# Genetic insights in Alzheimer's disease

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**In the search for new genes in Alzheimer's disease, classic linkage-based and candidate-gene-based association studies have been supplanted by exome sequencing, genome-wide sequencing (for mendelian forms of Alzheimer's disease), and genome-wide association studies (for non-mendelian forms). The identification of new susceptibility genes has opened new avenues for exploration of the underlying disease mechanisms. In addition to detecting novel risk factors in large samples, next-generation sequencing approaches can deliver novel insights with even small numbers of patients. The shift in focus towards translational studies and sequencing of individual patients places each patient's biomaterials as the central unit of genetic studies. The notional shift needed to make the patient central to genetic studies will necessitate strong collaboration and input from clinical neurologists.**

## Introduction

By 2030, a projected 66 million people worldwide will be living with dementia—a figure set to rise to 115 million by 2050.[1] Alzheimer's disease is the leading cause of dementia in the elderly. Clinically, Alzheimer's disease can be divided into early-onset (ie, patients younger than 65 years) and late-onset (ie, those older than 65 years), whereas pathologically it is characterised by the presence of plaques of amyloid β peptides and intraneuronal tangles of hyperphosphorylated forms of microtubule-associated protein tau (MAPT).[2] Both the early-onset and late-onset forms of Alzheimer's disease have a genetic component. Causal mutations in three genes have been identified in early-onset forms, establishing the central role of amyloid in Alzheimer's disease, which has become the most widely studied pathway since these discoveries.[3–7] Nonetheless, with the exception of a few families with autosomal dominant inheritance, the pattern of inheritance is not straightforward in most patients with Alzheimer's disease, and is most likely caused by the combination of several genetic and environmental factors (defined as a disease with a complex genetic background). Twin studies predicted the heritability of late-onset forms to be as high as 80%.[8] For many years, only one genetic risk factor, the *APOE* ε4 allele, was firmly implicated in Alzheimer's disease. Technological advances, such as large-scale genome-wide association studies, which identify millions of genetic variations simultaneously across the genome, have been the driving force in the identification of more than ten risk genes for late-onset disease.[9–13] These genes draw attention to additional disease pathways, such as lipid metabolism, the immune system, and synaptic functioning mechanisms.

More risk factors will probably be elucidated as a result of a meta-analysis of all genome-wide association studies of Alzheimer's disease.[9–13] Furthermore, next-generation sequencing techniques have broadened the potential to probe for the still-elusive causal and risk variants. These next-generation sequencing approaches can be applied in small families with unexplained Alzheimer's disease and unrelated patients (figure 1). In the clinical setting, these advanced technological approaches will have important implications in terms of sampling of patients' biomaterials and genetic diagnostic screenings. We review progress in the genetics of Alzheimer's disease by discussing new findings, their implications for neurologists and patients, and the challenges ahead.

## Early genetic discoveries

### Mendelian forms

Early-onset Alzheimer's disease families with autosomal dominant patterns of inheritance provided the initial insights into the molecular genetics of Alzheimer's disease. Highly penetrant mutations were identified in three genes: *APP*, *PSEN1,* and *PSEN2.*[3–7] Although most mutations in *APP* are heterozygous missense mutations in or near amyloid-β-coding exons 16 and 17,[3] whole-gene duplications,[14–17] rare recessive small deletions,[6] and recessive missense mutations[18,19] have also been identified, resulting in either altered amyloid β production, changes in the ratio of amyloid $\beta_{42}$ to amyloid $\beta_{40}$, or increased fibril formation. *APP* duplications might not be fully penetrant, suggesting the existence of protective genetic factors.[20] Mutations in *PSEN1* and *PSEN2* are also directly related to amyloid production; they impair the γ-secretase-mediated cleavage of APP, resulting in an increased ratio of amyloid $\beta_{42}$ to amyloid $\beta_{40}$.[21–24] Together, the discovery of these three causal genes suggested a central role for amyloid, upon which many drug development efforts and clinical trials have been based. However, none of these efforts has led to improved care. Of note, mutations in these genes explain disease in only about 13% of patients with early-onset Alzheimer's disease.[25]

### Genetically complex forms

Before the era of large-scale genome-wide association studies, little progress was made in identification of the genetic cause of late-onset Alzheimer's disease. The *APOE* ε4 allele was the only well established risk factor for late-onset and early-onset forms. People with one ε4 allele have a roughly three-times-increased risk of Alzheimer's disease, and those with two ε4 alleles have a roughly 15-times-increased risk, compared with those with the most common genotype, *APOE* ε3ε3.[26] The

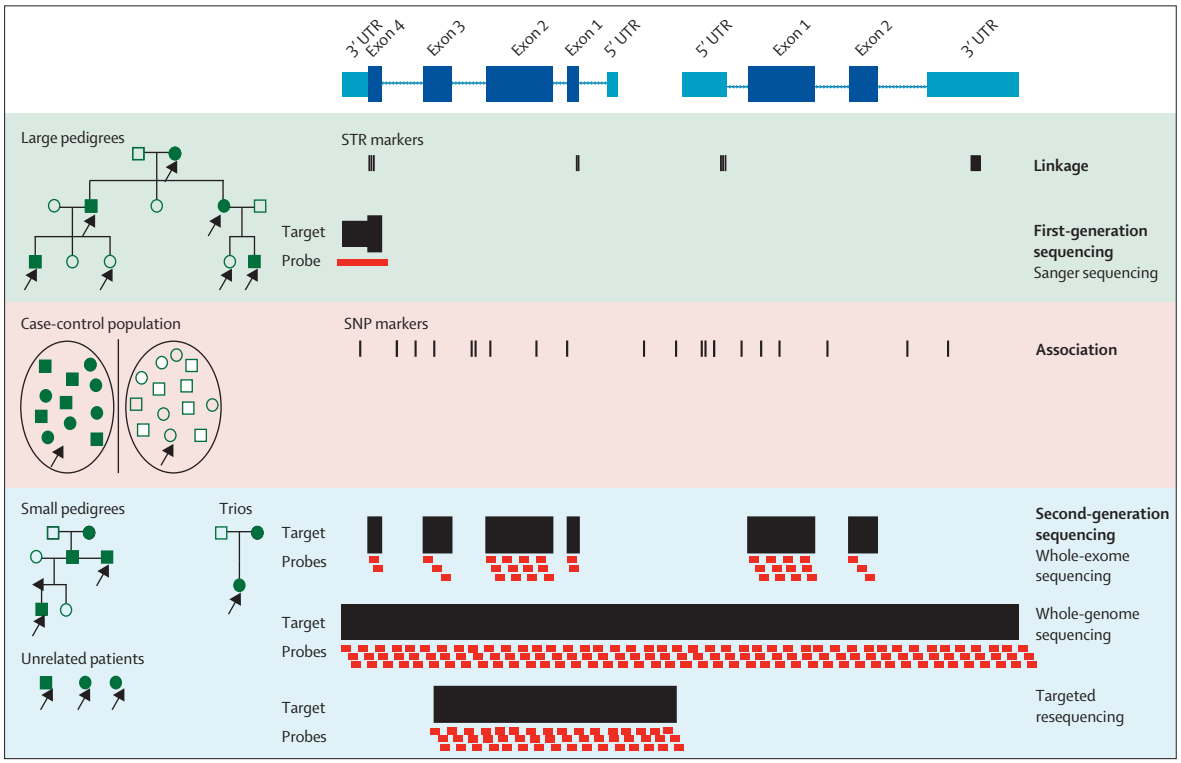


***Figure 1:*** **Evolution of investigation of genetic underpinnings of Alzheimer's disease**
Two genes are shown. Linkage studies of large pedigrees are based on STR markers (light green), whereas SNP markers are mainly used in association studies of case-control populations (pink). First-generation sequencing or Sanger sequencing consists of individual target amplification and sequencing (one primer set). Second-generation sequencing (also called massively parallel sequencing or next-generation sequencing) enables the sequencing of several target fragments simultaneously. Generally, the principle consists of shearing of genomic DNA into short pieces, ligation to adapters, hybridisation to probes, a capturing step (such as coding exons or fragments of choice), an enrichment step, and sequencing on commercially available platforms. Whereas Sanger was mainly applied in individuals from large families (indicated by arrows, light green), next-generation sequencing can be applied in case-control populations (arrows, pink) and in patients from smaller pedigrees, trios, or unrelated sporadic Alzheimer's disease patients (arrows, pale blue).

results of early studies suggested that this risk was greatest in patients who were of intermediate age (ie, 60–79 years) at onset, a notion confirmed by a study of more than 17 000 individuals in whom the risk of disease in *APOE* ε4ε4 carriers aged 60–69 years was as much as 35 times higher than that noted in *APOE* ε3ε3 carriers.[26–28] In *APOE* ε4 carriers, lifetime risk of Alzheimer's disease (an estimate independent of *APOE* ε3ε3 and the actual probability of developing disease between birth and a given age) at age 85 years was estimated to be as high as 35% for female *APOE* ε3ε4 carriers and 68% for female *APOE* ε4ε4 carriers.[28] Although *APOE* is usually thought to be a genetic risk factor (which tend to have a small contribution to disease), the very high risk estimates for *APOE* ε4 carriers seem similar to those associated with dominant genes. Therefore, *APOE* was proposed by Genin and colleagues[28] as a moderately penetrant gene with semidominant inheritance—a definition that acknowledges that not all ε4 carriers develop disease (hence the ε4 allele in this gene is not fully penetrant) and that heterozygous ε4 carriers have intermediate risk compared with homozygous carriers. Nonetheless, *APOE* is neither a prerequisite for, nor sufficient to cause, Alzheimer's disease.

The results of thousands of candidate-gene-based association studies searching for additional susceptibility factors were often not independently replicated. The main reason for the inconsistent findings was an absence of power due to insufficient sample sizes, which could lead to either false-positive or false-negative associations. Genome-wide association studies, allowing interrogation of hundreds of thousands to millions of polymorphisms simultaneously across the genome without the need for predefined hypotheses about the pathophysiological disease pathways, led to renewed hope, but such studies were initially inconclusive.

For the **candidate-gene-based association studies** see http://www.alzgene.org/

## Renewed genetic investigations

### New risk loci for genetically complex forms

Since 2009, large collaborative efforts have changed the face of the complex genetics of Alzheimer's disease. At least nine novel risk loci were uncovered as a result of European and international genome-wide association collaborations. *CLU* heads this list; its discovery instilled confidence in the value of genome-wide association

studies when the association was detected simultaneously in two independent studies.[9,11] Additionally, genome-wide association and replication have been noted for single nucleotide polymorphisms in or near *CR1, PICALM,* and *BIN1*; these were the genes detected in the first wave of large, collaborative genome-wide association studies. Continued concerted efforts identified the association with single nucleotide polymorphisms in *MS4A* cluster, *CD2AP*, *CD33*, *EPHA1*, and *ABCA7* (table 1).[9–13]

This list of common variants with small effects will probably be expanded by the International Genomics of Alzheimer's Project, which is pooling data from the four largest genome-wide association consortia and analysing these data with a range of approaches (eg, identification of genetic factors that affect age at onset, exploration of the possible effects of interactions between genes or entire physiological pathways on Alzheimer's disease). By contrast with findings from genome-wide association studies of Parkinson's disease and frontotemporal lobar degeneration, for which genes containing disease-causing variants are among the most common genome-wide association risk genes,[29–31] common variants in *APP*, *PSEN1*, and *PSEN2* are not risk factors for complex forms of Alzheimer's disease.[9,11]

## Revisiting genes for early-onset and mendelian forms

Technological advances in DNA sequencing that allow examination of the human genome at unprecedented resolution have given researchers the key to revisit the genetics of patients with early-onset or familial Alzheimer's disease, especially families that were too small to be investigated with traditional linkage analysis. Sequencing and comparison of the whole exomes or genomes of just a few related people is sufficient to uncover novel causal mutations in mendelian disorders, making individual patients rather than families central to the study of the molecular genetics of Alzheimer's disease (figure 1). So far, two whole-exome sequencing studies of people with Alzheimer's disease have been published. The first unexpectedly identified a missense mutation in *NOTCH3*,[32] a gene previously linked to cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy, and therefore not screened a priori in this family. Similarly, whole-exome sequencing in amyotrophic lateral sclerosis, which overlaps remarkably with frontotemporal lobar degeneration clinically, pathologically, and genetically, revealed a mutation in *VCP* in an autosomal dominant Italian family.[33] Mutations in *VCP* have previously been

| | Chromosome | Inheritance pattern | Location of mutations or main SNP | Effect size (odds ratio [95% CI]) | Proposed function* | Implicated pathway* |
|---|---|---|---|---|---|---|
| *APP* | 19 | Autosomal dominant or recessive | Exons 16 and 17 | ··† | Substrate of amyloid β peptide, cell signalling events, tau phosphorylation, GSK 3β activation | Amyloid β pathway, endocytotic receptor trafficking, tau pathway |
| *PSEN1* | 14 | Autosomal dominant | Whole gene | ··† | γ-secretase activity, transmembrane protein processing, intracellular signalling | Amyloid β pathway, synaptic plasticity, neuronal survival |
| *PSEN2* | 2 | Autosomal dominant | Whole gene | ··† | γ-secretase activity, transmembrane protein processing, intracellular signalling | Amyloid β pathway, synaptic plasticity, neuronal survival |
| *APOE* | 19 | Semi-dominant | Exon 4 | ε3ε4 3·2 (2·8–3·8)<br>ε4ε4 14·9 (10·8–20·6)‡ | Amyloid β aggregation and clearance, intracellular signalling through LRP | Lipid transport and metabolism, amyloid β pathway, synaptic plasticity, neuroinflammation |
| *CLU* | 8 | Risk gene | Intronic *rs1136000* | 0·89 (0·86–0·91) | Molecular chaperone, synapse turnover, amyloid β aggregation, clearance, and toxicity | Amyloid β pathway, lipid metabolism, immune system, inflammation, apoptosis |
| *CR1* | 1 | Risk gene | Intronic *rs6656401* | 1·19 (1·09–1·30) | Complement system activation, amyloid β clearance | Immune system, amyloid β pathway |
| *PICALM* | 11 | Risk gene | Upstream *rs3851179* | 0·88 (0·86–0·91) | Clathrin-mediated endocytosis | Synaptic cell functioning, amyloid β toxic effects, processing of APP |
| *BIN1* | 2 | Risk gene | Upstream *rs744373* | 1·17 (1·13–1·20) | Synaptic vesicle endocytosis, formation of tubular membrane structures | Synaptic cell functioning, caspase-independent apoptosis |
| *EPHA1* | 7 | Risk gene | Upstream *rs11767557* | 0·89 (0·83–0·96) | Synaptic development and plasticity | Immune system |
| *ABCA7* | 19 | Risk gene | Intronic *rs3764650* | 1·23 (1·18–1·28) | Transportation of substrates across cell membranes | Cholesterol metabolism, immune system, processing of APP |
| *MS4A4A, MS4A6E* | 11 | Risk gene | Intergenic *rs610932* | 0·90 (0·88–0·93) | No known functions (except MS4A2 β-subunit, which has high affinity for IgE receptors) | Immune system (MS4A2), cell surface signalling |
| *CD33* | 19 | Risk gene | Upstream *rs3865444* | 0·85 (0·86–0·92) | Clathrin-mediated endocytosis | Immune system, synaptic cell functioning |
| *CD2AP* | 6 | Risk gene | Intronic *rs9349407* | 1·12 (1·08–1·16) | Receptor-mediated endocytosis | Synaptic cell functioning, actin cytoskeleton |

Effect size for top SNPs in risk genes is based on AlzGene meta-analysis (http://www.alzgene/org). SNP=single nucleotide polymorphism. *Selection of proposed functions and pathways; the exact functional evidence of these loci in Alzheimer's disease is often sparse. †Effect size for mutations in causal variants is nearly complete penetrance. ‡Effect for *APOE* ε4 is based on Farrer et al.[26]

***Table 1:*** **Causal and risk genes in mendelian and non-mendelian Alzheimer's disease**

detected in patients with frontotemporal lobe dementia in association with the syndrome inclusion body myopathy, Paget's disease, and frontotemporal dementia.[33,34]

The finding that mutations in a single gene can underlie a broad phenotypic range of neurodegenerative disease is not novel (eg, *MAPT* Arg406Trp mutation in Alzheimer's disease,[35] *PSEN1* Gly183Val in Pick's disease,[36] *GRN* null mutation and missense mutations in Alzheimer's and Parkinson's diseases,[37,38] *C9orf72* repeat expansion in Alzheimer's disease[39]) but has important clinical implications, warranting diagnostic screening of all known neurodegenerative genes in patients with disease of unknown genetic cause.

Although the number of neurodegenerative genes is too large for Sanger sequencing for diagnostic purposes, next-generation sequencing techniques enable simultaneous screening of a whole battery of genes implicated in neurodegenerative diseases. In turn, this technology will greatly help with personalisation of treatment for neurodegenerative disorders, particularly when treatments that target the underlying molecular causes rather than the clinical symptoms become available.

The second whole-exome sequencing study done in patients with early-onset Alzheimer's disease identified missense and nonsense mutations in *SORL1*,[40] which encodes a neuronal sorting protein that binds APP and directs it towards the endosome-recycling pathways. Poor segregation evidence and the absence of functional evidence for these *SORL1* variants notwithstanding, the high frequency of coding *SORL1* variants in Alzheimer's disease is particularly noteworthy because this gene was previously suggested as a risk factor for complex forms of the illness.[41–43] This finding implies that both causal variants and common risk variants in one gene might have a role in disease.

Whole-exome sequencing is most often chosen for monogenic mendelian diseases, largely because of its low cost compared with whole-genome sequencing (the exome is 1–2% of the whole genome) and the notion that most sequence variations leading to a severe phenotypic effect are located in the coding part of the genome. However, increasing evidence suggests that non-coding variants (eg, intronic *SOD1* mutations in amyotrophic lateral sclerosis, *APP* promoter variants in Alzheimer's disease[44–46]) cause or increase the risk of neurodegenerative disease. Additionally, whole-genome sequencing provides better coverage of the exome than does whole-exome sequencing,[47] and with the cost of sequencing anticipated to drop and bioinformatics technology to advance, whole-genome sequencing will probably become the approach of choice in the future. On the basis of data from whole-genome sequencing of 1795 Icelanders, a protective *APP* missense variation (Ala676Thr) was noted near the *BACE1* cleavage site, leading to reduced formation of amyloidogenic peptides and better cognitive performance in individuals free from Alzheimer's disease compared with those who did not have the mutation.[48] This finding supports the notion that reduction of *BACE1* cleavage protects against disease and has the therapeutic potential to reduce amyloid burden and block amyloid toxic effects.

Studies of whole-genome sequencing still pose great challenges because of the amount of genetic variations for validation and follow-up (one genome harbours roughly 3 million single nucleotide polymorphisms compared with 20 000–50 000 in one exome), and thus very careful study designs and selection of patients (eg, based on shared clinical, biochemical, or neuropathological phenotypic characteristics) remain necessary. Close collaborations with clinicians, in terms of both collection of a wide range of clinical and biochemical phenotypes and follow-up of patients, is therefore essential. Collaborative initiatives, such as the Dominantly Inherited Alzheimer Network (DIAN) consortium, which aims to understand rare monogenic forms of Alzheimer's disease),[49] and the European early-onset dementia consortium,[50] which does clinical and genetic translational research in patients with early-onset dementia in Europe, will probably have major roles.

## From indirect association to genetic cause

### The search for underlying disease variants

Genome-wide association studies have confirmed the role of common genetic variants in complex Alzheimer's disease. Elucidation of the true risk variants might bring about important insights into pathomechanisms of disease. By design, however, the most strongly associated variants at the newly identified risk loci often have no apparent functional disease consequences and flag an unknown true risk factor in linkage disequilibrium (ie, non-random association of alleles at two or more loci). The most strongly associated single nucleotide polymorphism in *CLU*, for instance, is an intronic variant, which, at first, was thought to have no predicted consequences on *CLU* expression or function.[9,11]

Careful study of genome-wide association loci is necessary for identification of underlying risk alleles; not only the top genome-wide-associated single nucleotide polymorphisms, but also the complete genetic variability at these loci, including common, rare, and copy number variants, should be investigated. So far, most genetic follow-up studies have been based on the first-wave of genome-wide association genes—namely, *CLU*, *CR1*, *PICALM*, and *BIN1*[51–60]—and only a few on the second wave of genes.[61,62]

Particularly for *CLU*, several studies have confirmed associations or a trend towards association with Alzheimer's disease, most of which were with the top genome-wide-associated single nucleotide polymorphism.[51–58,63] A common polymorphism in linkage disequilibrium with the top single nucleotide polymorphism has been suggested as a possible functional variant because of its association with increased transcript concentrations of *CLU* in human temporal lobe samples (table 2).[65] Other studies, however, did not replicate the

| | Type of study | Genetic variants | Risk allele* | Biomaterial | Sample size | Main results |
|---|---|---|---|---|---|---|
| Nilselid et al[64] | Protein | .. | .. | CSF | 99 patients with Alzheimer's disease, 39 controls | Increased clusterin concentrations in CSF of patients with Alzheimer's disease |
| Szymanski et al[65] | Function | *rs9331908* | C allele | Post-mortem brain | 190 controls (61 patients with Alzheimer's disease and 54 controls in replication sample) | Risk allele increases clusterin transcript concentrations (NR_038335.1)<br>Higher transcript concentrations (NR_038335.1) in brains of people with Alzheimer's disease than in those of controls |
| Guerreiro et al[66] | eQTL | Several *CLU* variants | Several | Cortical brain tissue | 174 controls | Risk alleles have no effect on transcript expression in brain |
| Thambisetty et al[67] | Proteomic and neuroimaging | Several *CLU* variants | Several | Plasma; in-vivo brain imaging; RNA; TASTPM mice | Plasma of 78 patients with Alzheimer's disease and 17 with mild cognitive impairment (689 in the replication sample); RNA of 182 patients with Alzheimer's disease, 179 with mild cognitive impairment, and 207 controls | Increased clusterin plasma concentrations relates to increased risk of incident Alzheimer's disease and is predictive of increased amyloid β burden; no effect of genetic variants on clusterin messenger RNA or protein concentrations in blood; increased clusterin plasma concentrations in TASTPM mice |
| Schrijvers et al[68] | Case–cohort and prospective | .. | .. | Plasma | 60 patients with Alzheimer's disease, 926 controls (156 patients with Alzheimer's disease in replication sample) | Increased clusterin plasma concentrations related to increased prevalence and severity of Alzheimer's disease |
| Ijsselstijn et al[69] | Plasma | .. | .. | Plasma | 48 patients with Alzheimer's disease, 48 controls | No difference in clusterin concentrations between patients with Alzheimer's disease and controls |
| Schürmann et al[70] | Plasma | *rs11136000* | G allele | Plasma | 67 patients with Alzheimer's disease, 171 controls | Risk allele correlates to decreased clusterin plasma concentrations |
| Allen et al[71] | eQTL and brain gene expression | *rs11136000* and others | G allele | Post-mortem brain | 202 patients with Alzheimer's disease, 197 controls | Risk alleles correlates to decreased clusterin protein concentrations in temporal cortex |

eQTL=expression quantitative trait loci. TASTPM=double-mutant APPswe x PS10M146V transgenic. *Associated with increased risk of development of Alzheimer's disease on the basis of meta-analysis of all studies on AlzGene.

***Table 2:*** **Protein and biomarker studies of CLU**

association, showing that this polymorphism cannot, or at least not fully, explain the noted genome-wide association at *CLU*.[46,54,55] Several studies have resequenced the *CLU* coding regions (to various extents) in search of putative pathogenetic variants.[46,66] The largest revealed a pattern of clustering of rare predicted pathogenetic variants (minor allele frequency <5%; both non-synonymous substitutions and a 9 bp insertion or deletion) in the exons of the β-chain of CLU in patients with Alzheimer's disease, suggesting a functional role for this protein subunit in disease risk.[46]

Similar to those of *CLU*, the results from the first *CR1* follow-up studies were restricted to replication of the top genome-wide-associated single nucleotide polymorphism, which is located in a non-coding sequence with no obvious functional relevance.[51,52,54] The *CR1* locus is characterised by a high degree of repetitive sequences, making uncovering of the true genetic risk factor technically difficult. However, examination of the role of interindividual variations in the copy number of *CR1* for susceptibility to Alzheimer's disease identified an association between Alzheimer's disease and a functional copy number variation, which determines the length of the CR1 protein, and with that the number of C3b or C4b and cofactor activity binding sites, which are important in the complement cascade.[72] Association with this copy number variation could explain the single nucleotide polymorphism association noted in the original genome-wide association study.

For four of the novel Alzheimer's disease loci, the strongest association signals were noted upstream or downstream of the gene (table 1), which complicates the search for underlying pathogenetic variants. For *PICALM*,[9] for example, the genome-wide association signal was far upstream of the gene, a finding that was replicated in subsequent studies[13,51,52,54–56,73] but probably not explained by the reported coding *PICALM* variant.[60] Similarly, the initial association[13] upstream of *BIN1* was confirmed in sporadic and familial late-onset Alzheimer's disease[51,58,73] and meta-analyses of genome-wide association studies.[10,12] Although strong association does not necessarily imply causality for these single nucleotide polymorphisms, non-coding DNA variations upstream of a gene might directly contribute to the disease phenotype. In cholesterol metabolism, for instance, a non-coding variant 120 kb upstream of the *SORT1* promoter regulates expression of this gene.[74]

The labour-intensiveness of Sanger sequencing has precluded progress in the identification of true genetic risk factors in non-coding regions of the genome. By contrast, next-generation sequencing techniques enable identification of both common and rare variants with intermediate or large effects on disease and of copy number variations. Both of these are increasingly recognised as being implicated in complex disease.[75,76] To find the right algorithms and sufficiently large sample sizes to analyse the data is challenging, but the identification of rare *CLU* variant associations with

Alzheimer's disease has proved the worth of this strategy.[46] Furthermore, next-generation sequencing has shown an association between rare variants in *NCSTN* and late-onset Alzheimer's disease.[77] *NCSTN* shows association with familial early-onset Alzheimer's disease.[78] A functional genomic investigation of different nicastrin haplotypes precluded functional differences between different haplotypes and a role for single nucleotide polymorphisms in *NCSTN* in common forms of Alzheimer's disease.[79] Because risk genes for Alzheimer's disease, such as *CLU*,[46] might harbour rare disease variants, this shift in allele range calls attention to the need to sequence all novel risk genes in detail (eg, by targeted resequencing). Several rare variations might accumulate and cross the susceptibility threshold for disease. Large next-generation sequencing studies of unrelated patients with complex Alzheimer's disease will therefore have a crucial role in the identification of causal and risk variants. Functional studies will subsequently assess the pathogenic effects of these variants.

### Biological effects of genetic variations

All nine novel risk genes for Alzheimer's disease show patterns of functional relation. All except the *MS4A* cluster (the functions of which are largely unknown except for a possible role in signal transduction)[80] fit with hypotheses about lipid processing (*CLU* and *ABCA7*);[81,82] the complement system, inflammation, and immune system (*CLU, CR1, ABCA7, CD33,* and *EPHA1*);[83–87] and synaptic cell functioning, such as endocytosis (*PICALM,*[88] *BIN1,*[89] *CD33,*[90] and *CD2AP*;[91] table 1). Although the exact disease mechanisms cannot yet be established, speculations can be made. For instance, clusterin is a versatile protein, and disease-associated variants might affect a pleiotropy of functions, including amyloid $\beta_{42}$ clearance from the brain and roles in apoptosis, cholesterol trafficking, and inflammatory response.[92] Likewise, *CR1* variants are postulated to induce increased complement activation[93] and decreased opsonisation of amyloid plaques in the brain.[94] Genetic changes at the *PICALM* locus might lead to perturbations at the synapse, because PICALM targets VAMP2 trafficking, which is crucial to the fusion of synaptic vesicles (and hence memory function).[95] The finding that susceptibility genes for Alzheimer's disease are not random is underscored by pathway-based analyses (ie, computational methods for ranking of gene pathways in terms of implication in disease susceptibility and joint consideration of important and less important genome-wide-associated single nucleotide polymorphisms to discover new associated pathways based on information in medical literature) of genome-wide association data for Alzheimer's disease.[86,96]

Several approaches to establish the mode of action of Alzheimer's disease variants are under investigation (figure 2). Studies so far have focused on *CLU* and *CR1*. First, studies have investigated whether disease-associated variants affect amounts of gene product (at the RNA or protein level), which could be measured as an early biomarker of disease or reveal potential therapeutic targets. This notion is of particular interest for Alzheimer's disease genes that show strong association in regulatory regions and those with copy number variations. For *CLU*, several studies have assessed CLU protein concentration in disease[64,67,97] and whether these concentrations could be used as a biomarker,[67–70] or tried to correlate genetic associations with messenger RNA and protein concentration (table 2).[65,67,70,71] CLU concentrations are increased in the brain, plasma, and CSF of patients with Alzheimer's disease.[52,67,68] Nonetheless, findings correlating expression to genetic variants have not been fully consistent in the direction of effect (table 2). Further studies of larger cohorts of patients with Alzheimer's disease, use of the complete genetic *CLU* profile, and possibly refocusing on specific *CLU* isoforms[65] will probably reveal whether changes in expression of CLU are genuinely affected by Alzheimer's disease risk variants.[93]

Second, to reveal insights into disease mechanisms, studies have assessed the effect of genetic variants on amyloid $\beta_{1–42}$ and tau (t-tau and p-$tau_{181}$) in blood, CSF, and brain (table 3). In such studies of *CLU, CR1,* and *PICALM*, associations were noted between *PICALM* and decreased amyloid $\beta_{1–42}$ concentrations in the CSF[57] and between several *CR1* single nucleotide polymorphisms and increased amyloid $\beta_{1–42}$ concentrations in CSF (table 3).[72] In genome-wide association studies of CSF biomarkers in the Alzheimer's Disease Neuroimaging Initiative cohort, none of the novel loci was associated with biomarker concentrations.[101,102] No clear profile has emerged from studies of the relation between genotype and amyloid or tau phenotype (table 3). The inconsistent, at times even contradictory, findings might be partly because of issues in study design or small effect sizes, which have long hampered candidate-gene-based studies of Alzheimer's disease. Alternatively, identification of the true risk alleles might lead to more readily interpretable results.

Third, model systems have the potential to reveal unknown relations. A novel and direct link between the clathrin-mediated endocytosis genes *PICALM*, *BIN1*, and *CD2AP* and amyloid β toxic effects was noted in yeast, nematodes, and rat cortical neurons after study of the role of several novel genes in amyloid β and tau pathways.[103] Furthermore, results from human genome-wide association studies can be coupled to functional screening in model organisms such as *Drosophila*. The effect of genes identified from a genome-wide scan of Alzheimer's disease pathology on neurotoxic effects of tau was assessed in vivo by looking at which genes in cell lines could exacerbate or rescue the eye phenotype of transgenic tau *Drosophila* models.[104] Future assessment of the enhancer or suppressor effects on tau and amyloid β toxic effects for all significantly and suggestive associated genes will strengthen validity and suggest possible mechanisms of action.

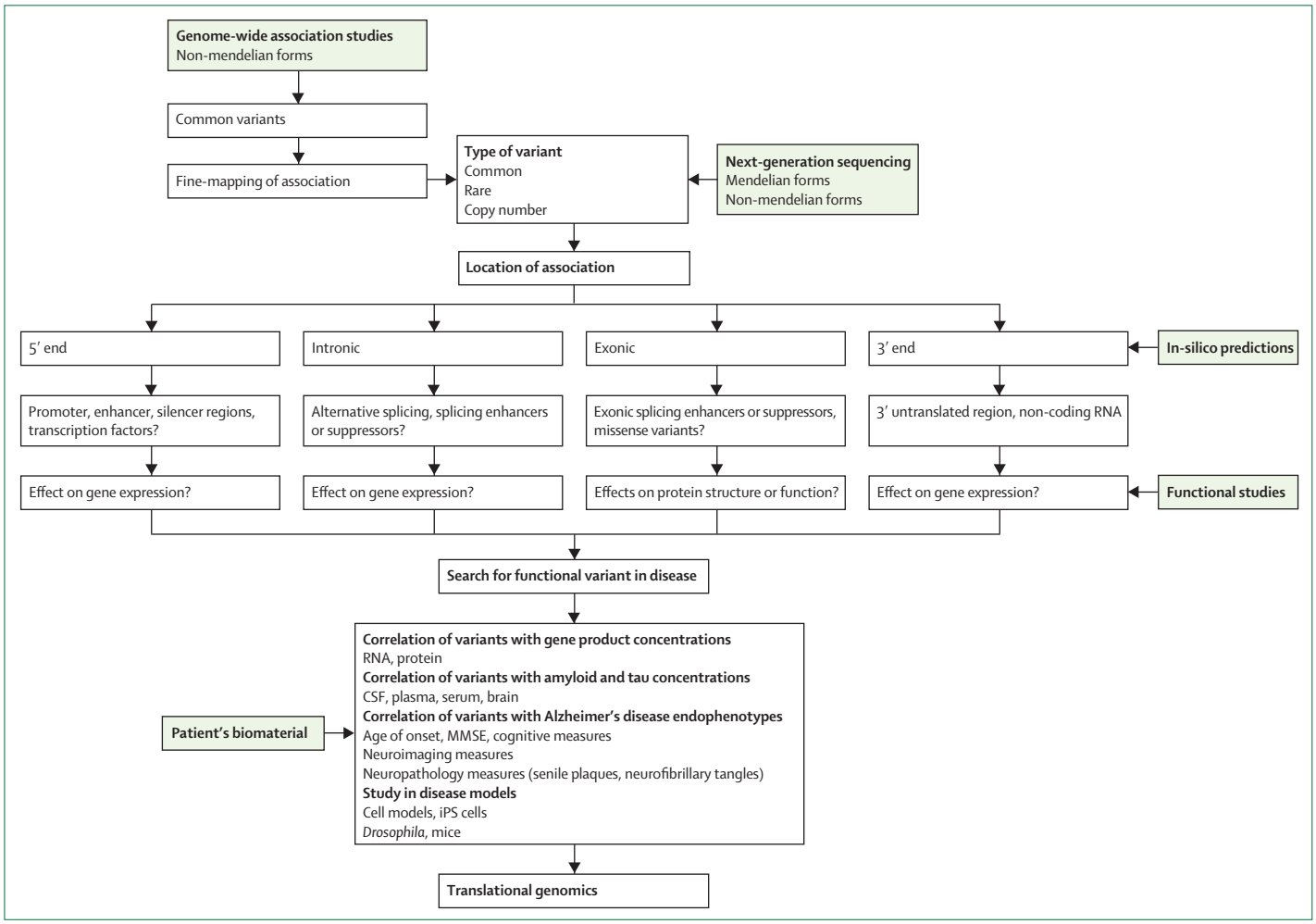


***Figure 2*: Identification of genetic association, underlying functional variants, and biological functions in Alzheimer's disease**
MMSE=mini-mental state examination. iPS=induced pluripotent stem cell.

Finally, correlation of genetic findings with endophenotypic data—eg, neuroimaging traits, age of onset, mini-mental state examination scores, and cognitive measurements—might further provide insights into disease mechanisms. Effects on neuroimaging correlates (such as entorhinal cortex thickness) and neuropathology burden of several Alzheimer's disease loci (*CLU*, *CR1*, *PICALM*, and *BIN1*) were noted, suggesting that these traits are, at least partly, determined by genome sequences at these loci (table 4). Such correlations will benefit from replication in large sample sizes.

Genome-wide association studies looking for variants affecting neuroimaging endophenotypes of Alzheimer's disease have been done in addition to those looking at concentrations of biomarkers. The novel Alzheimer's disease genome-wide association genes seem to overlap little with the genes affecting endophenotypes,[111] with the exception of the *PICALM* risk-decreasing allele *rs3851179*, which is associated with increased entorhinal cortical thickness.[112] Projects such as the Alzheimer's Disease Neuroimaging Initiative, which are designed to collect and validate Alzheimer's disease biomarker data, including blood and CSF tests, and MRI and PET scans, will continue to generate novel insights from associations between variants and endophenotypes. Multinational consortia that combine genome-wide single nucleotide polymorphisms and structural MRI data from large samples (eg, Enhancing Neuro Imaging Genetics through Meta-Analysis Consortium [ENIGMA]) seemed fruitful in identification of new associations between variants and hippocampal, intracranial, and total-brain volumes.[113] Additionally, the incorporation of clinical and cognitive measures, imaging, and biomarkers in cohorts of the mega meta-analysis of the International Genomics of Alzheimer's Project (IGAP) consortium will be beneficial to identify variants affecting these traits. Once the underlying variants are detected in the risk genes, the correlations with endophenotypes might be better addressed.

| | Genetic variants | Risk allele* | Biomaterial | Sample size | Main results |
|---|---|---|---|---|---|
| ***CLU*** | | | | | |
| Schjeide et al[57] | *rs11136000* | G allele | CSF | 202 patients with Alzheimer's disease, 187 controls | Not associated with amyloid $\beta_{1-42}$, p-tau$_{181p}$, or t-tau |
| Kok et al[98] | rs11136000 | G allele | Post-mortem brain | 603 controls | Increased amyloid burden (late-stage senile plaques), no effect on neurofibrillary tangles |
| Chibnik et al[99] | *rs11136000* | G allele | Post-mortem brain | 553 (both controls and patients with Alzheimer's disease) | No effect on amyloid burden or neurofibrillary tangles |
| Kauwe et al[100] | Several SNPs | Several | CSF | 256 patients with Alzheimer's disease, 408 controls | Not associated with amyloid $\beta_{1-42}$ or p-tau$_{181p}$ |
| ***CR1*** | | | | | |
| Schjeide et al[57] | *rs6656401* | A allele | CSF | 202 patients with Alzheimer's disease, 187 controls | Not associated with amyloid $\beta_{1-42}$ or t-tau |
| Brouwers et al[72] | Several SNPs, *CR1* copy number variant | Several, *CR1*-S† | CSF | 339 patients with Alzheimer's disease | Increased amyloid $\beta_{1-42}$, no effect on t-tau or p-tau$_{181p}$; *CR1*-S not associated with amyloid $\beta_{1-42}$ or t-tau |
| Kok et al[98] | *rs1408077* | T allele | Post-mortem brain | 603 controls | Increased amyloid burden (sparse senile plaques), no effect on neurofibrillary tangles |
| Chibnik et al[99] | *rs6656401* | A allele | Post-mortem brain | 553 (both controls and patients with Alzheimer's disease) | Increased Alzheimer's disease pathology and amyloid burden, no effect on neurofibrillary tangles |
| Kauwe et al[100] | Several SNPs | Several | CSF | 256 patients with Alzheimer's disease, 408 controls | Not associated with amyloid $\beta_{1-42}$ or p-tau$_{181p}$ |
| ***PICALM*** | | | | | |
| Schjeide et al[57] | rs541458 | T allele | CSF | 202 patients with Alzheimer's disease, 187 controls | Decreased amyloid $\beta_{1-42}$, no effect on t-tau |
| Kok et al[98] | *rs3851179* | G allele | Post-mortem brain | 603 controls | Decreased amyloid burden (moderate senile plaques), no effect on neurofibrillary tangles |
| Chibnik et al[99] | *rs7110631* | G allele | Post-mortem brain | 553 (both controls and patients with Alzheimer's disease) | No effect on amyloid burden or neurofibrillary tangles |
| Kauwe et al[100] | Several SNPs | Several | CSF | 256 patients with Alzheimer's disease, 408 controls | Not associated with amyloid $\beta_{1-42}$ or p-tau$_{181p}$ |

All main results are presented for the risk allele. SNP=single nucleotide polymorphism. *Associated with increased risk of development of Alzheimer's disease on the basis of meta-analysis of all studies on AlzGene. All main results are presented for the risk allele. †*CR1* isoform determined by the *CR1* copy number variant.

***Table 3:*** **Alzheimer's disease biomarker correlation studies of first-wave genome-wide association genes**

## Present and future implications

With a lifetime risk of 35% (higher for ε3ε4 carriers at 85 years), *APOE* remains the most important gene for complex Alzheimer's disease.[28] The risk-increasing effects of the novel Alzheimer's disease susceptibility variants are much smaller, and range from roughly 0·80 to 1·25 (table 1). The cumulative population attributable fraction (ie, the proportion of disease cases in a population that would be prevented if an exposure were eliminated) of the nine non-*APOE* loci is estimated to be as much as 35%.[12] This estimate will probably change with elucidation of the number, allele frequency, and risk-effect of the true functional variant at each locus, the detection of additional common risk loci by the International Genomics of Alzheimer's Project (IGAP), and patterns of epistasis.

But what effects have these genetic findings had on patients? Because of the small risk estimates, predictive or diagnostic testing for each of these loci individually or in combination does not have a role. The combination of *CLU* and *PICALM* variations, for instance, added very little to the prediction of risk and was thus not clinically relevant.[13] Future next-generation sequencing projects that screen for underlying functional variants and rare variants with larger (compared with the small effect size of risk variants) effects will probably lead to major changes in clinical practice.

With an increased focus on meticulous phenotyping, the availability of large volumes of genome-wide genotype data will ultimately allow accurate molecular reclassification of patients into subgroups. Because researchers are confronted with several genes in which variants might lead to different phenotypes, to screen not only genes related to a specific clinical phenotype, but also all genes related to neurodegenerative diseases is crucial, through use of targeted next-generation sequencing panels for a range of diseases, such as Alzheimer's disease, Parkinson's disease, frontotemporal lobe dementia, and amyotrophic lateral sclerosis. Detailed molecular genetic profiling of risk variants in patients might lead to the redefinition of clinical phenotype groups. For disorders such as Alzheimer's disease, which have heterogeneous causes, treatment ultimately needs to target underlying molecular pathomechanisms. Molecular subclassification will streamline clinical trials and ease future clinical decision making.

Although the effects will not be immediately perceptible, identification of the novel risk genes increases understanding of the molecular underpinnings of Alzheimer's disease by showing prominent roles of the lipid-processing pathway (*APOE*, *CLU*, and *ABCA7*), the immune system (*CLU*, *CR1*, *ABCA7*, *CD33*, and *EPHA1*), and the synaptic-cell-functioning pathways (*PICALM*, *BIN1*, *CD33*, and *CD2AP*), in addition to amyloid β (*CLU*, *PICALM*, *BIN1*, and *CD2AP*) and tau (*BIN1*) pathways. Despite the small effects of risk variants, investigation of functionality might be rewarding and could yield important clinical and biological insights. The study of cholesterol genetics provides a retrospective example. *HMGCR* is an important genome-wide significant locus for LDL cholesterol.[114–116] Statins, the most widely prescribed drugs to lower LDL cholesterol, act by inhibiting the HMGCR enzyme, showing that genetic risk factors with a small effect size can underlie major treatment targets. Effective treatment and targeting of Alzheimer's disease might become feasible when the underlying mechanisms of only a few risk factors are known and targeted. Additionally, understanding of the underlying biology and effects of risk variants—eg, variants that affect neuroimaging measures at early stages of disease—might be used for early diagnosis.[106]

Even though early-onset and familial Alzheimer's disease account for only a small percentage of all cases, most of our knowledge has resulted from research of these families, and hopes are high that renewed investment in this small subgroup of patients can lead to substantial advances in understanding the pathogenesis in Alzheimer's disease in general.

Genome-wide association studies have mostly been focused on single nucleotide markers that reach significance in genome-wide associations. Advanced statistical analyses might partly circumvent the limitations of genome-wide association studies and offer complementary approaches. Gene-based association studies in which genes rather than genetic markers are used can account for linkage disequilibrium structure and allelic

| | Type of study | Genetic variants | Risk allele* | Study population | Main results |
|---|---|---|---|---|---|
| ***CLU*** | | | | | |
| Biffi et al[105] | MRI | *rs11136000* | G allele | 168 patients with Alzheimer's disease, 357 with mild cognitive impairment, 215 controls | No association between six neuroimaging traits |
| Braskie et al[106] | Diffusion tensor imaging | *rs11136000* | G allele | 398 young controls | Decreased white matter integrity in brain regions |
| Lancaster et al[107] | Functional MRI | *rs11136000* | G allele | 43 controls | Increased activity in dorsolateral prefrontal cortex, hippocampus, and cingulate cortex during working memory |
| Erk et al[108] | Functional MRI | *rs11136000* | G allele | 109 controls | Reduced connectivity between hippocampus and dorsolateral prefrontal cortex during episodic memory |
| Bralten et al[109] | MRI | *rs11136000* | G allele | 430 young controls (492 controls in replication sample) | No effect on grey matter volume in entorhinal cortex and hippocampus |
| Thambisetty et al[110] | $^{15}$O-water PET; neuropsychological tests | *rs11136000* | G allele | 88 controls; 599 controls, 95 patients with mild cognitive impairment or Alzheimer's disease | Increase in resting state cerebral blood flow in hippocampus; increased rate of decline in memory performance |
| ***CR1*** | | | | | |
| Biffi et al[105] | MRI | *rs1408077* | T allele | 168 patients with Alzheimer's disease, 357 with mild cognitive impairment, 215 controls | Reduced entorhinal cortex thickness |
| Bralten et al[109] | MRI | *rs6656401* | A allele | 430 young controls (492 controls in replication sample) | Decreased grey matter volume in entorhinal cortex and hippocampus |
| ***PICALM*** | | | | | |
| Bralten et al[109] | MRI | *rs3851179* | G allele | 430 young controls (492 controls in replication sample) | No effect on grey matter volume in entorhinal cortex and hippocampus |
| Biffi et al[105] | MRI | *rs3851179* | G allele | 168 patients with Alzheimer's disease, 357 with mild cognitive impairment, 215 controls | Decreased entorhinal cortex thickness and hippocampal volume |
| ***BIN1*** | | | | | |
| Biffi et al[105] | MRI | .. | A allele | 168 patients with Alzheimer's disease, 357 with mild cognitive impairment, 215 controls | Reduced entorhinal cortex thickness and temporal pole cortex thickness |

All main results are presented for the risk allele. *Associated with increased risk of development of Alzheimer's disease on the basis of meta-analysis of all studies on AlzGene.

***Table 4:*** **Neuroimaging studies of several first-wave genome-wide association genes**

heterogeneity across populations, and novel genes can be identified without replication of individual markers.[117] Implementation of this gene-based algorithm has led to the discovery of the novel Alzheimer's disease loci *FRMD6* and *NARS*, the latter of which is immediately adjacent to *GAB2*.[118] Additionally, genome-wide haplotype-based association studies, which can characterise loci not detected by univariate analyses, have identified *FRMD4A*.[119] Furthermore, the combination of information about single nucleotide polymorphisms at different Alzheimer's disease susceptibility genes (*CR1*, *BIN1*, *CLU*, *PICALM*, and *APOE*) led to significantly associated genotype patterns that affected episodic memory performance.[120] Examination of regions with excess homozygous markers is an alternative approach for the detection of novel recessive causal genes for Alzheimer's disease or risk gene associations, when comparing patients with healthy controls. Although small studies have shown a high degree of homozygosity in a region of chromosome 9 in an Israeli–Arab community[121] and chromosome 8 in late-onset patients,[122] no homozygous tract associations were noted in recent genome-wide association data (including the nine novel risk genes).[123] Study of the genome-wide effect of copy number variations on predisposition to and modification of age of onset of Alzheimer's disease suggested a duplication affecting *CHRNA7*,[124] and an olfactory receptor region,[125] respectively.

Detection of gene–gene and gene–environment interactions between common single nucleotide polymorphisms or between single nucleotide polymorphisms and rare variants in genes that would not be detected by individual testing of single nucleotide polymorphisms adds another layer of complexity to the analysis of genome-wide association data. Evidence is accumulating that a pronounced part of the still-elusive genetic variability could be due to ignored epistatic effects.[126] To achieve adequate power for such interaction studies, large sample sizes are needed, such as that of the International Genomics of Alzheimer's Project mega meta-analysis. Furthermore, combination of genome-wide association data with gene expression profiling (eg, transcriptome analyses, expression quantitative trait loci analyses) is a strong strategy for the identification of novel risk genes for Alzheimer's disease, as shown in two genome-wide association studies of LDL cholesterol concentrations and heart disease.[74,116] Expression quantitative trait loci studies can provide new links between known Alzheimer's disease risk genes and changed brain expression,[71] whereas expression genome-wide association studies can provide complementary information on functional disease variants.[127]

The functional follow-up of variants with small effect sizes is challenging. Studies about induced neuronal cells, generated from fibroblasts of patients with Alzheimer's disease of differing genetic causes, might provide further insights into the underlying mechanisms of disease. Two studies have already noted that neurons derived from patients with familial or sporadic Alzheimer's disease showed Alzheimer's disease phenotypes.[128,129]

Patients' biomaterials, including genomic material, RNA, plasma, CSF, induced pluripotent stem cell lines, and brain samples will be crucial for further characterisation of the underlying variants in disease. Because technological progress puts patients more and more at the centre of molecular genetic research, with one patient's genome and his or her matching biomaterials sufficient, in principle, to uncover a new disease mechanism, the need for multidisciplinary research with strong input from clinical neurology is emphasised.

### Search strategy and selection criteria

We searched PubMed with the terms "Alzheimer disease", "genome-wide association studies AND Alzheimer", "*CLU* AND Alzheimer", "*CR1* AND Alzheimer", "*PICALM* AND Alzheimer", "*BIN1* AND Alzheimer", "*EPHA1* AND Alzheimer", "*MS4A* AND Alzheimer", "*CD2AP* AND Alzheimer", "*CD33* AND Alzheimer", "*ABCA7* AND Alzheimer", "CNV AND Alzheimer", and "Alzheimer AND replication" for papers published in English. Articles about genetic risk factors were further identified through specific searches in AlzGene for genome-wide association studies studies and other large-scale association studies of *CLU*, *CR1*, *PICALM*, *BIN1*, *ABCA7*, *CD2AP*, *MS4A*, *CD33*, and *EPHA1*. We also identified papers through searches of the authors' personal files. The final reference list, which features papers published between Jan 1, 1990, and Sept 30, 2012, was generated on the basis of originality and relevance to the broad scope of this Review.

## Conclusion

Over the past few years, high-throughput genome technologies have changed the genetic landscape of Alzheimer's disease. Genome-wide association studies of large cohorts resulted in substantial knowledge about the complex genetic forms. Nine risk genes with small effect sizes have been identified. In the near future, both hypothesis-free (eg, whole-genome, whole-exome) and hypothesis-driven (eg, targeted candidate resequencing of genome-wide assocation genes) next-generation sequencing approaches will probably disentangle much of the genetics of the disease, and the translational component of detailed genetic risk profiles will become crucial to effectively target the disease.

**Contributors**

All authors wrote the paper and did the search of published work. KB drew the figures.

**Conflicts of interest**

We declare that we have no conflicts of interest.

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