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The genetic landscape of Alzheimer disease: clinical implications and perspectives

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The search for the genetic factors contributing to Alzheimer disease (AD) has evolved tremendously throughout the years. It started from the discovery of fully penetrant mutations in *Amyloid precursor protein, Presenilin 1*, and *Presenilin 2* as a cause of autosomal dominant AD, the identification of the ε4 allele of *Apolipoprotein E* as a strong genetic risk factor for both early-onset and late-onset AD, and evolved to the more recent detection of at least 21 additional genetic risk loci for the genetically complex form of AD emerging from genome-wide association studies and massive parallel resequencing efforts. These advances in AD genetics are positioned in light of the current endeavor directing toward translational research and personalized treatment of AD. We discuss the current state of the art of AD genetics and address the implications and relevance of AD genetics in clinical diagnosis and risk prediction, distinguishing between monogenic and multifactorial AD. Furthermore, the potential and current limitations of molecular reclassification of AD to streamline clinical trials in drug development and biomarker studies are addressed.

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Alzheimer disease (AD) is a devastating neurodegenerative disease and the predominant form of dementia (50–75%). In 2015, ~44 million people worldwide are estimated to have AD or a related dementia. Each year, 4.6 million new cases of dementia are predicted with numbers expected to almost double by 2030.[1] AD is pathologically defined by severe neuronal loss, aggregation of amyloid β (Aβ) in extracellular senile plaques, and formation of intraneuronal neurofibrillary tangles consisting of hyperphosphorylated tau protein. The disease is clinically characterized by progressive deterioration of memory and cognitive functions, leading to loss of autonomy and ultimately requiring full-time medical care. Besides the strong impact of AD on the patient and primary caregivers, there is an enormous burden on society and public health due to the high costs associated with care and treatment of dementia. Aside from drugs that temporarily relieve symptoms, no treatment exists for AD.

Although the vast majority of patients develop clinical symptoms at age older than 65 years (late-onset AD), 2–10% of patients have an earlier onset of disease (early-onset AD). Rare autosomal dominant forms of AD exist, predominantly presenting as early-onset AD, although the majority of early-onset AD patients do not present with a clear autosomal pattern of inheritance. Nevertheless, the genetic predisposition of the non-Mendelian form of AD is considerable, even for late-onset AD patients, with a heritability estimate of 60–80%.[2]

The search for the genetic factors contributing to AD has evolved tremendously throughout the years, from the discovery of fully penetrant mutations in *Amyloid precursor protein (APP), Presenilin 1 (PSEN1)*, and *Presenilin 2 (PSEN2)* as a cause of autosomal dominant AD, and the identification of the ε4 allele of *Apolipoprotein E* (*APOE*) as strong genetic risk factor for both early-onset and late-onset AD one-quarter century ago, to the more recent identification of at least 21 additional genetic risk loci for the genetically complex form of AD in genome-wide association studies (GWAS) and massive parallel resequencing (MPS) efforts. Whereas the mutations in *APP*, *PSEN1*, and *PSEN2* have been instrumental in the current understanding of the pathological process underlying AD, the findings from GWAS and MPS re-emphasize the multifactorial nature of AD. Nevertheless, translation of genetic findings into functional mechanisms that are biologically important in disease pathogenesis and treatment design remains a challenge. In this review, we position advances in AD genetics in light of the current endeavor toward translational research and personalized treatment. In the first part, we briefly discuss the current state of the art of AD genetics. In the second part, we review the potential and limitations of AD genetics in diagnosis and risk prediction, distinguishing between monogenic and multifactorial AD, and in molecular reclassification of AD to streamline clinical trials in drug development and biomarker studies.

AUTOSOMAL DOMINANT AD

With an estimated prevalence of <1%, autosomal dominant AD is very rare, but the discovery of pathogenic mutations in

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autosomal dominant AD pedigrees has brought about a breakthrough in the understanding of the pathogenesis of AD that reaches far beyond this small subgroup of AD. High-penetrant mutations in three genes were identified to cause autosomal dominant AD: *APP*,[3,4] *PSEN1*,[5–7] and *PSEN2*.[8] Together, mutations in *APP*, *PSEN1*, and *PSEN2* explain 5–10% of the occurrence of early-onset AD. The identification of mutations in these genes has not only provided important insights in the molecular mechanisms and pathways involved in AD pathogenesis but also led to valuable targets currently used in diagnosis and drug development.[9]

Amyloid precursor protein

APP is proteolytically processed by α-, β-, and γ-secretases following two pathways: the constitutive (nonamyloidogenic) or amyloidogenic pathway, leading to the production of different peptides. In the constitutive pathway, proteolysis of APP by α- and γ-secretases results in nonpathogenic fragments (sAPPα and α-C-terminal fragment). However, in the amyloidogenic pathway, enriched in neurons, the subsequent proteolysis of APP by β-secretase and γ-secretase gives rise to a mixture of Aβ peptides with different lengths. There are two major Aβ species: $Aβ_{1–40}$ (90%) and $Aβ_{1–42}$ (10%). The $Aβ_{1–42}$ fragments are more aggregation-prone and are predominantly present in amyloid plaques in brains of AD patients. Of note, N- and C-truncated Aβ peptides also exist. A total of 39 *APP* mutations in 93 families are described, all of which affect proteolysis of APP in favor of $Aβ_{1–42}$ (http://www.molgen.ua.ac.be/ADMutations/)[10] (**Supplementary Table S1** online), although some mutations also appear to affect N- or C-truncated peptides.[3,4] In addition, *APP* duplications have been identified in autosomal dominant early-onset families.[11,12] In the Icelandic population a rare protective variant in *APP* (p.A673T) has been observed, located near the β-proteolytic cleavage site and resulting in an impaired cleavage of APP, a reduction of $Aβ_{1–40}$ and $Aβ_{1–42}$ in vitro, and reduced AD risk.[13] Interestingly, another mutation at the same position has been described (p.A673V) as pathogenic in the homozygous state but protective in the heterozygous state, suggesting a mixture of wild-type and mutant APP affects the aggregation properties of Aβ peptides.[14]

Presenilin 1 and presenilin 2

PSEN1 and *PSEN2* are highly homologous genes. Mutations in *PSEN1* are the most frequent cause of autosomal dominant AD known to date, whereas *PSEN2* mutations are least frequent (**Supplementary Table S1** online).[5–8,15] Both proteins are essential components of the γ-secretase complex, which catalyzes the cleavage of membrane proteins, including APP. Mutations in *PSEN1* and *PSEN2* impair the γ-secretase mediated cleavage of APP in Aβ fragments, resulting in an increased ratio of $Aβ_{1–42}$ to $Aβ_{1–40}$, either through an increased $Aβ_{1–42}$ production or decreased $Aβ_{1–40}$ production, or a combination of both.[16]

PSEN1 mutations cause the most severe forms of AD with complete penetrance, and the onset of disease can occur as early as 25 years of age.[10] The *PSEN1* mutations have a wide variability of onset age (25–65 years), rate of progression, and disease severity. Missense mutations in the *PSEN2* gene may show incomplete penetrance.[8] In comparison to *PSEN1* mutations, *PSEN2* mutation carriers show an older age of onset of disease (39–83 years), but the onset age is highly variable among *PSEN2*-affected family members.[5,8,17]

LATE-ONSET AD AND GENETIC RISK

Late-onset AD is considered to be multifactorial; however, it involves a strong genetic predisposition.[2] The genetic component itself is complex and heterogeneous, because there is no single model that explains the mode of disease transmission and gene mutations or polymorphisms may interact with each other and with environmental factors. For many years, *APOE* was the only major gene known to increase disease risk.[18,19]

Apolipoprotein E

APOE encodes a polymorphic glycoprotein expressed in liver, brain, macrophages, and monocytes. ApoE participates in transport of cholesterol and other lipids and is involved in neuronal growth, repair response to tissue injury, nerve regeneration, immunoregulation, and activation of lipolytic enzymes. The *APOE* gene contains three major allelic variants at a single gene locus (ε2, ε3, and ε4), encoding for different isoforms (ApoE2, ApoE3, and ApoE4) that differ in two sites of the amino acid sequence.[19] The *APOE* ε4 allele increases risk in familial and sporadic early-onset and late-onset AD, but it is not sufficient to cause disease.[18–20] The risk effect is estimated to be threefold for heterozygous carriers (*APOE* ε34) and 15-fold for ε4 homozygous carriers (*APOE* ε44), and has a dose-dependent effect on onset age.[18,19] The *APOE* ε2 allele is thought to have a protective effect (OR = 0.6) and to delay onset age.[20,21] Only 20–25% of the general population carries one or more ε4 alleles, where 40–65% of AD patients are ε4 carriers. ApoE binds to Aβ and effectuates the clearance of soluble Aβ and Aβ aggregations, and ApoE ε4 is thought to be less efficient in mediating Aβ clearance.[22] The effect of *APOE* ε4 accounts for 27.3% of the estimated disease heritability of 80%.[23] The part of the heritability that was yet unaccounted for has been the driving force behind decades of continued search for genetic risk factors.

Genome-wide association studies

Large-scale collaborative GWAS and the International Genomics of Alzheimer's Project have significantly advanced the knowledge regarding the genetic underpinnings of late-onset AD by identifying at least 20 additional genetic risk loci (**Table 1**).[23–28]

None of these risk loci has an effect on AD risk of the magnitude of *APOE* ε4. Effect estimates range from an OR of 1.1–2.0 per risk allele. In contrast to *APOE* ε4, the population-attributable fraction of the individual genetic risk loci therefore remains limited, and their cumulative population-attributable fraction may not exceed the population-attributable fraction of *APOE* by much (**Table 1**).[29] The real merit of GWAS in AD genetics is

Table 1 Overview of AD susceptibility loci defined by GWAS and meta-analysis

Gene	Location	SNP	Risk allele frequency controls	OR (95% CI)	Population-attributable fraction (%)	Potential functional variant
APOE (apolipoprotein E)	19q13.32	ε4	0.16	3.78 (2.60–5.48)	30.8[a]	ε4
SORL1 (sortilin-related receptor-1)	11q24.1	rs11218343-T	0.96	1.30 (1.22–1.39)	0.91[b]	Common and rare pathogenic variants[34,35]
BIN1 (bridging integrator 1)	2q14.3	rs6733839-T	0.41	1.22 (1.18–1.25)	8.2[a]	rs59335482, 3 bp insertion[40]
CR1 (complement component (3b/4b) receptor 1)	1q32.2	rs6656401-A	0.20	1.18 (1.14–1.22)	3.5[a]	Intragenic CNV resulting in different CR1 isoforms[41]
CLU (clusterin)	8p21.1	rs9331896-T	0.62	1.16 (1.12–1.19)	5.1[b]	Rare coding and common regulatory variants[30,31]
PICALM (phosphatidylinositol-binding clathrin assembly protein)	11q14.2	rs10792832-G	0.64	1.15 (1.12–1.18)	4.5[b]	—
ABCA7 (ATP-binding cassette transporter A)	19p13.3	rs4147929-A	0.19	1.15 (1.11–1.19)	2.8[a]	Loss-of-function variants[37,38]
FERMT2 (fermitin family member 2)	14q22.1	rs17125944-C	0.09	1.14 (1.09–1.19)	1.2[a]	—
CASS4 (Cas scaffolding protein family member 4)	20q13.31	rs7274581-T	0.92	1.14 (1.09–1.19)	1.0[b]	—
MS4A6A locus (membrane-spanning 4-domains, subfamily A)	11q12.2	rs983392-A	0.60	1.11 (1.09–1.15)	3.8[b]	—
EPHA1 (EPH receptor A1)	7q35	rs11771145-G	0.66	1.11 (1.08–1.14)	3.3[b]	—
HLA-DRB5, HLA-DRB1 locus (major histocompatibility complex, class II, DR beta 5/beta 1)	6p21.32	rs9271192-C	0.28	1.11 (1.08–1.18)	3.0[a]	—
PTK2B (protein tyrosine kinase 2 beta)	8p21.2	rs28834970-C	0.37	1.10 (1.08–1.13)	3.6[a]	—
CD2AP (CD2-associated protein)	6p12.3	rs10948363-G	0.27	1.10 (1.07–1.13)	2.6[a]	—
ZCWPW1 locus (zinc finger, CW type with PWWP domain 1)	7q22.1	rs1476679-T	0.71	1.10 (1.06–1.12)	2.5[b]	—
SLC24A4/RIN3 locus (solute carrier family 24/Ras and Rab interactor 3)	14q32.12	rs10498633-G	0.78	1.10 (1.06–1.14)	1.9[b]	—
INPP5D (inositol polyphosphate-5-phosphatase)	2q37.1	rs35349669-T	0.49	1.08 (1.05–1.11)	3.8[a]	—
MEF2C (myocyte enhancer factor 2C)	5q14.3	rs190982-A	0.59	1.08 (1.05–1.11)	2.8[b]	—
NME8 locus (NME/NM23 family member 8)	7p14.1	rs2718058-A	0.63	1.08 (1.05–1.11)	2.5[b]	—
CELF1 locus (CUGBP, Elav-like family member 1)	11p11.2	rs10838725-C	0.32	1.08 (1.05–1.11)	2.5[a]	—
CD33 (CD33 molecule)	19q13.41	rs3865444-C	0.69	1.06 (1.04–1.1)	1.8[b]	rs12459419 located in a putative SRSF2 splice site of exon 2, leading to alternative splicing of the IgV domain[44]

The most significant SNP in each gene/locus identified by meta-analysis[23] (http://www.genome.gov) are described with MAF in the control population, OR presented with 95% CI, and potential functional variant. Order of significant SNPs is according to effect size. Population-attributable/preventive fractions and type are described.[29] The population-attributable fraction (PAF) types are indicated with "a" for risk PAF and "b" for preventive PAF.

CI, confidence interval; CNV, copy-number variation; MAF, minor allele frequency; OR, odds ratio; PAF, population-attributable fraction; SNP, single-nucleotide polymorphism.

probably that these studies have shed light on the pathophysiological pathways involved in AD (**Table 2**). Although numerous GWAS-identified risk genes could be linked with the Aβ cascade and/or tau pathology, it was striking to note that the associated genes roughly cluster within three pathways: cholesterol and lipid metabolism; immune system and inflammatory response; and endosomal vesicle cycling.

Of note, the actual risk variants represented by these GWAS associations remain largely unidentified. This has implications for the translation of these findings into mechanistic insight, but also for obtaining accurate epidemiological estimates like population-attributable fraction and personal-level risk prediction (discussed in the next section). Moreover, several susceptibility loci are localized in gene-dense regions (**Table 3**),

Table 2 Overview of the single-locus AD-susceptibility genes identified by GWAS and meta-analysis: function and characteristics

Gene	Pathway	Function	Effect on APP pathway	Effect on tau pathway
SORL1	Endosomal vesicle cycling	Vesicle trafficking	Aβ generation and clearance	—
BIN1	Endosomal vesicle cycling	Clathrin-mediated endocytosis	—	tau toxicity
CR1	Immune response	Regulation of complement activation	Aβ clearance	—
CLU	Cholesterol and lipid metabolism	Chaperone function; regulation of cell proliferation	Aβ aggregation and clearance	—
PICALM	Endosomal vesicle cycling	Trafficking of synaptic vesicle proteins	APP trafficking and Aβ clearance	Co-localization in NFTs
ABCA7	Lipid metabolism and immune response	Efflux of phospholipids and phagocytosis	Aβ clearance	—
FERMT2	Cytoskeletal function and axonal transport	Actin assembly and cell shape modulation	—	Tau toxicity
CASS4	Cytoskeletal function and axonal transport	Scaffolding protein of unknown function (in Drosophila ortholog binds to CD2AP ortholog)	—	—
EPHA1	Endosomal vesicle cycling and immune system	Brain development, modulating cell migration, axon guidance, and synapse development and plasticity	—	—
PTK2B	Cell migration and synaptic function	Ion signaling and induction of long-term potentiation in the hippocampal CA1 neurons	—	—
CD2AP	Endosomal vesicle cycling	Cytoskeletal reorganization and vesicle movement	Aβ clearance	Protection against tau toxicity
INPP5D	Immune response	Regulation of gene expression and posttranslational modification of proteins, microglial and myeloid function	—	—
MEF2C	Immune response, neural development, synaptic function	Synaptic plasticity	—	—
CD33	Immune system and inflammatory response	Cell-cell interactions and cell functions in the innate and adaptive immune systems	Aβ clearance	—

For each gene, the pathway, gene function, and effect on the APP or tau pathway are described.

APP, amyloid precursor protein; Aβ, amyloid β; NFT, neurofibrillary tangles.

Table 3 Overview of the multigene susceptibility loci identified by GWAS and meta-analysis: function and characteristics

Gene	Genes in locus	Possible candidate genes	Function	Pathway	Effect on APP or tau
MS4A4A/*MS4A6E* locus (chr11:59,268,00-60,480,00)	17 genes	*MS4A2, MS4A3, MS4A4A, MS4A4E, MS4A6A*, *MS4A6E*	Signal transduction	Immune response	—
HLA-DRB5/*HLA-DRB1* locus (chr6:3,609,009-4,535,100)	17 genes	Not defined due to the complex genetic organization of the locus	Immunocompetence and histocompatibility	Immune response	—
ZCWPW1 locus (chr7:99,905, 955-100,093,149)	10 genes	*ZCWPW1*; *NYAP1*: affecting brain size, neurite elongation, neuronal morphogenesis	Epistatic regulation (*ZCWPW1*); brain and neural development (*NYAP1*)	Neural development	—
SLC24A4/*RIN3* locus (chr14:92,789,411-93,176,224)	2 genes	*SLC24A4*: brain expression; *RIN3*: known interactor of *BIN1* gene product	Neural development and regulation of blood pressure and hypertension	Neural development and synapse function	—
NME8 locus (chr7:37,779, 803-37,992,860)	4 genes	*NME8*: association signal adjacent to the gene	Ciliary functions	Cytoskeletal function and axonal transport	—
CELF1 locus (chr11:47,291, 161-47,666,021)	10 genes	*CELF1; MADD*: long-term neuronal viability in AD	RNA splicing, editing, and translation (*CELF1*); long-term neuronal viability (*MADD*)	Cytoskeletal function and axonal transport	Tau toxicity

For each locus, the number of genes in each locus is shown with the possible candidate genes. The pathway, function, and effect on APP or tau pathway are reported for each locus.

APP, amyloid precursor protein; GWAS, genome-wide association studies.

and it remains to be determined which gene in these regions is responsible for the association.

The GWAS have sparked a wealth of genotype-phenotype correlation studies, most of which use GWAS proxy single-nucleotide polymorphisms rather than an underlying real risk variant. Efforts to identify the culprit variants at each locus are still limited, with most progress made in the genes identified in the first wave of sufficiently powerful GWAS.

Clusterin (CLU) was the first novel risk gene for AD identified simultaneously by two independent GWAS.[24,25] CLU is a pleiotropic chaperone molecule that might be involved in AD pathogenesis through lipid transport, inflammation, and directly by influencing Aβ aggregation and clearance from the brain by endocytosis. A GWAS-identified single-nucleotide polymorphism has been proposed as a functional variant directly affecting alternative splicing of CLU,[30] and targeted resequencing of *CLU* led to the detection of an increased frequency of rare coding *CLU* variations in patients, independent of the common association signal identified in GWAS, suggesting that different variants (rare coding and common regulatory) within a single locus can exist and have independent effects on the disease.[31]

Similar observations have been made more recently for *Sortilin-related receptor-1 (SORL1)* and *ATP-binding cassette subfamily A member 7 (ABCA7)*, although for both genes the rare variants seem to have a higher penetrance than the rare variants observed in *CLU*.

SORL1 was identified as a risk factor for late-onset AD through a candidate gene approach,[32] and findings were confirmed in the recent International Genomics of Alzheimer's Project GWAS meta-analysis.[23] Although the functional variants underlying these association signals remain unclear, exploring the effect of a common risk haplotype at *SORL1* in human induced pluripotent stem cells suggests that these genetic variants might act as effect modifiers of the induction of SORL1 expression and APP processing by BDNF.[33] However, whole-exome sequencing revealed several *SORL1* nonsense and missense mutations in patients with autosomal dominant early-onset AD[34] and targeted resequencing in late-onset AD patients revealed additional missense mutations in *SORL1*, including a common variant, which segregated with disease and affected APP processing in vitro,[35] indicating that the mutation spectrum of *SORL1* in AD pathogenesis contains both common and rare variants. *ABCA7* was first identified as a risk gene for AD in the GWAS setting[28] and is highly expressed in hippocampal neurons, one of the earliest affected brain regions of AD patients, and in microglia. It remains unclear whether *ABCA7* influences AD risk via interacting with *APOE* and lipid metabolism, functioning as an immune system molecule and clearing of Aβ aggregates from the brain or a combination of both. Several studies have reported an association between AD risk variants and ABCA7 expression in brain,[36] albeit with discrepant findings. More recently, an increased frequency of rare loss-of-function mutations in *ABCA7* has been described in AD patients,[37] which may present with an autosomal dominant pattern of inheritance.[38]

Bridging integrator 1 (BIN1) is involved in clathrin-mediated endocytosis, a process essential for the recycling of synaptic vesicles after each synaptic release.[24,26] BIN1 expression levels are increased in human brain and are associated with later disease onset and shorter disease duration in AD patients.[39,40] A functional follow-up study identified a 3 bp insertion upstream of the *BIN1* gene as a putative functional candidate, increasing transcriptional activity in vitro, BIN1 expression levels in human brain, and AD risk. Further, increased P-tau$_{181P}$ has been described in cerebrospinal fluid of AD patients, and the Drosophila ortholog *Amph* modifies tau neurotoxicity, hypothesizing that BIN1 mediates AD risk through Tau pathology.[40]

Complement component (3b/4b) receptor 1 (CR1) has multiple functions, including the regulation of complement activation and functions as a mediator of the innate immunity. CR1 has the capacity to bind complement components C3b and C4b and is expressed in many cell types in particular cells of the circulatory system.[41] An intragenic functional copy-number variation in *CR1* has been proposed as the variant underlying the GWAS association between *CR1* and AD risk. This copy-number variation translates into two major isoforms, CR1-F and CR1-S, resulting in a variable number of C3b/C4b and cofactor activity binding sites at the receptor, which are important in the complement cascade.[41,42] Although the exact mechanism of action remains unclear, first explorations in brain samples suggest that the CR1-S isoform is expressed at lower protein levels than CR1-F, and therefore is probably linked to increased complement activation. Both isoforms show a different pattern of CR1 distribution in neurons, which could indicate that CR1-S and CR1-F isoforms are differentially processed in neurons.[42]

CD33 is an immune cell surface receptor promoting cell-cell interactions and regulating cell functions in the innate and adaptive immune systems through clathrin-mediated endocytosis.[27,28] CD33 is expressed on myeloid cells and microglia, and expression is specifically increased in brain microglial cells, which correlated with amyloid plaque burden and advanced cognitive decline.[39] Microglial cells expressing CD33 show impaired Aβ phagocytosis and are correlated with amyloid plaque burden in AD brains.[43] A variant, putatively involved in alternative splicing, has been proposed as a functional variant.[44]

Massive parallel resequencing

Through advances in MPS technologies different research groups have successfully identified low frequency and rare variants. Multiple rare, missense variants in *Triggering Receptor Expressed On Myeloid Cells 2 (TREM2)* have been reported to increase risk for late-onset AD.[45,46] The most common variant in European populations R47H was reported to increase AD risk threefold. The observed effect size of R47H is comparable to the effect of the *APOE* ε4 allele; the frequency of the variant, however, is low (MAF ~0.3%), meaning the impact on population level of the *TREM2* variant is much lower. These findings were replicated in different populations of European origin, but not in Asian subjects, demonstrating that certain risk variants

may be population-specific or that the same gene can harbor different disease-associated variants.

A family-based study design led to the detection of rare variants within the Phospholipase D3 (*PLD3*) gene associated with AD risk. Whole-exome sequencing identified variant V232M, which segregates with disease status in late-onset AD families[47] and is associated with a two- to threefold increase in disease risk. However, several independent studies were unable to replicate this observation.[48] Numerous large-scale MPS studies on AD are ongoing and are anticipated to bring about the next wave of gene discovery for AD.

CLINICAL IMPLICATIONS AND RELEVANCE OF GENETIC FINDINGS

Genetics in diagnosis and risk prediction of autosomal dominant AD

Although our knowledge of the genetic causes and risk factors of AD is advancing, the question arises how to translate and implement these insights into improved public health. The most direct implementation, which is already available to patients and relatives today, is genetic diagnostic and predictive screening for causal mutations in *APP*, *PSEN1*, and *PSEN2*. These causal mutations, however, are only responsible for a small portion of AD patients. For a significant number of patients for whom genetic diagnostic screening is requested, the tests will therefore be negative without excluding a genetic cause of disease. For instance, in our Diagnostic Service Facility (http://www.molgen.ua.ac.be/DNADiagnostics/), pathogenic mutations are identified in only 4, 1.4, and 1% of patients referred for diagnostic screening for *PSEN1*, *APP*, and *PSEN2*, respectively, despite prior evidence for a monogenic cause of AD. Nevertheless, the ability to identify an explanation for the clustering of AD in a family and the ability to use this toward predictive testing in subsequent generations provide an important step toward autonomy of patients and at-risk individuals. Comprehensive genetic counseling protocols are available for AD diagnostic and predictive testing to provide a framework for clinicians and geneticists to evaluate which patients may benefit from genetic testing.[49] Important ethical considerations should be taken into account, including the disclosure of AD diagnosis and genetic test results, the social stigma, employment, and family planning.[50] Moreover, the identification of a mutation is not a certain predictor of disease or onset age, given that these mutations can vary in terms of penetrance and gene expression.[10,51] Several studies have attempted to discover onset age modifiers. A dose-dependent effect of *APOE* ε4 on onset age has been described, showing a lower mean onset age in AD patients for each additional copy of the ε4 risk allele. The mean onset age decreased from 84 to 68 years.[19] The effect of *APOE* ε4 on onset age of autosomal dominant AD was confirmed in large pedigrees.[52] A review of demographic data on 387 autosomal dominant AD families, however, suggests that factors like parental onset age, mean onset age of family members, and mean onset age of carriers with the same mutation may be stronger predictors of onset age in a mutation carrier than *APOE* ε4.[51] In a Columbian kindred carrying the *PSEN1* p.E280A mutation, a protective haplotype was recently identified through whole genome sequencing. This haplotype, associated with a 10-year delay in onset age, harbors a missense mutation in the *CCL11* gene encoding eotaxin-1.[53] Evidence exists for additional onset age modifiers detected through linkage analyses; however, the specific genes driving these linkage signals remain unknown.[54] Gene–environment interactions and epigenetic changes can also result in significantly different disease outcomes.[55]

With the advent of high-capacity MPS, genetic diagnostic testing is entering a new era. Multiple genes can now be screened simultaneously using disease-oriented or disease spectrum–oriented gene panels, obviating the need of decision trees based on clinical parameters. For AD, a disease-spectrum approach may be particularly relevant. For example, the mutation p.R406W in *MAPT*, a known causal gene for FTLD, has repeatedly been reported in pedigrees with a clinical presentation of AD.[56] Mutations in two other FTLD genes, *GRN* and *C9orf72*, have also been described in clinical AD cohorts.[57,58] It may be important to include screening of these genes in the genetic diagnostic work-up of high genetic load AD patients, particularly in light of the fact that *APP*, *PSEN1*, and *PSEN2* account for only a small proportion of autosomal dominant AD.

These advancing techniques could represent fast and cost-effective tools in a clinical setting, but with the incorporation of new technologies, both a greater complexity in the interpretation of genetic results and additional ethical issues arise. For instance, prior to the availability of MPS, mutation screening of *APP* focused only on the exons encoding the Aβ peptide and its cleavage sites. Now that screening of the full locus has become highly feasible, this may reveal variants of unknown significance. This complicates the interpretation and impact of the information on patients and society. An additional challenge will be the prospect of secondary or incidental findings, particularly when whole exome or genome sequencing is performed rather than gene panel-based sequencing. These findings might be relevant for patient management but not related to the phenotype. Incidental findings have great implications concerning the return of genomic information to patients or research participants. This necessitates extensive guidelines and genetic counseling.[59] A recent study investigated the opinion and expectations of stakeholders concerning an opportunistic screening of data sets by researchers and the return of incidental but health-related findings to the patient. The results indicated that 88% of the stakeholders thought that incidental findings should be made available to research participants. Despite the interest in access to data results, 69% of the responders did not expect researchers to actively search for incidental findings not relevant to their research in exome or genome data sets.[60]

Genetic risk prediction in complex AD

The role of genetics in diagnosis and risk prediction in late-onset complex AD is much less straightforward. Despite the

established evidence of *APOE* ε4 as a risk factor for AD, its value in disease prediction in a clinical setting is limited, not only due to the restriction of current therapeutic consequences but also because *APOE* ε4 is neither necessary nor sufficient to cause the disease. Up to 75% of individuals heterogeneous for *APOE* ε4 do not develop AD during life, and up to 50% of people with AD do not carry the high-risk ε4 allele.[20]

The relevance of clinical testing for common genetic variations identified in GWAS is even more limited, because these variations confer smaller relative risks and collectively explain only a small proportion of the underlying genetic contribution. Moreover, genetic testing would only provide an assumption of an individual's risk of developing AD since these variants represent indirect markers of association and not the true disease-related functional variants. Combining multiple susceptibility loci into a global genetic risk score (GRS) might improve the prediction of individuals at risk. For example, an 8-single-nucleotide polymorphism GRS was associated with an accelerated progression from mild cognitive impairment to AD.[61] Another study reported that a GRS including nine non-*APOE* alleles in a model allowing for interaction between loci significantly improved the ability to predict AD compared to *APOE* alone; however, the improvement did not reach the sensitivity or specificity necessary for clinical diagnostic use.[29] We investigated a GRS combining 22 AD susceptibility loci. The best model was a weighted GRS that took into account the different risk effects of *APOE* in different age groups.[62,63] This model performed significantly better than *APOE* alone in discriminating AD patients from healthy elderly. Despite the fact that this model incorporated all reliable GWAS association signals reported thus far, as well as the established rare risk variant in *TREM2*, the model only achieved a sensitivity of 55% and a specificity of 78%, impeding use in clinical practice.[63] More gain in predictive ability is to be expected from inclusion of true functional variants rather than GWAS signals, from incorporation of epistatic effects,[29] or combination with nongenetic biomarkers.

Impact of genetic testing on individuals

Genetic and susceptibility testing brings concerns regarding the impact of test results on individuals. Both survey data and clinical research have shown that the majority of individuals at risk for AD are interested in knowing their genetic profile.[64] Furthermore, since the emergence of personal genomics companies offering direct-to-consumer testing for various neurodegenerative diseases, it is of great importance to examine the possible implications of these test results. Direct-to-consumer companies do not provide genetic counseling or exclude psychologically vulnerable consumers, which increases the potential for inadequately understanding the meaning or implications of test results.[55]

A series of clinical studies, part of the REVEAL trial, has been conducted to investigate the psychological and behavioral impact of genetic screening results. In the REVEAL study, risk estimates for AD were established based on age, sex, family history, and *APOE* genotype. No evidence of fatalism was observed among participants receiving *APOE* ε4–positive results; however, modest evidence of false reassurance was detected among participants receiving *APOE* ε4–negative results. Further, *APOE* testing in at-risk individuals with a positive family history does not pose significant psychological risks like depression or anxiety when they are provided by proper pre- and posttest counseling. A multisite clinical trial evaluated the impact of susceptibility testing with *APOE* and further assessed distress of deterministic genetic testing by disclosing *PSEN1* or *PSEN2* information to individuals at risk for AD. This study suggested that the test-related distress experienced by those receiving positive results for a deterministic mutation is similar to the distress experienced by those receiving positive results from genetic susceptibility. The most common behavioral change reported in the REVEAL study was the use of nutritional supplements, suggesting that genetic susceptibility testing may enhance the preference for biological interventions like medication over health behavior changes like a lifestyle change.[55]

Drug development and clinical trials

Development of disease-modifying and symptomatic therapeutics for AD to date has mostly focused on early insights on the molecular mechanisms and pathways involved in AD and specifically followed three AD hypotheses: the cholinergic, amyloid cascade, and tau hypotheses.[9] Therapeutics based on the enhancement of the cholinergic system show consistent, but modest, clinical effects in late-phase trials.[9] A substantial portion of the field focused its efforts on the amyloid cascade hypothesis, highlighted by the identification of pathogenic mutations in *APP*, *PSEN1*, and *PSEN2*. This approach led to human clinical trials potentially decreasing the production or aggregation of Aβ or enhancing Aβ clearance from the brain. Recent passive and active anti-tau immunization studies in mouse models have been proven effective at preventing and improving tau pathology.[65] The progression of neurofibrillary tangles pathology throughout the brain correlates strongly with synaptic and neuronal loss and cognitive decline, and makes it a potential therapeutic target to interrupt progression of tau pathology early in disease. The spread of tau pathology and neuronal tau release is thought to be a regulated process through active secretion and interneuronal transfer of tau, which could facilitate transneuronal spread of tangle pathology.[66]

Despite considerable advances in the knowledge of AD pathogenesis, the AD field has struggled to move druggable targets from preclinical research into effective therapies. Although there may be numerous pharmacological reasons for this, it might be partly explained by the study design and patient recruitment in phase III clinical trials. Evidence accumulates that AD pathology is progressing silently for decades before the appearance of clinical symptoms, and neuronal loss is already present at the stage of mild cognitive impairment.[67] Treating symptomatic patients with full-blown disease may not be effective, and therapeutics applied

earlier in the course of AD would be more likely to achieve effective disease modification. This concept has been supported by several preclinical research successes in AD mouse models.[68] Although the prospect for preventing neurodegenerative AD processes seems challenging at the moment, this approach is well-accepted and successful in other medical fields, like cardiovascular disease.[69]

Moreover, the majority of these trials have enrolled patients with a clinical diagnosis of late-onset AD. The success rate of therapeutic and biomarker trials might considerably be improved if trial design would foresee preselection of study participants, representing an etiologically more homogeneous group that has the highest chance of benefiting from specific treatments and fewer chance of showing adverse effects.

Genetic testing can play an important role in this endeavor. To investigate early disease changes, patients cohorts such as the Dominantly Inherited Alzheimer's Network can be specifically selected for mutation carriers of *APP*, *PSEN1*, and *PSEN2*.[70] Furthermore, clinically normal individuals thought to be on the trajectory toward the symptomatic stages of AD can be detected through advances in genetic testing for *APOE* or more comprehensive genetic risk scores alongside the more generally accepted biomarkers like amyloid and tau in cerebrospinal fluid and PET neuroimaging.[67]

Several clinical trials have been initiated that focus on presymptomatic or early symptomatic, more homogenous study cohorts on the basis of their genetic profile. For instance, a phase II/III clinical trial of two drugs is ongoing for preclinical carriers of mutations in *APP*, *PSEN1*, and *PSEN2* in the context of the Alzheimer Prevention Initiative (ClinicalTrials.gov). The TOMMORROW trial is a phase III trial examining the effect and ability of pioglitazone, a peroxisome proliferator–activated receptor γ agonist, to delay the onset of mild cognitive impairment of AD in individuals at high risk. Risk prediction is based on an algorithm including *APOE* and *TOMM40* genotypes. A poly-T repeat in *TOMM40* has been described to increase risk of AD.[71] However, *TOMM40* is in very strong linkage disequilibrium with *APOE*; therefore, the independent role of *TOMM40* as a risk gene is still under discussion. This trial may give further insight regarding the role of *TOMM40* in AD risk.[72] A phase II trial of the Aβ-specific antibody bapineuzumab stratified individuals based on *APOE* ε4 status. Analysis suggested that *APOE* ε4–negative subjects had a better response compared with placebo than the *APOE* ε4–positive study participants, who showed worse Aβ pathology and an earlier onset of symptoms.[73]

Although no clinical trials are yet incorporating full genetic risk profiles into their design, genetic risk profiles are a promising tool in genetic classification. A high GRS was found predictive of positive family history, younger disease onset, and lower cerebrospinal fluid $A\beta_{1-42}$ levels,[63] implying that a genetic preselection may be a fast and cost-efficient way to identify individuals at increased risk for AD for clinical or biomarker trials, prior to more cost-intensive and labor-intensive methods like amyloid imaging. In addition, molecular subclassification will be possible by determining pathways enriched for risk alleles within an individual, at least to the extent to which associated single-nucleotide polymorphisms can currently be assigned to single genes within a locus. This will be particularly relevant when testing therapeutics targeting a specific pathway.[63,74]

More complex risk profiling by integrating additional nongenetic information concerning AD endophenotypes, such as cerebrospinal fluid biomarkers and neurofibrillary pathology, into a genetic risk profile may aid in the selection of individuals at high risk and the prediction of future cognitive decline.[75] As with personal genetic and susceptibility testing, the identification of high risk individuals for research purposes has important ethical implications, which requires careful attention.

CONCLUSION

Shifting research toward genetic molecular profiling using integrated -omics approaches has led to considerable progress in complex diseases such as those addressed by the cancer research field. Advances in genetics and clinically relevant NGS applications including whole-genome sequencing, whole-exome sequencing, transcriptome profiling, as well as epigenomic and proteomic characterization have paved the way to molecular profiling of cancer subtypes and provide important instructions for other complex diseases like AD. Although there is still a long way to go in the AD field before precision medicine is achieved, there is reason for cautious optimism with the continued elucidation of novel genes involved in AD and the impact that genetic profiling can have can shift toward prediction and prevention.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/gim

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DISCLOSURE

The authors declare no conflict of interest.

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