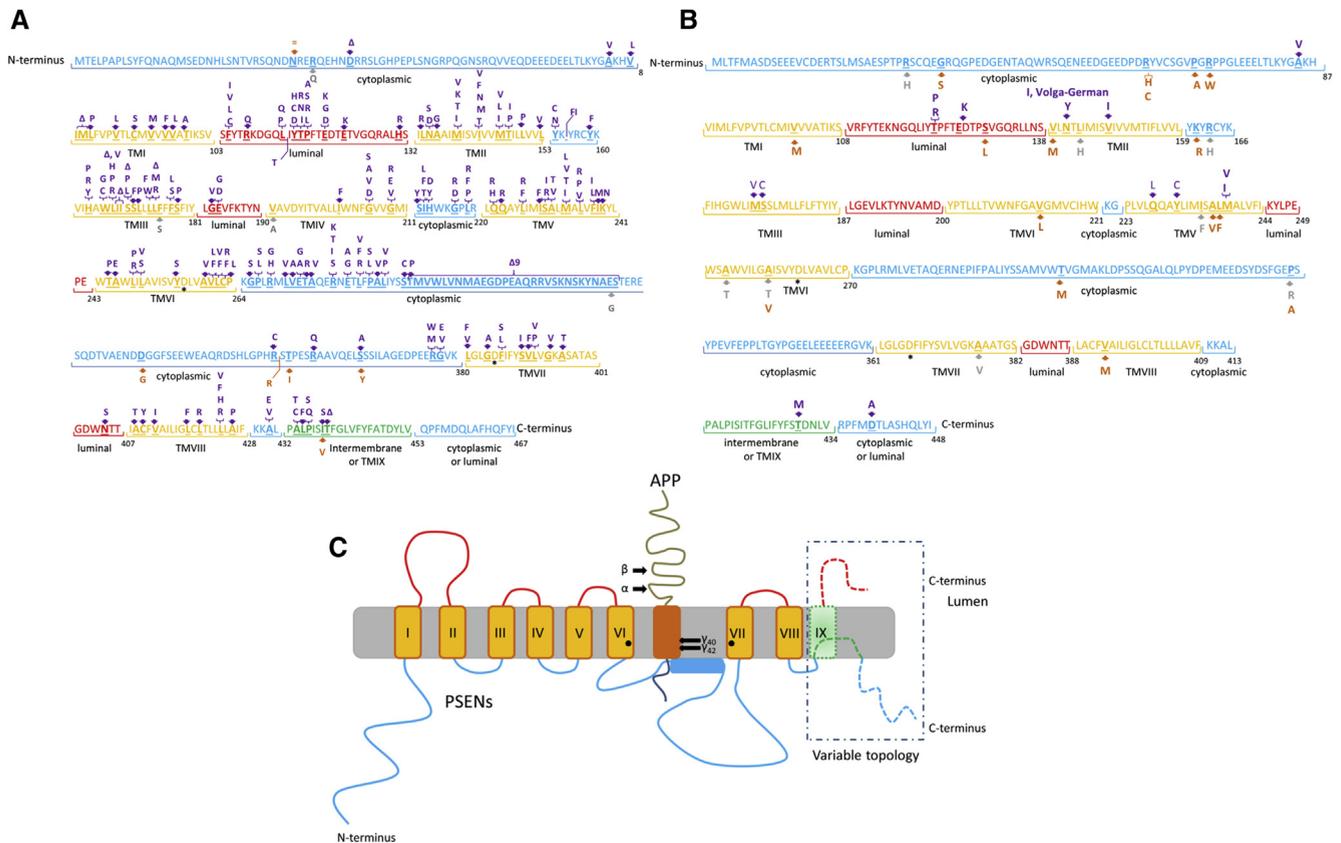


Fig. 2. PSENs protein mutations and structure. (A) PSEN1 and (B) PSEN2 protein sequences. In blue are marked the cytoplasmic domains, in yellow the transmembrane domains, in red the luminal domains, and in green the one intermembrane/IX transmembrane domain. Pathogenic or predicted pathogenic mutations 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 autaptic pathologic and immunohistochemistry analysis of the brain showed pick-type tauopathy, normally caused by MAPT mutations, in absence of extracellular A β 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

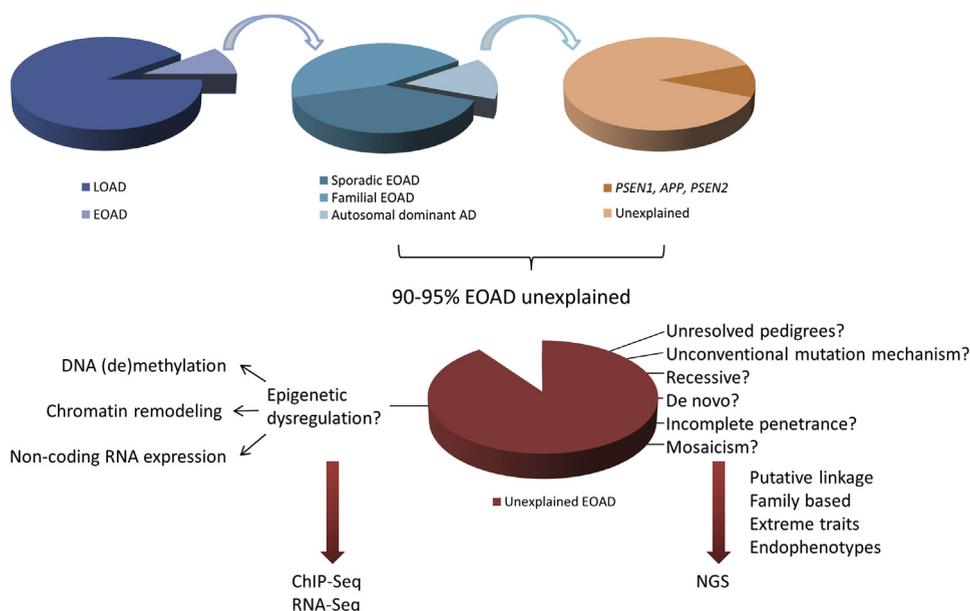


Fig. 3. Missing genetic etiology of early-onset Alzheimer's disease (EOAD). The pie charts indicate the distribution of EOAD and late-onset Alzheimer's disease (LOAD) patients (dark blue), the fraction of sporadic and familial EOAD patients with the sub-fraction of autosomal dominant patients (light 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 A β 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 $\epsilon 4$ 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* $\epsilon 4$ allele increased the risk to develop LOAD by a factor 3 up to 15-fold in a dose-dependent manner [66,67].

The *APOE* $\epsilon 4$ allele also increased risk for EOAD in carriers of at least one $\epsilon 4$ allele and was highest in those with a positive family history (Table 2) [12]. In carriers, the homozygosity for the *APOE* $\epsilon 4$ allele was sufficient to significantly increase risk for EOAD independent of other genetic factors. In contrast, in carriers, heterozygous for the *APOE* $\epsilon 4$ allele risk was only significantly increased in the presence of a positive family history of disease, indicating that the presence of one $\epsilon 4$ allele was insufficient to increase risk for AD before the age of 65 years. It also suggested that the *APOE* $\epsilon 4$ 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
<i>PSEN1</i>	Promoter SNVs	Increased risk	[68,69]
<i>APOE</i>	$\epsilon 4$ allele	Increase risk—highest in the presence of positive family history—and earlier AAO	[12,70,71]
	$\epsilon 2$ allele	Delays AAO	[70–73]
<i>CCL11</i>	p.A23T	Delays AAO	[74]
<i>PRNP</i>	p.M129V	Increased risk—VV homozygosity and highest in presence of positive family history	[75]
	Octapeptide repeat insertions	Earlier AAO	[76]
<i>SORLI</i>	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 $\epsilon 4$ allele have an increased risk in the presence of a positive family history. Contrary to the risk-increasing effect of the *APOE* $\epsilon 4$ allele, it was demonstrated that the $\epsilon 2$ allele exerted a protective effect [72,73]. Different from the mutations in *APP*, *PSEN1*, and *PSEN2*, the *APOE* $\epsilon 4$ 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 $\epsilon 4$ allele in mutation carriers, whereas those with the $\epsilon 2$ allele had later onset ages [70,71]. A larger study of samples of the *PSEN1* (p.E280A) family [73] confirmed the *APOE* $\epsilon 4$ allele as an age-at-onset (AAO) modifier (~ 12 years of delay) compared to the $\epsilon 4$ 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 (*PSEN2* 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 $A\beta$ 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 (sAPP α), 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 (sAPP β). 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 $A\beta$ species that differ in protein length including $A\beta 38$, $A\beta 40$ (most common fragment), $A\beta 42$ (self-aggregative fragment), and $A\beta 43$ [93]. Genetic mutations in *APP* are located in or near the $A\beta$ 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 $A\beta$ levels or increased production of $A\beta 42$ (and $A\beta 43$) peptide over $A\beta 40$, triggering $A\beta$ aggregation [93]. From its postulation, the “amyloid cascade hypothesis” has been supported and questioned (as reviewed by Morris et al. [94]) acknowledging that $A\beta$ 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 $A\beta$ deposits, defined as “suspected non-AD pathology” (SNAP) [95]. In a clinical trial of anti- $A\beta$ 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
<i>GRN</i>	17q21.31	FTLD	<2%	[80–82]
<i>MAPT</i>	17q21.31	FTLD	<1%	[83,84]
<i>C9orf72</i>	9p21.2	FTLD	<1%	[85–87]
<i>PRNP</i>	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 A β 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 A β 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 A β 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 A β trafficking [111]. One of the EOAD missense mutations in *SORL1* (p.G511R) that cosegregates 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 A β , 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 A β 40 and/or A β 42 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 cosegregation 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* ϵ 4 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 non-coding 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 mutation 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-A β 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-A β antibodies (gantenerumab and solanezumab) that target different A β species [151]. A β ₄₂-reducing approaches may have more efficacy in familial patients, because the increased production of A β 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 A β 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 *PSEN*s 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 (A β 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 β_{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 β_{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 mitochondrial 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 processes 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.

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