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C. R. Biologies 328 (2004) 119–130



<http://france.elsevier.com/direct/CRASS3/>

Neurosciences

## Molecular biology and genetics of Alzheimer's disease

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Received 26 July 2004; accepted after revision 19 October 2004

Available online 4 January 2005

Presented by Nicole Le Douarin & Henri Korn

### Abstract

Like several other adult onset neurodegenerative diseases, Alzheimer's disease is a multifactorial illness with both genetic and non-genetic causes. Recent genetic studies have identified four genes associated with inherited risk for AD (presenilin 1, presenilin 2, amyloid precursor protein, and apolipoprotein E). These genes account for about half of the total genetic risk for Alzheimer's disease. It is suspected that several other Alzheimer's disease-susceptibility genes exist, and their identification is the subject of ongoing research. Nevertheless, biological studies on the effects of mutations in the four known genes has led to the conclusion that all of these genes cause dysregulation of amyloid precursor protein processing and in particular dysregulation of the handling of a proteolytic derivative termed A $\beta$ . The accumulation of A $\beta$  appears to be an early and initiating event that triggers a series of downstream processes including misprocessing of the tau protein. This cascade ultimately causes neuronal dysfunction and death, and leads to the clinical and pathological features of Alzheimer's disease. Knowledge of this biochemical cascade now provides several potential targets for the development of diagnostics and therapeutics. **To cite this article: P.H. St George-Hyslop, A. Petit, C. R. Biologies 328 (2004).**

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### Résumé

**Biologie moléculaire et génétique de la maladie d'Alzheimer.** Comme de nombreuses autres maladies neurodégénératives débutant à l'âge adulte, la maladie d'Alzheimer est un syndrome multifactoriel dont les causes sont à la fois génétiques et épigénétiques. Des études ont permis l'identification de quatre gènes associés à un risque héréditaire de maladie d'Alzheimer (gènes de la préséniline 1, préséniline 2, de la protéine précurseur du peptide amyloïde et de l'apolipoprotéine E). Les mutations de ces gènes sont responsables d'environ la moitié des cas génétiques de maladie d'Alzheimer. De nombreux autres gènes de susceptibilité à cette maladie existent certainement, et leur identification fait actuellement l'objet de recherches. Néanmoins, les études biologiques sur les effets des mutations portées par ces quatre gènes ont permis de conclure qu'elles étaient toutes à l'origine de la dérégulation de la maturation protéolytique de la protéine précurseur du peptide amyloïde et plus particulièrement de la modification du devenir d'un de ses fragments, appelé A $\beta$ . L'accumulation d'A $\beta$  semble être l'élément précoce à l'origine

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d'une série d'événements incluant l'agrégation de la protéine tau. Cette cascade aboutit au dysfonctionnement et à la mort des neurones, responsables des caractéristiques pathologiques et cliniques de la maladie d'Alzheimer. Aujourd'hui, la connaissance de cette cascade biochimique fournit plusieurs cibles potentielles pour la mise au point de nouveaux diagnostics et thérapies.

**Pour citer cet article :** P.H. St George-Hyslop, A. Petit, C. R. Biologies 328 (2004).

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**Keywords:** Alzheimer's disease; Amyloid precursor protein; Presenilin; Apolipoprotein E; Tau; Genetics

**Mots-clés :** Maladie d'Alzheimer ; Protéine précurseur du peptide amyloïde ; Préséniline ; Apolipoprotéine E ; Tau ; Génétique

## 1. Introduction

This review focuses upon recent discussions on the biological basis of Alzheimer's disease. Alzheimer's disease (AD), together with Lewy Body Variant of Alzheimer's disease (LBV), and the fronto-temporal dementias (FTD) represent the commonest causes of adult onset dementia. These diseases present in mid to late adult life with progressive defects in memory and higher cognitive functions such as performing complex learned motor tasks (apraxias), reasoning etc. In the fronto-temporal dementias, the clinical syndrome can be overshadowed by behavioural disturbances (disinhibition, aggressivity, etc.) and speech disturbances (aphasia), which arise from involvement of the frontal neocortex. The FTD symptom complex frequently also includes additional features such as muscle rigidity, tremor, bradykinesia (Parkinsonism), and motor neuron induced muscle weakness (amyotrophy). In contrast, the clinical features of AD and LBV (recent and immediate memory deficits, deficits in praxis, reasoning and judgement, etc) are those stemming from involvement of the temporal lobe, hippocampus, and the parietal association cortices, with lesser involvement of frontal lobes until late in the disease. LBV overlaps with AD, sharing most of the clinical and neuropathological features of AD, but being differentiated by the presence of prominent visual hallucinations, sensitivity to phenothiazine tranquilizers, and the presence of Lewy bodies ( $\alpha$ -synuclein containing intraneuronal inclusions) in neocortical neurons. In all three diseases there is prominent loss of neurons in selected cerebral cortical regions (e.g. hippocampus and temporoparietal neocortices in AD and LBV; frontal neocortices in FTD). In AD and LBV, a second prominent neuropathological feature is the complex, extracellular, fibrillar deposits in the cortex termed senile or amyloid plaques. These plaques

contain a number of proteins including apolipoprotein E and  $\alpha$ 1-anti-chymotrypsin, but the principal protein component is amyloid- $\beta$ -peptide ( $A\beta$ ) derived from a longer precursor ( $\beta$ -amyloid precursor protein –  $\beta$ APP). Finally, AD, LBV and FTD are characterized by the presence of neurofibrillary tangles – intraneuronal inclusions composed of hyperphosphorylated forms of tau, a microtubule-associated protein. These intranuclear phosphorylated tau aggregates have been termed neurofibrillary tangles. The overlap between AD and LBV is considerable not only in their major clinical and neuropathological features [1], but also in their genetic bases (i.e. both show associations with the apolipoprotein E  $\epsilon$ 4 variant – see below). A detailed discussion of the clinical and neuropathologic attributes of these diseases can be found in any standard textbook of neurology and neuropathology.

In the past several years, a number of genetic epidemiology studies have been undertaken on probands with AD and their families. Cumulatively, these studies (see review in Ref. [2]) strongly argue that the familial aggregation of AD is not due simply to the high frequency of AD in the general population. These studies reveal that the overall lifetime risk for AD in first-degree relatives of AD probands is about 38% by age 85 years [3]. These studies also demonstrate that the majority of cases of familiarly aggregated AD probably reflect a complex mode of transmission such as: (1) one or more common independent, but incompletely penetrant, single autosomal gene defects; (2) a multi-genic trait; or (3) a mode of transmission in which genetic and environmental factors interact. Nevertheless, there are a small proportion of AD cases (~ 10%) that appear to be transmitted as pure autosomal dominant Mendelian traits with age-dependent but high penetrance. Molecular genetic studies on pedigrees with the latter type of FAD with molecular genetic tools has led to the discovery of four different

genetic loci associated with inherited susceptibility to AD. Molecular genetic studies have identified four different genes associated with inherited susceptibility to Alzheimer's disease (AD). It is suspected that at least two, and possibly several additional AD susceptibility loci remain to be identified because only about 50% of FAD cases are associated with the four FAD loci known to date.

### 1.1. The Amyloid Precursor Protein

The first gene to be identified in association with inherited susceptibility to AD was the amyloid precursor protein gene ( $\beta$ APP). The  $\beta$ APP gene encodes an alternatively spliced transcript which, in its longest isoform, encodes a single transmembrane spanning polypeptide of 770 amino acids [4]. The  $\beta$ APP precursor protein undergoes a series of endoproteolytic cleavages [5]. One of these is mediated by a putative membrane-associated  $\alpha$ -secretase, which cleaves  $\beta$ APP<sub>695</sub> in the middle of the A $\beta$  peptide domain and liberates the extracellular N-terminus of  $\beta$ APP. The identity of  $\alpha$ -secretase is not yet known with certainty, although members of the disintegrin metalloprotease family, TACE and ADAM17, are strong candidates.

The other cleavage pathway involves sequential cleavages by  $\beta$ - and  $\gamma$ -secretases, and generates the 40–42 amino acid A $\beta$  peptide. The first cleavage occurs at the beginning of the A $\beta$  domain, and is mediated by  $\beta$ -secretase (BACE 1), a Type 1 transmembrane glycosylated aspartyl protease, resident in post-Golgi membranes and at the cell surface [6]. The second set of cleavages occurs at residues +40, 42 by a putative enzyme activity termed  $\gamma$ -secretase (some recent data suggests that there may be a necessary preceding cleavage at residues +49, +50 and 51 – termed  $\epsilon$ -cleavage). Both  $\gamma$ -cleavage and  $\epsilon$ -cleavage require the presence of the presenilin proteins (see below, and both cleavage events occur mainly in a post-Golgi compartment and/or following re-entry of  $\beta$ APP from the cell surface). The N-terminal product of  $\gamma/\epsilon$ -secretase is A $\beta$ , while the C-terminal product is a labile fragment termed amyloid intracellular domain (AICD), which might act as a signal transduction molecule. The  $\gamma/\epsilon$ -secretase cleavage actually generates a mixture of A $\beta$  peptides, containing 39 to 43 residues. A $\beta$  peptides ending at residue 42 or 43 (long tailed A $\beta$ ) are thought to be more fibrillogenic and

more neurotoxic than A $\beta$  ending at residue 40, which is the predominant isoform produced during normal metabolism of  $\beta$ APP [7–10]. Little is currently known about the physiological role (if any) of A $\beta$ . A $\beta$  is removed by several pathways, including degradation by neutral endopeptidase cleavage (e.g., by neprilysin [11]) or by insulin degrading enzyme (IDE) [12].

The function of  $\beta$ APP is currently unknown. Knockout of the murine  $\beta$ APP gene leads only to minor weight loss, decreased locomotor activity, abnormal forelimb motor activity, and non-specific degrees of reactive gliosis in the cortex [13]. In vitro studies in cultured cells suggest that secreted  $\beta$ APP ( $\beta$ APPs) can function as an autocrine factor stimulating cell proliferation and cell adhesion. Other studies have implied a role for  $\beta$ APP in: (1) signal transduction by association of  $\beta$ APP with heterotrimeric GTP-binding proteins; (2) a receptor for kinesin-1 during the fast axoplasmic transport of vesicles containing BACE and presenilins, and cleavage of APP [14] and/or (3) a signal transduction molecule in a manner similar to the role of the Notch Intra-Cellular Domain, which mediates Notch signalling following binding of delta to Notch at the cell surface during dorsal axis development in embryogenesis [15–17].

Several different missense mutations have been discovered in exons 16 and 17 of the  $\beta$ APP gene in families with early-onset AD (<http://molgen-www.uia.ac.be/ADMutations>). All of these mutations either alter APP processing and A $\beta$  production, or alter the propensity of A $\beta$  to aggregate into  $\beta$ -sheet amyloid fibrils. Some of the missense mutations in the  $\beta$ APP gene result in the relative (APP<sub>717</sub>) or absolute (APP<sub>670/671</sub>) over-production of full length A $\beta$  species ending at residue 42. Other mutations cause the over-production of N-terminally truncated species of A $\beta$  ending at residue 42 (APP<sub>715</sub>); or the production of A $\beta$  species that have increased propensity to assemble into neurotoxic fibrils (APP<sub>692</sub>, APP<sub>693</sub>). Multiple molecular mechanisms have been proposed to explain the neurotoxic effects of A $\beta$  (and especially of small fibrillar aggregates called protofibrils). These include inducing apoptosis by direct effects on cell membranes and by indirect effects, such as potentiating effects of excitatory amino acids, oxidative stress, and increases in intracellular calcium and free radicals [18–20]. However, A $\beta$  may not be the only cytotoxic product of  $\beta$ - and  $\gamma$ -secretase cleavage because the cy-

toplasmic C-terminal stub (C31- $\beta$ APP) is also toxic when over-expressed [21].

### 1.2. Apolipoprotein E

The association of APOE with inherited susceptibility to AD was uncovered by the concurrence of three lines of investigation. Genetic linkage studies in pedigrees with predominantly late-onset, familiarly aggregated AD provided suggestive evidence ( $z = +2.5$ ) for an AD susceptibility locus on chromosome 19q12–q13 near the APOE gene [22]. Second, analysis of proteins from the CSF which were capable of binding the A $\beta$  peptide revealed that one of the proteins was apolipoprotein E (APOE) [23]. Finally, APOE is a component of the senile plaque of AD.

The APOE gene in humans contains three common polymorphisms –  $\epsilon 2$  (cysteines at codon 112 and codon 158),  $\epsilon 3$  (cysteines at codon 112), and  $\epsilon 4$  (arginine at codon 112). Analysis of these polymorphisms in normal control populations and in patients with AD has shown that there is an increase in the frequency of the  $\epsilon 4$  allele in patients with AD (allele frequency in AD is approximately 40%, compared to 15% in normals) [24], and that there is a smaller reduction in the frequency of the  $\epsilon 2$  allele (from 10% to about 2% in AD) [25]. More significantly, there is a dose-dependent relationship between the number of copies of  $\epsilon 4$ , and the age-of-onset of AD such that  $\epsilon 4/\epsilon 4$  subjects have an earlier onset than do heterozygous  $\epsilon 4$  subjects [26]. Subjects with an  $\epsilon 2$  allele, on the other hand, have a later onset [25]. The association between  $\epsilon 4$  and AD has been robustly confirmed in numerous studies and in several different ethnic groups. The association is weaker with advanced age of onset, and the putative protective role of the  $\epsilon 2$  allele is less clear at younger ages of onset (where it may even be associated with a more aggressive course) [27,28]. This association of APOE  $\epsilon 4$  with AD has been replicated in numerous studies and in numerous ethnic groups with the possible exception of black Americans and American Hispanics, which have generated conflicting results [29,30].

Although the association between APOE  $\epsilon 4$  and AD is robust, it is not entirely specific. Observations in patients with head injury [31,32]; spontaneous intracerebral haemorrhage [33], and in patients undergoing elective cardiac bypass surgery [34], all suggest

a poorer outcome for patients with the  $\epsilon 4$  allele. There is a confirmed association between the  $\epsilon 4$  allele and the Lewy body variant of AD (see above) [35].

The mechanism by which the  $\epsilon 4$  allele is associated with an earlier onset of AD, and by which the  $\epsilon 2$  allele is associated with a later onset is unclear. The most obvious hypothesis is that APOE might influence the production, distribution, or clearance of the A $\beta$  peptide. This hypothesis is supported by observations that the genotype at APOE modulates age-of-onset in subjects carrying the  $\beta$ APP Val717Ile mutation (but not the APP<sub>692</sub> mutation), suggesting a direct biochemical interaction between APOE and  $\beta$ APP (or its metabolic products) [36]. Second, subjects with one or more APOE  $\epsilon 4$  alleles have a higher A $\beta$  peptide plaque burden than do subjects with no  $\epsilon 4$  alleles [37]. In vitro studies suggest that delipidated APOE  $\epsilon 4$  binds A $\beta$  more avidly than APOE  $\epsilon 3$  [23,38]. There is also evidence that both APOE and A $\beta$  may be cleared through the lipoprotein-related (LRP) receptor and that APOE  $\epsilon 4$  and the A $\beta$  peptide may compete for clearance through the LRP receptor [39]. Finally, transgenic mice expressing the  $\beta$ APP<sub>V717F</sub> mutation (PDAPP mice) develop profound cerebral A $\beta$  deposition when bred on an APOE<sup>+/+</sup> background, but have very little A $\beta$  deposition on an APOE<sup>-/-</sup> background [40].

An alternate hypothesis concerns changes in cholesterol metabolism. Both epidemiological and direct experimental evidence in cell culture models suggests that cholesterol metabolism and APP metabolism are functionally intertwined. Specifically, reduction in cellular cholesterol availability results in significant changes in APP trafficking and processing, with the resultant reduction in A $\beta$  formation [41]. In addition, patients who have taken statins for hypercholesterolemia appear to have a reduced incidence of Alzheimer's disease [42–44].

Finally, there is a good correlation between the degree of clinical dementia and the decrease in synaptic density in AD [45], and it has been suggested that APOE may be involved in synaptic plasticity during regeneration and repair, and that the  $\epsilon 4$  allele is less efficient in this role. This is in accord with clinical epidemiological data suggesting that the presence of APOE  $\epsilon 4$  is associated with a poorer outcome after a variety of unrelated CNS injuries including head injury, stroke, and coronary artery bypass grafting. It has therefore been suggested that the association between

APOE  $\epsilon 4$  and AD may not determine whether AD occurs, but rather, the clinico-pathologic response to other causative factors by modulating a variety of effects including A $\beta$  processing and regeneration-repair, etc. Indeed, these putative effects of APOE on several different mechanisms need not be mutually exclusive.

### 1.3. Presenilin 1

Genetic mapping studies located a third Alzheimer susceptibility locus (AD3) to a region of approximately 10 centiMorgans on the long arm of Chromosome 14. The actual disease gene (presenilin 1) was isolated using a positional cloning strategy [46].

The presenilin 1 (PS1) gene, is highly conserved in evolution, being present in *C. elegans* [47] and *D. melanogaster* [48]. PS1 encodes a polytopic integral membrane protein. Currently favoured models have 8 transmembrane domains, with a large, hydrophilic, acidically charged loop domain between the putative sixth and seventh transmembrane domains. The PS1 protein is approximately 50 kDa in size and is predominantly located within intracellular membranes in the endoplasmic reticulum, the perinuclear envelope, the Golgi apparatus and at the cell surface as well as in some as yet uncharacterized intracytoplasmic vesicles [49,50]. Only very small amounts of the PS1 holoprotein exist within the cell at any given time [51,52]. Instead, the holoprotein undergoes endoproteolytic cleavage near residue 290 within the TM6-TM7 loop domain. The biologically active form of the presenilins are the N- and C-terminal fragments (NTF and CTF), which are tightly associated with each other in a high molecular weight, intracellular protein complex (> 450 kDa). This protein complex co-elutes with  $\gamma$ -secretase activity in cellular fractionation studies. Several other components of the presenilin complexes have been identified. These include nicastrin (*a* ~ 110 kDa Type 1 transmembrane glycoprotein [53]); APH-1 (a polytopic transmembrane protein that may act as the initial assembly molecule for the complex [54]); and PEN2 (a short hydrophobic protein with two transmembrane domains, but with unknown function).

The presenilins play either a direct catalytic or indirect (facilitatory) role in the proteolytic processing ('Regulated Intramembranous Proteolysis') of several Type-1 transmembrane proteins including APP, p75,

LRP, ErbB4, and notch. Null mutations in the presenilins result in severe developmental defects, which directly arise from defective proteolysis of notch with the subsequent failure to generate the notch intracellular domain (NICD) [47,55–57]. Loss of presenilin function, or loss of nicastrin, APH-1, or PEN-2 has similar effects on notch processing and on the processing of APP. The latter defect causes a profound reduction in the secretion of A $\beta$  and the accumulation of the substrate for  $\gamma/\epsilon$ - cleavage (i.e. C83 ( $\alpha$ -stubs) and C99 ( $\beta$ -stubs), which are the derivatives of  $\alpha$ - and  $\beta$ -secretase cleavage respectively). It is currently unclear whether the presenilins have a direct enzymatic activity [58], or whether the presenilins are indirectly involved perhaps through modulating trafficking and/or activation of various substrates or other components of the  $\gamma$ -secretase complex. Further details on this controversy are in Refs. [59,60].

To date, more than 128 different mutations have been discovered in the PS1 gene (<http://molgen-www.uia.ac.be/ADMutations>). The majority of these mutations are missense mutations giving rise to the substitution of a single amino acid. A few in-frame splicing, deletion or insertion defects have also been identified [61–63]. However, nonsense mutations resulting in truncated proteins that would cause loss-of-function mutations have yet to be found in AD-affected subjects.

All of PS1 mutations associated with AD increase  $\gamma$ -secretase cleavage of  $\beta$ APP and preferentially increase the production of toxic long-tailed A $\beta$  peptides ending at residue 42 [64–67]. However, some investigators believe that like the  $\beta$ APP mutations, PS1 and PS2 mutations may also cause neurodegeneration by modulating cellular sensitivity to apoptosis induced by a variety of factors, including staurosporine, A $\beta$  peptide, serum withdrawal, etc.

### 1.4. Presenilin 2

During the cloning of the presenilin 1 gene on chromosome 14, an homologous sequence (Presenilin 2) was identified on chromosome 1 [68]. PS2 encodes a polypeptide whose open reading frame contains 448 amino acids, with substantial sequence similarity to PS1 (overall identity approximately 60%), and a very similar structural organization. Despite this similarity, PS1 and PS2 are likely to have distinct but overlapping



functions. For instance, PS2 does not functionally replace either the APP or Notch processing defects in PS1<sup>-/-</sup> animals [69], yet PS2 mutations, like PS1 mutations, increase the secretion of long-tailed A $\beta$  peptides [65,67].

Mutational analyses have uncovered a small number of missense mutations ( $\sim 9$ ) in the presenilin 2 gene in families segregating early-onset forms of Alzheimer's disease (<http://molgen-www.uia.ac.be/ADMutations>). The phenotype associated with PS2 mutations is much more variable [70,71]. Thus, the vast majority of heterozygous carriers of missense mutations in the  $\beta$ APP and PS1 genes develop the illness between the ages of 35 and 55 for PS1 mutations, and between 45 and 65 for  $\beta$ APP mutations. In contrast, the range of age-of-onset in heterozygous carriers of PS2 mutations is between 40 and 85 years, and there is at least one instance of apparent non-penetrance in an asymptomatic octogenarian transmitting the disease to affected offspring [70,72,73]. Furthermore, in contrast to APP mutations, the effect of APOE  $\epsilon$ 4 on the age-at-onset in PS2 mutations is either absent or less profound. Modifier loci other than APOE probably account for much of this variation.

### 1.5. Other Genes for 'AD'

Several large surveys of patients with familial Alzheimer's disease have indicated that the APP, APOE, PS1 and PS2 genes account for only about half of the genetic risk factors for Alzheimer's disease. It is therefore likely that there are several additional AD susceptibility genes. Some of these loci will be associated with additional rare, but highly penetrant defects similar to those seen with mutations in PS1 and APP. Other genes may result in incompletely penetrant autosomal-dominant traits like those associated with PS2. However, it is likely that a significant proportion of the remaining genes will be genes with low-effect sizes similar to APOE, and in which the ultimate phenotype is likely to be influenced by the presence or absence of other genetic and environmental risk factors.

There have been multiple strategies deployed to try to map these additional AD susceptibility genes. Genetic linkage studies and family-based association analyses have been employed on datasets with pedigrees multiply affected with Alzheimer's disease, and

have led to the suggestion that there may be additional susceptibility loci in: (1) the pericentromeric region of chromosome 12 (Alzheimer Type 5) [74,75]; and (2) the long arm and pericentromeric region of chromosome 10q (Alzheimer Type 6) [76–78]. However, to date, the exact genes in these regions that cause susceptibility to AD have not been identified. Weaker evidence, in single studies have also implicated chromosome 20p [79], 15q22 [80], and 9p [81]. The Glutathione S-transferase omega-1 (GSTO1) gene on distal chromosome 10q has also been implicated as a gene modulating age-of-onset in both AD and Parkinson disease [82], but this has not yet been replicated in other datasets.

A second strategy to identify AD genes has been to use cohorts of sporadic AD cases and age/sex matched controls in a case: control association design. This has led to a long list of potential candidate genes, most of which have not been robustly replicated. A partial list of candidate genes provisionally identified as putative AD susceptibility loci includes homozygosity for the AA allele of an intronic polymorphism in  $\alpha$ 1-chymotrypsin, A5 repeat allele of an intronic insertion–deletion polymorphism in the very low density lipoprotein receptor, neutral coding sequence and intronic polymorphisms in low density lipoprotein receptor related protein, homozygosity for intronic polymorphisms in presenilin 1 or presenilin 2, K-variant of butyrylcholinesterase, homozygosity for the Val/Val variant of the Val443Ile polymorphism in bleomycin hydroxylase; IL4, etc. However, most of these candidate genes have not received the same widespread confirmation, as did APOE  $\epsilon$ 4 when tested in independent but comparable datasets. Interestingly, a number of obvious candidate genes involved in APP processing, including BACE ( $\beta$ -secretase involved in A $\beta$  generation) and neprilysin and insulin degrading enzyme (involved in A $\beta$  degradation) have been screened, but as of this writing have not been widely found to have genetic variants associated with increased risk for AD.

## 2. Practical implications

### 2.1. Predictive genetic testing

The discovery that mutations in specific genes are associated with inheritable susceptibility to late onset

dementias, together with the increasing public awareness both of genetics as a cause of these illnesses, and of familial aggregation of AD in particular, lead to the frequent need for physicians to consider the merits of genetic counselling and genetic testing. At the present time, in the absence of clearly effective preventative or curative treatments without significant side effects, the main reason for genetic counselling and testing is to provide information only. However, while such information can be empowering, it obviously also has the potential to be misused to the patient's disadvantage.

There is currently relatively little practical experience with genetic counselling of members of families multiply affected with AD or the other dementias. Consequently, most of the paradigms used for genetic counselling of members of families with dementia are based upon similar paradigms used in the counselling of subjects with Huntington's disease [83]. The Huntington's disease model is actually quite useful for counselling of members of families with early-onset familial Alzheimer's disease (FAD) associated with mutations in PS1, PS2 or  $\beta$ APP and of FTD associated with mutations in tau because the age-of-onset is often similar (30–65 years of age) and has a similar pattern of transmission (highly penetrant, age-dependent penetrance, autosomal-dominant segregation). Thus, in members of families with mutations in the  $\beta$ APP, PS1 and Tau genes, it is possible to screen at-risk family members for the presence of mutations detected in affected individuals, and to counsel these family members based upon the concept that  $\beta$ APP, PS1 and tau mutations are highly penetrant (approximately 95%) with typical age-of-symptom-onset between 35 and 65 years. PS2 mutations on the other hand have a lower penetrance and a more variable age-of-onset (45–85 years). Using the Huntington's disease paradigm, we and several other groups have had the opportunity to counsel a small number of members of families with PS1 and  $\beta$ APP mutations without significant problems. We have also shown that screening for PS1 mutations is cost-effective when done on symptomatic cases with a positive family history of AD and onset before the age of 60 years [84].

Although use of the Huntington's disease paradigm will probably work well for PS1, tau and  $\beta$ APP mutations, mutations in these genes are a comparatively rare cause of familial dementia, cumulatively accounting for about 50% of early-onset FAD (which itself

accounts for  $\sim$  5% of all AD) and 10–40% of familial frontotemporal dementia. A much more common clinical experience is the presence of two or three affected family members with late-onset dementia in a small nuclear pedigree. Frequently, in these 'multiplex' late-onset dementia pedigrees, the disease does not inherit as a classic autosomal-dominant trait. As a result, in any given family, it is frequently unclear whether the multiplex pedigree structure reflects an incompletely penetrant autosomal-dominant trait, or a more complex mode of transmission involving either the additive effects of several genes or the interaction of genes and environment. Empirical counselling of pedigrees of this type is difficult and, in the case of AD where the data are the more robust, must largely be based upon recent epidemiological studies such as those of Lautenschlager et al. While the use of molecular genetic studies would clearly facilitate counselling in such pedigrees, the only locus that has shown robust association with late-onset AD is the apolipoprotein E (APOE) gene. Retrospective studies, in autopsy and clinical series, have suggested that the cumulative lifetime risk for AD in subjects homozygous for APOE  $\epsilon$ 4 may be as high as 90% by age 90 years. However, even from these retrospective studies, it is apparent that there is a huge variation in the age-of-onset of AD even in subjects who are homozygous for APOE  $\epsilon$ 4 (50–90 years). To confound matters further, a small number of limited prospective studies suggest that the APOE genotype is a relatively poor predictor of the onset of AD even in high risk groups such as those with age-associated memory loss [85]. Consequently, a number of research groups have recommended that the APOE genotype not be used for presymptomatic testing [86]. The question of whether the APOE genotype may be of assistance in establishing the diagnosis of AD in demented patients undergoing diagnostic work-up is currently a matter of some discussion. Most experts agree that while the APOE genotype studies might form a part of the diagnostic armamentarium, they are unlikely by themselves to be the only test which should be done even in individuals with classical clinical features of AD [87]. Thus Mayeux and colleagues have shown that a clinical diagnosis of AD has a sensitivity of  $\sim$  93% and specificity of 55%, whereas the APOE  $\epsilon$ 4 allele confers sensitivity and specificity of 65% and 68%, respectively. The addition of information about the APOE genotype increased

the overall specificity to 84% in patients who met the clinical criteria for Alzheimer's disease, but the sensitivity decreased 61%. However, it is important to note that the patients studied in the report by Mayeux et al. were referred to tertiary centres, and it remains to be determined whether genotype would provide similar levels of sensitivity and specificity in more typical clinical settings [87,88].

## 2.2. Pharmacogenomics

Another role of genetic testing is in the design of a therapeutic program for subjects affected with AD and other dementias. Given the obvious genetic heterogeneity of AD (and the other dementias), it would not be unreasonable to suspect that this etiologic heterogeneity would be reflected in different biochemical pathogenic pathways and therefore differences in response to specific treatments. It is conceivable, therefore, that some subsets of AD (or the other dementias) might respond better to specific therapeutic agents than other subtypes. For instance, there was provisional evidence that the APOE genotype may be associated with differences in response to the cholinesterase inhibitor Tacrine [89]. However, this does not seem to have been born out as a general phenomenon because as additional AChEase inhibitors have been developed, there has been no predictive value for APOE [90].

It is apparent, from the discussion on the molecular genetics of AD and related disorders, that a significant proportion of familiarly aggregated dementia cannot be related to polymorphisms or mutations in any of the known genes (PS1, PS2,  $\beta$ APP, tau and APOE). It is likely that in the next several years, additional AD and FTD susceptibility genes will be identified. However, until most or all of these genes are identified, and their relative frequencies as a cause of AD or FTD in specific populations can be defined, genetic testing of at-risk family members can only be reasonably performed if there is a testable, clinically-affected member available. If an affected pedigree member is available, their DNA can be screened for mutations in the known susceptibility genes. If mutations are found, this information can then be used for the testing in at-risk family members. Where no mutations in the affected subject's DNA are found, it can be reasonably assumed that the disease is caused by mutations or

polymorphisms in other AD/FTD genes yet to be identified, and mutation screening studies in at-risk family members would not be indicated. However, in the absence of a living affected member who can be tested, the screening for mutations in the DNA of at-risk family members is currently likely to be a fruitless task because, until all of the disease-causing genes for that trait are known, the failure to discover disease-related polymorphisms or mutations in the known genes can give no reassurance that the at-risk family member is not a carrier of a mutation in another susceptibility gene.

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