

If these simple guidelines are followed, then discussions of how best to analyze the data can more fruitfully be directed towards the question of how best to maximize power to detect linkage with these pedigrees, and towards the difficult question of how to actually get the relevant computations done.

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The molecular genetics of familial Alzheimer's disease

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Familial Alzheimer's disease is genetically heterogeneous. In some families, early onset of disease is caused by mutations in the APP gene. In other families, a second gene on chromosome 21, a gene on chromosome 19, or a gene located somewhere else in the genome causes Alzheimer's disease.

THE role of inheritance in the occurrence of Alzheimer's disease (AD) has come under intense scrutiny during the past decade. Epidemiologic survey studies where family history was studied have repeatedly shown that families of AD probands have more cases of AD in close relatives than families of controls¹. Twin studies not only show that many identical twins are often concordant for AD² but also that the families of

concordant twins have more AD cases than the families of twins discordant for AD³. These types of studies are highly suggestive that the inheritance of defective genes is important in the pathogenesis of AD. However, population-based studies cannot clearly determine what mode of inheritance is responsible for the observed family clustering.

Some authors have suggested that all AD is autosomal dominant⁴. This hypothesis is difficult to prove by family studies since the onset of AD typically occurs late in life and relatives of probands often die of other causes before they reach the age of appropriate risk (age censoring). Thus even if all AD was the result of autosomal dominant inheritance, in most cases of randomly ascertained AD probands, secondary cases in close relatives would not be observed due to age censoring⁴. Alternatively, single cases in a family could

be the result of somatic cell mutations and thus would have a genetic basis but would not be heritable and close relatives would not be expected to have an increased risk for AD. An equally plausible model is that only some cases of AD are genetic while most are caused by other factors such as infectious agents or environmental factors. Moreover, mechanisms related to intrinsic biological aging may be important in AD pathogenesis. The fact that AD is a common disease in the elderly further complicates the study of AD genetics. Thus family clustering could occur by chance or multiple cases in a kindred could be due to a mixture of genetic and non-genetic cases.

The most convincing evidence that defective genes are involved in AD comes from the many early-onset families described in the literature⁵⁻⁹ in which the disease appears to be inherited as an autosomal dominant trait. In some of the most dramatic kindreds, family average ages-of-onset between 30 and 40 years have been reported⁷. In these rare kindreds, approximately 50% of those at risk develop the disease. Since AD with an onset in 3rd and 4th decade of life is rare, clustering of AD in multiple generations in these families is clearly the result of inheritance and not chance.

The goal of molecular genetic studies of familial Alzheimer's disease (FAD) is to identify the genes responsible for the disease. In the past year, remarkable progress has been made in identifying mutations responsible for FAD in at least some early-onset families¹⁰⁻¹⁵. This success has come in part since the mode of inheritance is unambiguous in these rare but important families. Late-onset families are also being studied with some encouraging results^{16,17}. Though study of these kindreds is more difficult due to the problems outlined above, late-onset AD is tremendously important since it is the most common form of AD.

Amyloid precursor protein gene

The neuropathologic feature most characteristic of AD are amyloid deposits in the brain either associated with blood vessels or found as extracellular deposits termed plaques. A principle component of AD amyloid is the A β peptide, a 39-43 amino acid peptide whose partial sequence was first determined by Glenner and Wong in 1984 (ref. 18). This same peptide is also a component of the amyloid deposits found in Down's syndrome¹⁹⁻²⁰. The A β amino acid sequence was used to obtain a cDNA (c=copy) clone²¹ derived from the messenger RNA transcript of the amyloid precursor protein (APP) gene. This gene encodes at least 4 isoforms²¹⁻²⁵ which are normal cellular proteins found in the central nervous system as well as in many other peripheral tissues. Three of these isoforms contain the A β sequence in the

carboxyl terminal portion of the protein. The APP gene maps to chromosome 21 (ref. 26); this observation has profound though poorly understood implications for the high prevalence of AD in Down's syndrome subjects (who have an extra copy of chromosome 21).

Cloning of the APP gene permitted it to be tested as a candidate gene for FAD. Initial work appeared to eliminate the gene as being responsible for FAD^{7,27-29}. DNA polymorphisms associated with the gene did not show the same pattern of inheritance as the disease in a number of large early-onset families and obligate recombinants between APP polymorphisms and FAD were observed^{7,27}. When results for either early or late-onset families were pooled, the results were quite negative for the two groups indicating that, in most FAD families, mutations in the APP gene were not responsible for AD. Because of these negative findings, the APP gene was ignored as an FAD candidate gene until Levy *et al.*³⁰ identified a mutation in this gene responsible for hereditary cerebral hemorrhage with amyloidosis of Dutch type (HCHWA-D). In this disease, amyloid deposits containing the A β peptide are found associated with cerebral blood vessels³¹. Polymorphisms in the APP gene co-segregate with the disease³² and a point mutation in codon 698 (codon numbering based on the APP₇₇₀ transcript) was found in all affected subjects studied³⁰. The mutation results in a glutamate to glutamine change at amino acid position 22 of the A β peptide. Identification of the HCHWA-D mutation demonstrated that a mutation in the APP gene could cause A β deposition.

In 1991, Goate *et al.*¹⁰ re-evaluated the APP gene in a family in which APP polymorphisms co-segregated with the disease. Direct sequencing of APP exon 17 (which encodes part of the A β peptide sequence) revealed a mutation at codon APP₇₁₇ which results in a valine to isoleucine change 3 amino acids C-terminal to the end of the A β sequence. This mutation has subsequently been found to co-segregate with AD in a total of 6 early-onset kindreds¹⁰⁻¹² and has not been found in a large number of 'sporadic' AD subjects or normal controls^{10,33,34}. The absence of this mutation in normal controls indicates that it is not a rare polymorphism. The fact that this mutation is only observed in early-onset FAD kindreds clearly demonstrates that it is sufficient to initiate the pathogenesis of FAD. Two other APP mutations have been subsequently identified in single early-onset FAD kindreds. These mutations are also in codon APP₇₁₇; one results in a valine to glycine substitution¹⁵ and the other a valine to phenylalanine change¹⁴. Another APP mutation has recently been described in a Dutch family with both presenile dementia and cerebral hemorrhage in cognitively normal subjects. The mutation in this family is at codon 692 and results in the substitution of a glycine in place of the normal alanine at this position. The

relationship of this disorder to FAD remains to be determined as only biopsy material has been studied to date.

Additional FAD loci

The identification of the three and possibly 4 FAD mutations clearly demonstrates that mutations in the APP gene can cause AD in at least some early-onset families. However, linkage data^{7,27-29,35} and mutation analysis^{10,34,36} also indicate that numerous early-onset kindreds do not have mutations in the APP gene. Perhaps 5-10% of early-onset kindreds have APP mutations (unpublished data). No APP mutations have been identified in late-onset families. Thus the combined data indicate that FAD is genetically heterogeneous and that genes at locations other than the APP gene are capable of causing FAD.

For early-onset kindreds, there is evidence that a second chromosome 21 FAD locus, distinct from the APP gene, exists in the centromeric region of the chromosome^{8,37}. One family of French-Italian origin gives a peak 2-point LOD score of 2.94 with marker D21S52³⁸ which is located in a chromosomal region of considerable genetic and physical distance centromeric to the APP gene. Other early-onset families such as the Volga German kindreds³⁵ are clearly not linked to markers in this region nor to the APP locus. Thus non-chromosome 21 FAD loci for early-onset families remain to be identified.

Recently, we described localization on chromosome 14 of a new FAD locus³⁹. Linkage analysis of 9 early-onset families (onset means <60 years) was positive for markers in the 14q24.3 region. A peak 2-point LOD score of 9.15 was obtained using the polymorphic locus D14S43. In 1 single family, a maximum LOD score of 4.89 was observed.

Significant progress in mapping the late-onset FAD gene(s) has been achieved by Pericak-Vance and co-workers¹⁶. Using both conventional linkage analysis and sib-pair and affected pedigree member methods, this group has identified a region on chromosome 19 q13 which appears to be linked to FAD. The location which is most likely to contain a late-onset FAD locus is the region containing the ATP1A3 gene which encodes for one of the subunits of the Na⁺/K⁺ ATPase. Though these findings need to be independently confirmed, the initial results are quite promising.

Discussion

The work summarized above demonstrates that FAD is genetically heterogeneous. A number of kindreds have mutations in codon 717 of the APP gene but most early-onset kindreds do not have these mutations and many

are unlikely to have mutations at other locations within the APP gene. The centromeric region of chromosome 21 may contain a second early-onset locus. A gene for late-onset FAD may be located on chromosome 19. Other loci for early-onset FAD remain to be identified and a genomic linkage search is underway to identify the chromosomal location of additional FAD loci. For late-onset families, loci at locations other than chromosome 19 may also contribute to susceptibility to the disease. Hopefully the identification of additional loci and the identification of a variety of specific FAD mutations will help us to understand the contribution of inheritance plays in the most common form of the disease, the late-onset 'sporadic' cases. The identification of additional FAD genes will contribute to our understanding of the genetic etiology of all forms of AD.

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