

## Genetic aspects of Alzheimer's disease

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Review

**Abstract.** Alzheimer's disease (AD) is a neurodegenerative disorder with a complex etiology and pathogenesis. Mutations in presenilin 1 gene (*PSEN1*), located on chromosome 14, more rarely in amyloid- $\beta$  protein precursor (*APP*) on chromosome 21, and presenilin 2 genes (*PSEN2*) on chromosome 1, underlie the pathogenesis of most cases of familial early onset of AD (EOAD). The genetics of late-onset AD (LOAD) have been more enigmatic and the only confirmed risk factor for LOAD remains the apolipoprotein E4 allele (*ApoE4*) on chromosome 19. In this review, we discuss the genetics of AD with a focus on the role of the APP and presenilins.

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## INTRODUCTION

Alzheimer's disease (AD) is a clinically and neuropathologically heterogeneous disorder. The clinical diagnosis can at best be "probable" AD as there is no specific diagnostic test for the disease. At autopsy, however, the diagnosis may be confirmed by a histopathological examination of the brain. The characteristic neuropathological findings for "definitive" AD are: neuronal loss leading to cerebral atrophy, extracellular amyloid deposits (amyloid plaques, senile plaques, SPs), and intracellular neurofibrillary tangles (NFTs). Amyloid plaques mainly consist of amyloid  $\beta$ -peptides ( $A\beta$ ), primarily a 40-residue  $A\beta_{40}$  form and a 42-residue  $A\beta_{42}$  form.  $A\beta$  peptides are proteolytic cleavage products of the amyloid- $\beta$  protein precursor (APP). APP is a class I transmembrane glycoprotein, whose biological functions remain largely unknown. NFTs, are intraneuronal deposits of paired helical filaments, mainly composed of hyperphosphorylated tau protein and frequently associated with ubiquitin (Gomez-Ramos et al. 2004). Tau protein is normally present in the adult brain, where it serves as a microtubule stabilizing protein, maintaining neuronal cell structure and axonal transportation.

Neither SPs nor NFTs are absolute hallmarks of AD since cognitively-intact aged individuals may show both SPs and NFTs upon post-mortem brain examination (Jellinger and Bancher 1998, Nussbaum and Ellis 2003).

The exact relationship between APP/ $A\beta$  and abnormally sequestered tau protein in AD pathogenesis is unclear (Ling et al. 2003). The amyloid hypothesis suggests that the accumulation of  $A\beta$  in specific brain regions (hippocampus, and cerebral cortex) is the primary pathogenic process which triggers a cascade of various physiological events such as microglial and astrocytic activation, oxidative damage, formation of tau pathology, synaptic loss and progressive cognitive decline (Brzyska and Elbaum 2003, Mattson 2002). Studies supporting the hypothesis indicate that amyloid fibrils are toxic to neurons *in vitro* (Hartley et al. 1999). Another study reported that soluble oligomers of  $A\beta$  peptides (dimers and trimers) could be the primary neurotoxic factors, causing damage to synapses (Ellis and Pinheiro 2002, Haass and Steiner 2001). Further, it has been suggested that long-term potentiation (LTP), the key element in memory and learning, is particularly sensitive to  $A\beta$  oligomers and that  $A\beta$  can induce demyelination and oligodendrocyte injury *in vivo* (Roher et al. 2002, Walsh et al. 2002).

However, the amyloid cascade hypothesis is still controversial. Recent studies on transgenic mice suggest that synaptic dysfunction, including LTP, precedes the formation of plaques and tangles indicating that the histopathological lesions of AD develop after irreversible neuronal damage has occurred (Oddo et al. 2003). Synaptic dysfunction may thus be more significant than either tangles or plaques in the early stages of cognitive decline. Also, there is a weak correlation between the distribution of  $A\beta$  in the brain and the degree of dementia (Thal et al. 2002). The progressive distribution of tangles, starting in the entorhinal cortex, *via* the hippocampus, to the cortical association regions, however, correlates better with the early memory dysfunction and progressive loss of higher cortical functions seen in AD patients (Braak and Braak 1991).

NFTs are also found in familial form of frontotemporal dementia, caused by mutations in the tau protein gene (*MAPT*). These mutations lead to abnormal hyperphosphorylation of tau similar to the findings in AD, suggesting that NFTs or hyperphosphorylated soluble tau have a neurotoxic potential. Thus, there is strong genetic evidence that tau protein dysfunction is sufficient to cause dementia. Some experimental data suggests that hyperphosphorylated tau protein exerts a cytotoxic effect in cell cultures, which is associated with the induction of apoptosis and sensitization to apoptotic signals (Fath et al. 2002). In addition, there are several other clinical entities besides AD that have intraneuronal accumulations of hyperphosphorylated tau: e.g., frontotemporal lobe dementia, Pick's disease, and progressive supranuclear palsy. However, there is no data showing that genetic variations in *MAPT* predispose for AD. Possibly, abnormal tau phosphorylation is an effect of pathological APP processing, thus participating in the destructive events leading to AD.

The major risk factor for AD is age, and AD is usually classified according to its age of onset. The majority (>95%) of patients have an age of onset higher than 65 years of age (late-onset AD, LOAD), whereas 1-5% have an earlier onset (early-onset AD, EOAD). LOAD and EOAD are clinically indistinguishable, however EOAD is generally more severe with a more rapid rate of progression, as clinical practice indicate (Rademakers et al. 2003).

A second important risk factor for AD is a positive family history. Both twin and family studies suggest an important heritable constituent of AD. In cross-sectional, epidemiological studies the presence of a positive family history of LOAD is a risk factor for

late-onset AD. The proportion of LOAD being attributable to genetic factors ranges between 37% and 83% (Warwick Daw et al. 2000), however, in the vast majority of LOAD cases there is no clear pattern of Mendelian inheritance.

The search for genes involved in LOAD has proved to be a difficult task. AD is a common disease, so it is possible that a familial clustering could be purely incidental and also that multiple risk factors (genetic, and non-genetic) could coexist. Additionally, due to late age of onset, a subgroup of potential AD patients die before the first symptoms are recognized. This effect could hamper the identification of families with a true genetic component of AD.

Only a part of EOAD cases with a family history of the disease (approximately 10% of total EOAD) appear to be inherited as an autosomal dominant trait (familial AD, FAD), which is equivalent to less than 1% of all AD cases. Therefore, most AD cases (sporadic i.e., with no family history, as well as familial) have a more complex etiology (Breteler et al. 1992).

### CAUSATIVE GENES OF FAMILIAL, AUTOSOMAL-DOMINANT AD FORM

In 30-50% of all autosomal-dominant FAD cases single mutations in one of three causative genes have been identified to date (Van Gassen and Van Broeckhoven 2000). These genes code for APP (*APP*), presenilin 1 (*PSEN1*), and presenilin 2 (*PSEN2*) (Campion et al. 1999). However, the reported contribution of these mutations to EOAD varies from 1-50%, depending on the diagnostic criteria used to define autosomal dominant inheritance, and the age limit used to define EOAD (Rogaeva 2002). In the Polish population, we detected mutations in all three causative genes, and the frequency of mutated alleles was 17% (Żekanowski et al. 2003). The prevalence of the mutations was almost identical to those reported in other screening programs which only included patients that had at least one relative with EOAD (Croes et al. 2000, Sleegers and Van Duijn 2001).

### AMYLOID PRECURSOR PROTEIN (APP) GENE

The *APP* gene is located on chromosome 21p21, contains 18 exons, and as a result of alternative splicing of exons 7, 8, and 15 codes for at least eight APP isoforms. The APP695 isoform lacks exon 15 and is expressed

predominantly in neurons, whereas APP751 and APP770 (the longest isoform) are expressed ubiquitously. At least five less abundant isoforms are generated by alternative splicing of exons 7, 8, and 15.

APP is an N- and O-glycosylated type I integral membrane protein, with a long N-terminal, extracellular domain, and a short cytoplasmic C-terminal tail. APP undergoes a series of endoproteolytic cleavages during processing (Ling et al. 2003). One pathway involves  $\alpha$ -secretase, a membrane-associated protein, which cleaves APP between residues Lys<sub>687</sub> and Leu<sub>688</sub>, in the middle of the A $\beta$  domain, and releases extracellular N-terminal fragment of APP (soluble APP, sAPP). This cleavage is non-amyloidogenic, since it prevents formation of A $\beta$ . The second, less frequent pathway, involves  $\beta$ -secretase, which cleaves between residues Met<sub>671</sub> and Asp<sub>672</sub>, leading to formation of the N-terminal end of A $\beta$ . The third proteolytic event involves  $\gamma$ -secretase cleaving APP in the transmembrane domain, after Ile<sub>712</sub>, Thr<sub>714</sub>, or Val<sub>715</sub>, which, in combination with  $\beta$ -secretase cleavage, generates extracellular A $\beta$ <sub>1-40</sub>, A $\beta$ <sub>1-42</sub>, or A $\beta$ <sub>1-43</sub>. The short version of A $\beta$  is the most common form of the peptide (90%) and is less amyloidogenic when compared to the longer forms A $\beta$ <sub>1-42</sub> or A $\beta$ <sub>1-43</sub>, which are less soluble and more neurotoxic and tend to aggregate rapidly to form amyloid deposits (Trzeźniewska et al. 2004). Very recently, an additional cleavage site in the APP transmembrane domain has been discovered. This cleavage has been named  $\epsilon$ -cleavage and occurs mainly after position 49 in A $\beta$  (Yu et al. 2001). The C-terminal fragment formed by  $\epsilon$  cleavage is called APP intracellular domain (AICD). AICD has been proposed to regulate gene transcription, and it can be hypothesized that altered AICD production and nuclear signaling is also disturbed in AD. In most cells, the  $\alpha$ -secretase pathway is the main APP processing pathway. However, in neurons amyloidogenic processing releasing predominantly A $\beta$ <sub>40</sub> is the main route.

APP processing takes place in different cell compartments. The  $\alpha$ -secretase pathway occurs in the cell membrane and the  $\beta$ -secretase cleavage takes place in endosomal-lysosomal compartments. The  $\gamma$ -secretase cleavage, generating A $\beta$ <sub>42(43)</sub>, occurs mainly (but not exclusively) in endoplasmic reticulum, and requires APP endocytosis from the cell surface in clathrin-coated vesicles (Marquez-Sterling et al. 1997). Recent studies indicate that APP trafficking is regulated by PS1, and that this process could be impaired in familial AD caused by *PSEN1* mutations. This could add another level of com-

plexity, to the existing hypotheses concerning the effects of *PSEN1* mutations (Cai et al. 2003).

Cholesterol is another important factor, modulating production of A $\beta$  (Gibson Wood et al. 2003, Wolozin 2001). Sites of  $\gamma$ -secretase activity and A $\beta$  production are colocalized with cholesterol-rich membrane structures (lipid rafts). In contrast,  $\alpha$ -secretase activity is associated with regions with low cholesterol content (Kojro et al. 2001). Changes in cholesterol metabolism have been suggested to be confined only to specific regions of brain or membranes since the total amount of brain cholesterol is relatively constant (Ehehalt et al. 2003, Kirsch et al. 2003). It was hypothesized that redistribution of cholesterol from the cytoplasmic leaflet of the membrane to the exofacial one promotes A $\beta$  production (Gibson Wood et al. 2003). However, some observations indicate that the  $\gamma$ -cleavage required for generating A $\beta$  occurs in rafts, and that its activity is virtually cholesterol-independent (Wada et al. 2003).

On the other hand, A $\beta$  influences membrane fluidity. However, there are conflicting results as to whether this molecule increases or decreases fluidity. Additionally, A $\beta$  modulates many different neuronal functions associated with membrane structure like ion flux, signal transduction, or calcium homeostasis, all of which could be consequences of disruption of the membrane structural properties. A $\beta$  also influences cholesterol homeostasis (e.g., estrification, transport, uptake, and release). All those factors are interconnected and could play a role in the promotion and development of LOAD.

There are 16 different pathogenic mutations and 4 nonpathogenic polymorphisms in the *APP* gene (see: AD Mutation Database, <http://molgen-www.uia.ac.be/ADMutations>). *APP* mutations account for 4-6% of all FAD cases. Families carrying mutations in *APP* have ages of onset generally within the range of 40-65 years. The pathogenic effect of the mutations is not entirely understood, however, all missense mutations causing EOAD are clustered in exons 16 and 17 in the proximity to one of the three cleavage sites. The mutations change the normal APP cleavage pathway in different ways. For instance, mutations in codons 714-717 increase the production of A $\beta_{42}$  which form amyloid deposits more easily than A $\beta_{40}$  (Eckman et al. 1997, Suzuki et al. 1994). The double Swedish K670N/M671L mutation influences  $\beta$ -secretase cleavage, and elevates levels of both A $\beta_{40}$  and A $\beta_{42}$  (Mullan et al. 1992). Some other mutations (e.g., L723P) can affect  $\gamma$ -secretase cleavage by al-

tering the structure of transmembrane architecture of APP. In contrast to *PSEN1* mutations, there is no data showing that APP mutations can lead to changes in AICD formation (Bergman et al. 2003).

Seven missense substitutions are associated with a non-AD phenotype. These mutations are generally located near the  $\alpha$ -secretase cleavage site, changing the A $\beta$  peptide sequence. These mutations are not associated with a classical AD phenotype. For example, the Flemish mutation (A692G) reduces  $\alpha$ -secretase cleavage, and increases the heterogeneity of secreted A $\beta$  species (Haass et al. 1994, Roks et al. 2000). Mutations V715M, E693G reduce total A $\beta$  production, but at the same time increase the A $\beta_{42}$ /A $\beta_{40}$  ratio (Ancolio et al. 1999, Nilsberth et al. 2001). The E693Q mutation is associated with hereditary recurrent cerebral hemorrhages with amyloidosis of the Dutch type. A692G and D694N were identified in patients with cerebral hemorrhages with congophilic amyloid angiopathy but were also identified in EOAD patients from affected families (Grabowski et al. 2002, Roks et al. 2000).

The presented facts suggest that the relative proportions of different A $\beta$  species can be more important in AD etiology than the absolute levels of A $\beta_{42}$  or total A $\beta$ . Also *in vitro* studies reveal a phenotypic heterogeneity of APP mutations at the cellular level, which may explain the observed clinical heterogeneity.

All enzymes involved in APP processing could potentially be regarded as possible targets of AD causing mutations. Recently, the  $\beta$ -secretase gene (*BACE1*) was cloned. Genetic analysis suggests, however, that *BACE1* is not associated with AD, neither causally, nor as a risk factor. Several proteins with  $\alpha$ -secretase activity have been identified, including disintegrin and metalloproteinases belonging to adamalysin family (e.g., ADAM9, ADAM10, and ADAM17) (Allinson et al. 2003). Additionally, mutations in genes leading to disturbances of various processes involved in clearance and degradation of neurotoxic A $\beta$  are also potential biological candidate genes for AD.

## PRESENILIN 1 AND PRESENILIN 2 GENES

The precise biochemical nature of  $\gamma$ -secretase remains unresolved. However, a growing set of evidence suggests that PS1 and, to some extent, PS2 are active sites of the  $\gamma$ -secretase complex (Farmery et al. 2003, Tandon and Fraser 2002). Mutations in the PS genes di-

rectly influence APP processing, causing an increased production of A $\beta$ <sub>42</sub>.

The *PSEN1* gene is located on chromosome 14q23.3 and consists of 13 exons with three alternatively spliced variants. Exons 3 to 12 code for a 467 residue protein. The *PSEN2* gene, located on chromosome 1q31-42 was identified based on its extensive homology to *PSEN1* and codes for a 448 residue protein. Both presenilins (PSs) share 80.5% homology, highest in the eight transmembrane (TM) domains. The hydrophobic amino acid sequences in the TM domains are also highly evolutionary conserved among various presenilin homologs. The N-terminal cytoplasmic loop is the region of highest variability and harbours the most pronounced differences between PSs. Both presenilins are translated as an unstable holoprotein, which undergoes autocatalytic endoproteolysis, forming a stable and biologically active heterodimer comprising an N- and a C-terminal fragment. Both fragments are incorporated into a high molecular weight oligomer containing three other proteins: nicastrin, APH-1 and PEN-2 (Kimberly and Wolfe 2003). Other proteins like caveolins, may play a role in switching between alternative APP cleavage pathways, however, none of the PS-interacting proteins have been shown, to date, to play a direct role in the enhanced production of A $\beta$ <sub>42</sub> mediated by mutant PS1.

Presenilins are involved in a range of biological processes, such as NOTCH, WNT and G-protein mediated signaling, Fas-induced apoptosis, cell adhesion, and protein trafficking (Fortini 2002). It can be hypothesized that transcription factors released after presenilin-mediated cleavage of signaling proteins could affect some unknown mechanisms of AD pathology. For example, AICD generation is also presenilin-dependent and the *PSEN1* mutation (L166P) not only results in increased levels of A $\beta$ <sub>42</sub>, but also decreased formation of AICD (Moehlmann et al. 2002). On the other hand, A $\beta$  peptides destabilize endogenous levels of  $\beta$ -catenin and induce a loss of function of the Wnt signalling pathway in rats (De Ferrari et al. 2003).

The high structural similarity between PS1 and PS2 suggests that their physiological functions overlap or may, at least partly, be redundant. PS1 and PS2 knockout mice have reduced A $\beta$  amounts in neuronal tissue. Double knockout mutants have no  $\gamma$ -secretase activity. However, PS1-knockout mice display embryonic/neonatal-lethal phenotype, while PS2-knockouts are viable (Herreman et al. 1999). Also, PS transcription and PS posttranslational modification patterns during develop-

ment are different, indicating separate roles at least in embryogenesis (Hong et al. 1999). Functional and clinical analyses of identified human mutations also indicate differences between the presenilins.

Examination of membranes from brain cortex of AD patients, and from cases with *PSEN1* missense mutations, does not indicate any change in  $\gamma$ -secretase complex mobility. However, higher molecular mass PS1-reactive species were detected in brains containing the *PSEN1* exon 9 deletion mutation. This observation was confirmed in cells transfected with the same presenilin deletion mutation.

Mutations in *PSEN1* are the most frequent cause of familial, autosomal-dominant AD, accounting for 18-50% of EOAD in various populations. To date more than 100 mutations have been identified in *PSEN1*, almost all being missense mutations (see: AD Mutation Database, <http://molgen-www.uia.ac.be/ADMutations>). Mutations in *PSEN2* are substantially less frequent: only nine missense mutations have been identified. The majority of mutations are located in or near the transmembrane domains. It has been proposed that they may influence the architecture of the whole protein. A few *PSEN1* mutations located outside those regions, in the cytoplasmic hydrophilic loop, have been reported and are associated with later age of onset. This class of mutations may affect the amino acid residues involved in the oligomerization of presenilin or interactions with other proteins.

Most *PSEN1* mutations are fully penetrant. Two silent missense substitution has been reported (F175S and E318G). *PSEN1* mutations are scattered over all exons and conserved flanking intronic sequences (except for exon 3). Mutations in *PSEN2* are variably or partially penetrant, as the age of onset varies from 40-90 years of age, and the course of the disease is generally less severe (Binetti et al. 2003, Ezquerra et al. 2003). This could be explained by the lower brain expression of *PSEN2*, compared to *PSEN1*. In contrast to *PSEN1*, silent polymorphic substitutions are relatively frequent in the coding region of *PSEN2*.

Almost all mutations in the presenilins characterized so far increase A $\beta$  levels *in vivo* and *in vitro*, and this fits with the amyloid cascade hypothesis. However, the molecular mechanism by which mutated presenilins exert their pathogenic effects is complex. Recently, a *PSEN1* mutation, located in the large hydrophilic loop associated with a frontotemporal dementia-like phenotype, was identified (Amtul et al. 2002). This mutation inhib-

its  $\gamma$ -secretase cleavage of APP and NOTCH. Another example of the complex genotype-phenotype correlation is the *PSEN1* mutation associated with the familial form of spastic paraparesis (SpPa) with AD signs (Crook et al. 1998). The clinical and histopathological presentation of SpPa is different from AD showing deposits of A $\beta$  fibrils in the form of large diffuse, cotton wool plaques (CWP). These plaques do not show amyloid fibril deposition in the core and are not associated with surrounding dystrophic neurites and inflammatory reactions. These signs are present in some LOAD patients, suggesting that both neurodegenerative conditions could be caused by the same main mutation in *PSEN1* and additional, unidentified genetic modifiers (Le et al. 2001). In the case of SpPa/AD and CWP phenotypes, such modifiers could influence the kinetics of A $\beta$  polymerization, which is a rather slow process, needing some kind of crystallization center (Jarret and Lansbury Jr. 1993). Another explanation is that A $\beta$  deposition is not the key element in the pathogenesis of SpPa/AD, but rather that the neurotoxic events occur before the formation of amyloid deposits. Possible mechanisms could involve disturbances in signaling pathways, calcium homeostasis, or increased production of free radicals. There is also some evidence of possible modifications, concerning the interaction of PS1 with other proteins (Chen and Schubert 2002).

## SUSCEPTIBILITY GENES

In most families EOAD coexists with LOAD, and the pedigrees display a diffuse mode of inheritance. This could be the result of interactions between several, susceptibility-conferring genotypes or incompletely penetrant loci. Even in monogenic disorders, genetic modifying effects can be substantial (Badano and Katsanis 2002). The conflicting results of linkage studies may be a result of such oligogenic or multigenic modes of inheritance. In multigenetic modes of inheritance, development and progression of the disease may be influenced by both environmental and genetic factors. In such cases, the separate risk factors will not be sufficient to develop AD (Finckh 2003).

The only well-established risk factor for complex forms of AD (mainly LOAD) is the  $\epsilon$ 4 allele of the apolipoprotein gene (*ApoE*) on chromosome 19q13 (Cedazo-Minguez and Cowburn 2001). Patients with the *ApoE4/ApoE4* genotype account for 10-15% of all AD cases compared to 2-5% in the general population.

However, the *ApoE4* allele is absent in 40-50% of non-familial AD patients, and only about 30% of individuals with the *ApoE4/ApoE4* genotype develop AD, indicating that additional susceptibility genes or factors are involved in the pathogenesis of the disease. *ApoE4* is not a disease causing allele, and it should be remembered that a person with two susceptibility-conferring alleles may not develop AD. Only 12-18% of all AD cases appear to be attributable to the *ApoE4* allele (Tol et al. 1999). The *ApoE4* effect is also ethnic-specific, and some studies have questioned ApoE4 as a risk factor in African Americans (Hedera 2001).

Several studies suggest that the *ApoE* genotype may not be a classical risk-factor, but rather a modifier, lowering the age of onset of symptoms. This effect is particularly pronounced between 60-70 years of age, and decreases after 70 or 80 years of age (Chapman et al. 2001, Juva et al. 2000). The maximum risk-effect of the ApoE4 allele for AD is in the sixth decade, when almost 70% of patients with dementia carry at least one *ApoE4* allele. However, only 40% of patients aged >75 are ApoE4 positive (Meyer et al. 1994). In fact, 85% of people over 65 years of age with the *ApoE4/ApoE4* genotype have no symptoms of cognitive impairment. These observations have been reported in sporadic AD, as well as in familial AD caused by mutations in *APP* and *PSEN1*, but not *PSEN2* (Pastor et al. 2003).

ApoE is a serum protein, involved in cholesterol transport. The largest production of ApoE is found in the liver, followed by the brain. In the central nervous system, ApoE is produced by astrocytes and microglia and may enter neurons. There is some data indicating that neurons can also produce ApoE (Baskin 1998). ApoE in the brain is involved in nerve development and regeneration after trauma. After brain injury, ApoE levels increase, first in neutrophils and macrophages and then in astrocytes (Seitz et al. 2003). It has been suggested that this stimulates the formation of amyloid fibrils and plaques (Burns et al. 2003). It was shown that the *in vitro* formation of A $\beta$  fibrils is faster in the presence of ApoE4 than ApoE3. It seems that this effect is a result of a loss of a protective function of ApoE3, and not pathological gain of function of ApoE4 (Esler et al. 2002). Some studies suggest that the ApoE3 isoform lowers the rate of modification of the tau protein. The ApoE4 isoform seems to be less protective against oxygen radicals. Mutations and polymorphisms in at least 50 other genes have been proposed to be genetic risk factors for LOAD based on case-control, family and

twin linkage or association studies (Retz et al. 2001, Rocchi et al. 2003). Polymorphisms have been identified in untranslated, regulatory regions of *APP*, *PSEN1*, and *ApoE* genes (Athán et al. 2002, Lambert et al. 2002, Theuns et al. 2003). Exonic and intronic polymorphisms have also been reported in genes coding, e.g., LDL receptor-related protein, VLDL receptor protein,  $\alpha_2$ -macroglobulin,  $\alpha_1$ -antichymotrypsin, interleukins 1 $\alpha$ , 1 $\beta$  and 6, TNF, TGF- $\beta_1$ , butyrylcholinesterase, bleomycin hydrolase,  $\alpha$ -T catenin, insulin degrading enzyme (IDE), and cholesterol-24 hydroxylase (CYP46). Almost all the reported associated genes code for proteins interacting with the  $\gamma$ -secretase complex, proteins involved in cholesterol metabolism or A $\beta$  clearance (McGeer and McGeer 2001, Papassotiropoulos et al. 2003). Whether this should be indicative of a true general association between these types of proteins and AD or purely an effect of the bias towards studies in these biological candidate genes remains unclear.

Associated polymorphisms identified so far have been found not only in AD patients but also in the general population, and, in most cases, there is only a slight statistical difference in their frequency between case and control groups. Most associations between various polymorphisms and AD are restricted to specific populations or ethnic groups. It has sometimes been possible to relate the polymorphism to a biological effect with measurable consequences. As an example, a polymorphic substitution in CYP46 is correlated with decreased hydroxylase activity and increased levels of brain cholesterol. In a case-control study, a link between these polymorphisms and increased amounts of A $\beta$  brain-load, increased levels of A $\beta_{42}$  and increased levels of hyperphosphorylated tau protein in cerebrospinal fluid were found in AD patients (Papassotiropoulos et al. 2003, Wolozin 2003).

It is possible that combinations of several polymorphisms in different genes increases risk to develop AD. If that is the case, a simultaneous detection of several or more biochemical and genetic markers involved in different biological processes implicated in AD could improve the clinical diagnosis (Blennow and Vanmechelen 1998, Emahazion et al. 2001).

However, the nature of association between polymorphic substitutions in specific genes and the function of the proteins coded by those genetic variants still remains largely unresolved. It is also possible that the polymorphisms are not causally connected to AD and that they instead are markers of unidentified genetic

variants in linkage disequilibrium with associated polymorphisms (Weiss 2000).

It is possible that a dozen of quite different pathological processes, connected with different genetic backgrounds, lead to the same clinical presentation, which we call AD. Thus it may be speculated that in some patients factors that influence tau phosphorylation, differences in inflammatory responses, toxic endovascular factors, changes in caspases or other regulators of apoptosis in neurons, may be more important than disturbances in A $\beta$  metabolism. Molecular association and linkage studies may thus only be possible after a careful selection of etiologically homogenous study-groups, suggesting that a biochemical and physiological delineation of AD should be done prior to genetic studies.

Recent discoveries of non-coding RNAs (ncRNA), present in nuclei of all higher organisms, add a new dimension to genetic analysis. Disease associated polymorphisms and mutations may be causally connected with those non-coding regions of "junk" DNA (Krichevsky et al. 2003, Mattick 2003). It should finally be stated that genetics is not the only risk factor for complex phenotypes. There are known cases of monozygotic twins discordant for AD and with different presentation of the disease (Raiha et al. 1998) suggesting that non-genetic and environmental causes could be as important as genetic ones in determining the risk for AD.

## CHALLENGES OF A MOLECULAR DIAGNOSIS

Recent progress in revealing the genetic basis of AD has created a lot of excitement (Hedera 2001). However, this may create misconceptions and confusion among the general public and health care professionals. None of the susceptibility genes identified so far, including *ApoE*, render themselves suitable for any kind of presymptomatic or diagnostic genetic testing. Further, our partial knowledge about the genetic basis of familial EOAD makes molecular diagnosis and presymptomatic genetic testing of AD possible only in limited cases. Such testing requires careful and comprehensive counseling to minimize possible harm for tested individuals and their families. Novel mutation identified in a family is particularly difficult to counsel since the pathogenicity of the mutation is unknown. It is difficult to assess the relevance of a novel mutation on disease pathogenesis in general, and on AD in particular. An ex-

ample is the E318G mutation in *PSEN1*, which was initially reported as pathogenic and associated with a variable age of onset for familial AD. Later the E318G mutation was identified in a number of healthy control individuals in addition to early- and late-onset sporadic AD (LOAD) patients and familial AD patients. Presently, E318G is regarded as a silent, polymorphic substitution found in several populations, including the Polish one (Żekanowski et al. 2004).

Thus it is essential to know the nature of a novel "mutation" before any counseling is performed. A list of criteria for reporting and classifying novel mutations has been suggested (Cotton and Scriver 1998), the purpose being to delimitate between disease causing, phenotype-modifying, and neutral (silent) mutations. The type of mutation (stop, frame shift, deletion, insertion), mutation prevalence, segregation analysis, the amino acid(s) affected (if it is a conserved amino acid), and finally expression studies should be used to classify novel mutations in order to define their phenotypic significance. Functional *in silico* studies using today's extensive bioinformatics tools may also be useful. In the case of the presenilin mutations, homology between both proteins makes the *in silico* analysis easier. Some mutations in *PSEN2* have their counterparts in *PSEN1*, affecting amino acid residues conserved in both proteins. For instance Q228L in *PSEN2* and Q222R in *PSEN1*, affect homologous locations in the fifth transmembrane domain. However, the phenotypic presentation is different. The Q222R mutation is associated with classical EOAD (Rogaeva, pers. comm.). On the other hand, mutation Q228L in *PSEN2* was identified by our group in a patient with a clinical diagnosis of mild cognitive impairment, at the age of 60 years. The disease developed slowly for 2 years and, at present, a possible AD diagnosis is established. The patient's mother has LOAD, which developed around 75 years of age.

Our preliminary data suggests that the Q228L mutation is a disease-causing mutation. While it is absent in a large cohort of LOAD patients, as well as healthy controls, it affects an evolutionary conserved residue, and the substitution changes the biochemical character of the amino acid. To confirm the possible influence of the Q228L mutation on PS2 structure, we performed comparative modeling studies of PS1 and PS2 fragments containing Q222R and Q228L mutations respectively. We analyzed both transmembrane helices and the loop between them, using four proteins most similar to the analyzed fragment as template: bacteriorhodopsin, rho-

dopsin, ABC transporter, and multidrug efflux transporter. For each template 10 alignments were prepared, five for the wild type fragment and analogously five for the mutant fragment. We observed that wild-type PSEN1 Gln<sup>222</sup> interacts with Arg<sup>220</sup> *via* hydrogen bonds. In the Q222R mutation construct both arginins (Arg<sup>222</sup> and Arg<sup>220</sup>) repel each other. The repulsion is so strong that the backbone of the loop fragment changes its location and Arg<sup>222</sup> moves into an unfavorable hydrophobic environment that can result in distortion of the cytoplasmic loop and inability to bind other proteins in a complex. On the other hand, in the PS2 Q228L, an identical residue like that in PS1, is mutated into the hydrophobic amino acid, leucine. Since Gln<sup>228</sup> doesn't interact with any other residues in the investigated fragment of PS2, the change in structure after a mutation is not so strong. However, the backbone also changes its location. This is caused by the mutated Leu<sup>228</sup> residue that tends to point toward the membrane, whereas in wild types, the hydrophilic amino acid is directed away from the membrane. Such alteration in the structure can influence the binding of proteins important for proper presenilin function.

The above example shows some of the problems connected with molecular diagnosis of familial EOAD. Classification of a novel mutation as a pathogenic one is a complex task. It should be stressed that identification of a novel mutation (especially in *PSEN2* and *APP*) does not predict the age of onset and severity of AD.

## CONCLUSIONS

During the last 15 years, molecular genetics has offered an insight into the genetic aspects of AD. Numerous mutations in three causative genes, associated with rare, familial AD have been detected. Also, a variety of susceptibility-conferring loci have been examined. However, only one well established risk factor for AD (*ApoE4*) has been described. Genetic segregation modeling suggests that there are at least four more LOAD genes remaining to be identified (Warwick Daw et al. 2000). A number of genome screens have been performed on affected LOAD sib-pairs and the results indicate that the most promising location for new loci involved in LOAD pathogenesis are on chromosomes 9, 10 and 12 (Blacker et al. 2003, Kehoe et al. 1999, Myers et al. 2000, Pericak-Vance et al. 2000). Several candidate-gene association studies of genes located in the linkage regions of those chromosomes have been reported (Combarros et al. 2002). However, due to limited



sample sizes, population stratification and/or population admixture, the results have often been conflicting.

Also a recent large-scale association study performed on 54 genes, including 13 previously claimed to be risk factors for AD, failed to provide highly significant associations with the disease (Prince et al. 2001). The abundance of conflicting results concerning the genetic background of sporadic, as well familial AD, may be explained by a heterogeneous etiology of AD, being perhaps a syndrome not a disease, strongly dependent on broadly defined environmental factors. Additionally, conflicting reports could also emerge from simple overestimation of real risk or protective effects of SNPs polymorphisms of preselected genes, whose products are known or suggested to be involved in AD pathogenesis. This could, to some extent, be overcome by meta-analysis studies performed on large, clinically homogenous groups of patients, and genetic profiling based on simultaneous analysis of large numbers of polymorphisms.

Finally, it has been suggested that neurodegenerative disorders may be proteinopathies, affecting complicated protein networks, and whose effects cannot be reduced to a simple set of DNA polymorphisms (Christen 2003). It seems that an interdisciplinary approach, combining results obtained by molecular biology, biochemistry, cell biology, neurobiology, and medicine, will be most fruitful in future research investigating the genetic aspects of Alzheimer's disease (Weiss and Terwilliger 2000). In consequence genetics together with other approaches can lead to the development of more effective therapeutic strategies for AD than those available at present (Religa and Winblad 2003).

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