The Genetic Defect Causing Familial Alzheimer's Disease Maps on Chromosome 21

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Alzheimer's disease is a leading cause of morbidity and mortality among the elderly. Several families have been described in which Alzheimer's disease is caused by an autosomal dominant gene defect. The chromosomal location of this defective gene has been discovered by using genetic linkage to DNA markers on chromosome 21. The localization on chromosome 21 provides an explanation for the occurrence of Alzheimer's disease–like pathology in Down syndrome. Isolation and characterization of the gene at this locus may yield new insights into the nature of the defect causing familial Alzheimer's disease and possibly, into the etiology of all forms of Alzheimer's disease.

ALZHEIMER'S DISEASE (AD) IS A DEgenerative disorder of the human central nervous system characterized by progressive impairment of memory and intellectual function beginning in middle to late adult life (*1*). Victims of AD develop profound mental and physical disability that frequently necessitates institutional care. Death usually occurs within 5 to 10 years after the onset of symptoms. AD has become a major public health problem in many Western societies where age-dependent incidence rates of up to 127 cases per 100,000 people per year and age-dependent prevalence rates of up to 5.8 cases per 100 people per year have made the disorder and its associated medical complications the fourth leading cause of death (*1*). In the United States alone, an estimated $25 billion per year are spent on the institutional care of demented patients (*1*). In view of the continuing increase in the relative size of the elderly population in Western societies, AD is likely to assume even greater importance in the future.

No treatment effective in preventing or arresting the neurodegenerative process of AD has yet been discovered. An understanding of the biochemical basis of the disorder would clearly facilitate attempts to develop useful therapies. The primary cause of AD remains unknown, although both genetic and environmental factors have been considered (*1*). The occasional observation of more than one affected member in a single family does not necessarily imply that AD is inherited. However, several large families have been reported that display autosomal dominant transmission of the disorder (*2*). The familial form of AD (FAD) observed in these pedigrees is clearly caused by a genetic defect. Apart from the evident pattern of inheritance and a somewhat younger age of onset, the disease phenotype exhibited by members of these FAD pedigrees is indistinguishable from that of "sporadic" cases with respect to its clinical, pathological, and biochemical features (*3*). Indeed, an incompletely penetrant autosomal dominant gene defect may underlie even "sporadic" AD (*4, 5*). If the primary genetic defect in FAD could be identified, it could lead to a clearer understanding of both familial and nonfamilial cases of AD.

A high incidence of Alzheimer-like neuropathologic changes in older patients with Down syndrome (DS) (trisomy 21) has led to the suggestion that a gene on chromosome 21 may be important in the pathogenesis of AD (*5*). This hypothesis is weakened, however, by two considerations. First, it is not clear that all DS patients invariably develop clinically apparent dementia despite the high incidence of AD-like neuropathology (*6*). Second, some aspects of the neurochemistry and neuropathology of AD are also mimicked by a heterogeneous group of other conditions that includes normal aging, parkinsonism dementia, and dementia pugilistica (*7*). The tentative association of the AD phenotype with trisomy 21 thus represents an intriguing, though inconclusive, clue to the possible location of the FAD gene.

The existence of large FAD pedigrees has raised the prospect that the chromosomal location of the FAD gene could be discovered by identifying cosegregation (genetic linkage) of a polymorphic marker with the disorder. In recent years, the power of this strategy has increased dramatically, because large numbers of DNA markers detecting restriction fragment length polymorphisms (RFLPs) in human DNA have been characterized (*8*). Linkage with DNA markers has already been used to map the defects in Huntington's disease, adult polycystic kidney disease, and cystic fibrosis, as well as several X-linked disorders (*9*).

We have applied the strategy of genetic linkage analysis with DNA markers to four large kindreds with histologically proven FAD. We have established and banked cell lines from particular individuals to act as an inexhaustible source of DNA for RFLP studies. Our results indicate that, although the FAD gene does map to chromosome 21, it is not located in the 21q22 region associated with the DS phenotype (*10*). Instead, our data provide strong evidence for the location of the FAD defect near two marker loci closer to the centromere in the region 21q11.2→ 21q22.2. This location does not contradict previous work since DS patients trisomic for only band 21q22 are rare, and it is not known whether these patients express AD-like neuropathology.

Pedigree and clinical data from four large FAD kindreds (Fig. 1) were collected by members of the research group, who were blind to the results of the molecular genetic studies (*11*). The diagnosis of FAD for deceased family members was documented by examination of medical records and by neuropathologic examination of postmortem brain tissue. Diagnosis of FAD in living members of the kindreds was established with standard clinical and laboratory criteria (*12*) that are very accurate (*13*). Wherever feasible, independent clinical information was also obtained from another, usually local, neurologist. During the course of the

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investigation, autopsies have been performed on seven patients who had an antemortem diagnosis of FAD. The neuropathologic examination confirmed the clinical diagnosis in all cases.

Carriers of the FAD gene generally do not develop symptoms until the fifth decade of life, and consequently the genetic status of asymptomatic individuals remains uncertain. In these families, however, there is a well-defined period during which symptoms usually appear. It is therefore possible to construct a cumulative age of onset curve depicting the age-dependent risk of developing AD. The age-risk curve can be used to assess the likelihood that a currently asymptomatic "at risk" individual is actually a presymptomatic carrier of the FAD gene. This age-corrected risk is then incorporated into the likelihood calculations performed during linkage analysis.

Several individuals in these pedigrees relate subtle clinical symptoms which, although suggestive of early FAD, are insufficient to establish a definitive diagnosis. Because diagnosing an individual without FAD as having the disease will lead to spurious results from the linkage analysis, we have chosen the conservative approach of considering these individuals as presently asymptomatic, with a risk determined by their age.

FAD1, the first pedigree in Fig. 1, is a large kindred of British descent whose ancestors emigrated to eastern Canada in 1837 (*2*). Fifty-four cases of AD have been described in eight generations. Although the frequency of DS and hematologic malignancy may be increased in FAD families (*3*), no cases of either have been observed in this pedigree. Postmortem neuropathologic studies on seven affected members showed typical features of AD with neuronal loss, prominent neurofibrillary tangles, senile plaques, and granulovacuolar changes in the cerebral cortex. We also observed no specific abnormalities in the cerebellum, brainstem, or spinal cord. Accurate data concerning the age of onset was available for 25 individuals (52 ± 6.23 years; mean ± SD); an age-dependent risk curve was then compiled for the development of AD in this family.

FAD2 is a pedigree of German origin with 20 cases of FAD in six generations. No cases of DS or hematologic malignancy have been recorded. The diagnosis of FAD was confirmed by neuropathology in three individuals. In one of these diagnosed individuals, in addition to the classical changes of AD in the cerebral cortex, amyloid plaques were observed in the cerebellum. Such cerebellar amyloid plaques, however, have been described in both familial and nonfamilial cases of AD (*14*). Reliable data for age of onset was known for 12 cases (48.7 ± 5.73 years) and was similar to that for FAD1.

FAD3, a large kindred of Russian origin (*2*), includes 23 definite cases of FAD in six generations and a single case each of DS, amyotrophic lateral sclerosis (ALS), and a myeloproliferative disorder. Nine of the 23 positive diagnoses have been confirmed by neuropathology, and in all nine cases the changes observed were exclusively those of AD. Accurate data on age of onset was available in 18 cases (49.8 ± 4.84 years).

FAD4 includes 48 cases in eight generations (*2*). The pedigree originated in Calabria, Italy, although branches of the family have emigrated to the United States and France. Clinical features of the disease in this family are similar to those of the other pedigrees except that myoclonus and seizures appear more frequently in the terminal stages of the illness. Both myoclonus and seizures have been reported previously in familial and nonfamilial cases of AD (*15*). No instances of DS or hematologic malignancy were recorded in FAD4. Histologic examination of either antemortem biopsy or postmortem material (six cases) from the European and American branches of the family showed prominent neurofibrillary tangles, senile plaques, extracellular amyloid, and in some cases, amyloid angiopathy in the cerebral cortex. In addition, neurofibrillary tangles were also observed in the brainstem, dentate and lentiform nuclei, and spinal cord. The age of onset in this family (39.9 ± 7.18 years), based on 21 cases, was younger than in the other three pedigrees. Consequently, genetic data from this kindred were analyzed with both the overall age-risk curve generated for all four families (normal distribution, 50 ± 7 years) as well as with the age-risk curve already generated for this family.

Available members of the four FAD kin-

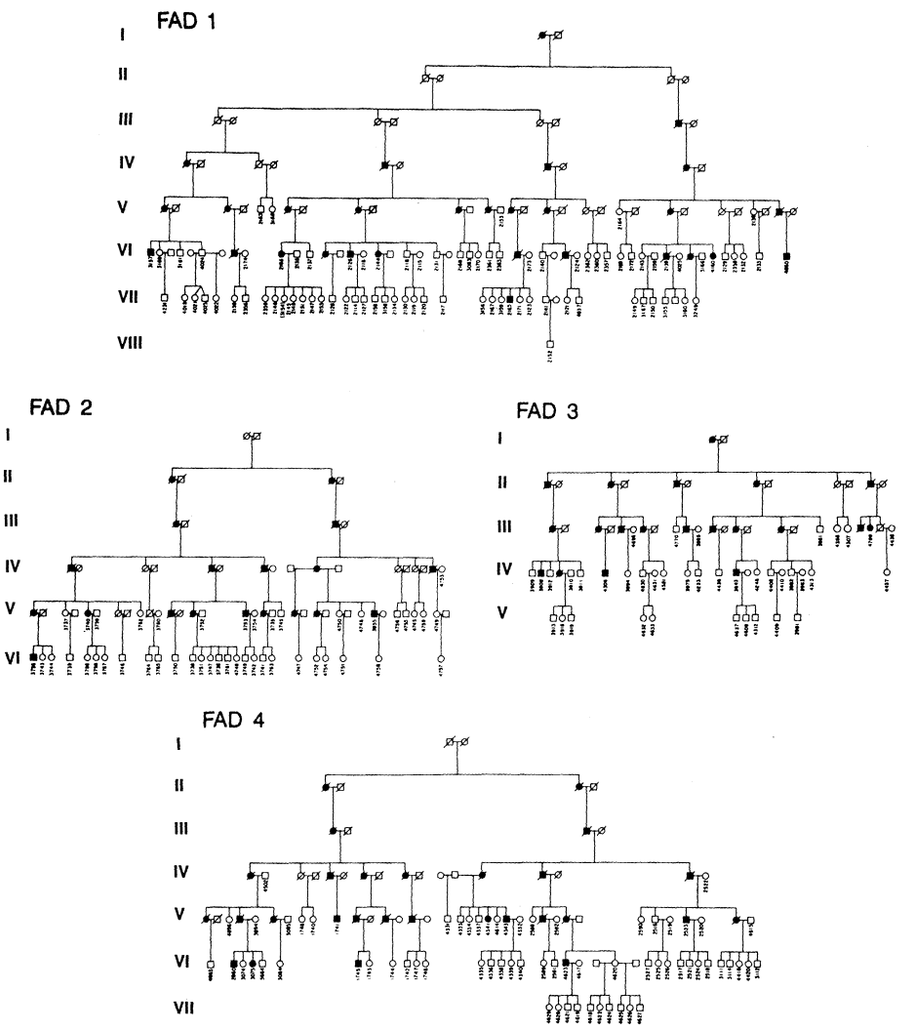

Fig. 1. Familial Alzheimer's disease pedigrees—FAD1, FAD2, FAD3, and FAD4. The following symbols are used: □, male; ○, female; solid symbol, affected with FAD; slashed symbol, deceased. Four digit numbers beneath these symbols indicate members who have been examined for signs of FAD, and for whom fibroblast and Epstein-Barr virus transformed lymphoblast cell lines have been established and stored in liquid nitrogen (*27*).

dreds were typed with ten DNA markers (Table 1) detected by anonymous DNA probes that have been regionally mapped on chromosome 21 (Fig. 2) (*16*). Seven markers are simple two allele systems. The other three loci are multiple allele markers for which haplotypes can be constructed by monitoring variation at more than one restriction enzyme site. For seven of the DNA markers, relative linkage relationships have been previously determined by multilocus analysis in families without FAD (*17, 18*). The order of the loci and frequency of recombination between them have been combined to generate a linkage map that spans the long arm of chromosome 21 and covers approximately 73 recombination units. For several loci, regional physical mapping has also been performed, either by somatic cell hybrids that contained a portion of human chromosome 21 (*19*) or by in situ hybridization (*20*). The relation of the linkage and physical mapping data summarized in Fig. 2 demonstrates that recombination occurs relatively more frequently in the distal portion of the long arm (*17*). For three markers, D21S16, D21S55, and D21S53, there is insufficient data to determine their precise order on the linkage map. The physical locations of these markers, however, permit the portion of the linkage map in which they reside to be approximated (Fig. 2).

For each marker locus, data from the FAD pedigrees was analyzed with the computer program LIPED (*21*) [with an age of onset correction (*22*)] to calculate the likelihood of linkage to FAD at various recombination fractions (θ = 0.00, 0.05, 0.10, 0.20, 0.30, 0.40) relative to the likelihood of nonlinkage (θ = 0.50). The logarithm of each likelihood ratio (lod score) (log of the odds; z) is shown in Tables 1 and 2. A lod score less than −2 is considered to exclude linkage to the disease locus, while a lod score greater than +3 is generally considered proof of linkage. For convenience, the markers can be considered separately in two groups based on their being either in band 21q22, or above 21q22.

Band 21q22 contains the region of chromosome 21 that must be trisomic for full expression of the DS phenotype (*10*). The linkage scores for FAD to each of the markers in this region are uniformly negative (Table 2). The two allele systems, D21S15, D21S17, D21S19, and D21S58 were weakly informative and provided exclusion of FAD from only a short interval on either side of the marker locus. Notably, D21S58 is tightly linked to *SOD1* (*18*), the locus encoding superoxide dismutase 1, in subband 21q22.1 and provides exclusion of the FAD defect from the immediate vicinity of this gene. The two markers for which haplotypes of individual RFLPs could be constructed were considerably more useful. These provided substantial regions of exclusion that extrapolate to approximately 14% and 7% recombination on either side of D21S53 and D21S55, respectively.

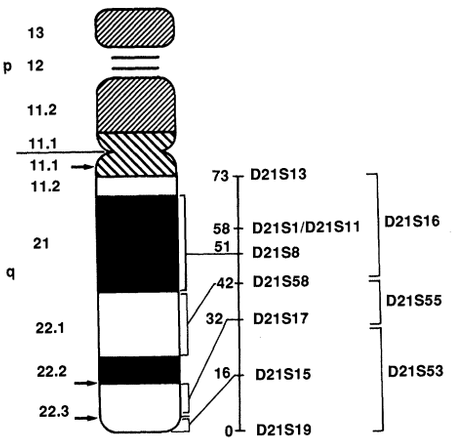

Fig. 2. Location of DNA markers on chromosome 21. The linkage relations of seven DNA loci on chromosome 21 previously established from multipoint analysis of these and other markers is given by the vertical line to the right of the chromosome. Numbers on the line represent recombination units (1 recombination unit = 1% recombination) derived from the frequency of recombination observed between these markers in a large reference pedigree (*17*). Physical locations of individual markers are denoted by the bracketed regions.

The four DNA markers mapping above 21q22 yielded mixed results (Table 2). Although close linkage was excluded for markers D21S13 and D21S8, the loci D21S16 and D21S1/D21S11 gave positive scores. The recombination value $\hat{\theta}$) at which the maximum lod score ($\hat{z}$) is obtained provides

Table 1. Polymorphic DNA markers on chromosome 21. Chromosome 21 DNA markers and the DNA fragments characteristic of each allele have been described (*16*). Allele or haplotype frequencies were based on 75 to 150 chromosomes from unrelated individuals of Western European or North American origin, including all unrelated spouses in the FAD pedigrees. Alleles for single RFLPs were named "+" or "−," based on the presence or absence of cleavage at the variable site. D21S1/D21S11 represents a composite of the two loci D21S1 and D21S11 detected by the anonymous DNA probes pPW228C and pPW236B, respectively. It is feasible to construct haplotypes from the four RFLPs detected by these two probes because the two loci are very tightly linked with no crossovers detected in over 150 informative events (*17, 18*). Linkage disequilibrium for the RFLPs supports the view that they are located in close proximity on chromosome 21 but limits the informativeness of the marker. As a result, only 3 of the 16 possible haplotypes comprise more than 90% of chromosomes tested. Haplotypes based on the Msp I, Bam HI, Eco RI, and Taq I sites, respectively, are: A, −+−−; B, ++++; C, +−++; and D, which represents all other possible haplotypes. Haplotypes for D21S53 were assigned RFLPs with Bcl I, Sac I, and Apa I, detected by independent subclones from cosmid clone 512: A, −−−; B, −−+; C, −+−; D, −++; E, ++−; and F, others. D21S55 displays four haplotypes named in order of the sizes of the bands resulting either from variation at two different Xba I sites or from an insertion-deletion mechanism.

Locus	Probe name	Restriction enzyme	Frequency		Haplotype	
			− Allele	+ Allele		
D21S8	pPW245D	Hind III	0.31	0.69		
D21S13	pGSM21	Taq I	0.29	0.71		
D21S15	pGSE8	Msp I	0.54	0.46		
D21S16	pGSE9	Xba I	0.25	0.75		
D21S17	pGSH8	Bgl II	0.57	0.43		
D21S19	pGSB3	Pst I	0.80	0.20		
D21S58	pPW524-5P	Pst I	0.46	0.54		
D21S1/ D21S11	pPW228C	Bam HI			A	0.26
		Msp I			B	0.40
	pPW236B	Eco RI			C	0.25
		Taq I			D	0.09
D21S53	pPW512-16P	Bcl I			A	0.06
	pPW512-6B	Sac I			B	0.20
	pPW512-18P	Apa I			C	0.59
					D	0.08
					E	0.04
					F	0.03
D21S55	pPW518-1R	Xba I			A	0.47
					B	0.28
					C	0.22
					D	0.03

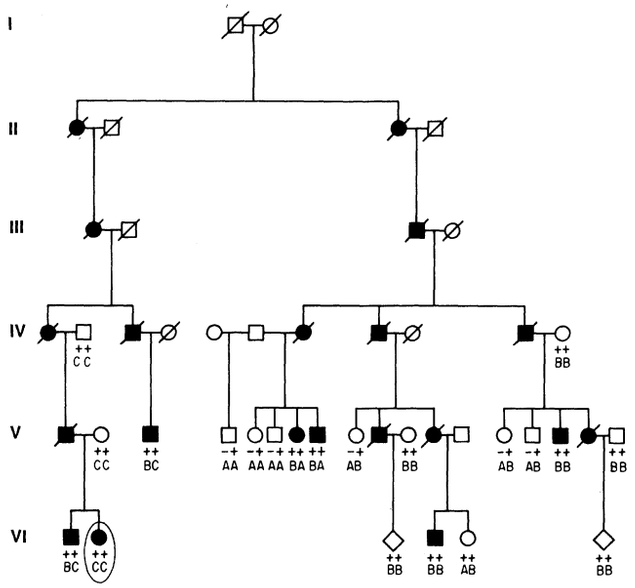

Fig. 3. Segregation of D21S16 and D21S1/D21S11 in FAD4. Individual genotypes at the loci D21S16 (alleles, − or +) and D21S1/D21S11 (haplotypes A, B, C, or D) are displayed for members of pedigree FAD4 in whom the phase of the marker alleles is unambiguously known. To preserve confidentiality, where the inclusion of at-risk individuals has been necessary to show the reconstructed genotype of a deceased affected parent, a symbol (◇) has been used to indicate one or more individuals who display a given genotype. A single recombination event between FAD and the D21S1/D21S11 locus was observed in both pedigree FAD4 (circled) and in pedigree FAD1 (not shown). The genotype of each available member of the pedigrees was ascertained by digesting 5 μg of genomic DNA with the appropriate restriction enzyme. The resultant DNA fragments were resolved according to size by horizontal agarose gel electrophoresis in TBE buffer (90 m*M* tris, 90 m*M* borate, 1 m*M* EDTA), and, after staining with ethidium bromide for direct visual inspection, the DNA fragments were denatured in situ in the gel with 1*N* NaOH. The denatured DNA fragments were transferred to a nylon filter support (Zetapor–AMF/Cuno, Meriden, CT) by Southern blotting, and fixed to the filter by baking. DNA probes were labeled with [α-^{32}P]adenosine triphosphate (Amersham) by random oligonucleotide priming (*28*) and then hybridized to the filter for 48 hours at 65°C in 6× SSC (1× SSC = 0.15 *M* sodium chloride and 0.15*M* citrate), 1× Denhardt's solution, 0.3% SDS, and salmon testis DNA (100 μg/ml). The filters were washed in 0.5× SSC at 65°C, and exposed to x-ray film (Kodak XAR-5) with a Dupont Cronex intensifying screen at −80°C for 48 hours. Alleles present in each individual were deduced from the sizes of bands on the developed film.

the best estimate of the recombination frequency separating the defect from the marker locus. For D21S16, the positive lod score derives mainly from family FAD4. It has its maximum ($\hat{z}$ = 2.32) at $\hat{\theta}$ = 0.00 indicating that there are no obligate recombination events between the marker locus and the defect in any of the pedigrees. D21S1/D21S11 yielded positive scores with all four families, although FAD2 provided a negligible contribution to the total because of limited informativeness of the available matings. The maximum lod score, $\hat{z}$ = 2.37, occurred at $\hat{\theta}$ = 0.08. Representative data for both D21S16 and D21S1/D21S11 are shown (Fig. 3) for a portion of pedigree FAD4, which contains one obligate crossover between the latter marker and the gene defect.

Table 2. Lod scores for linkage of FAD with DNA markers. (**A**) Lod scores (z) summed over all families for linkage of FAD with individual marker loci in band 21q22. The region from which the FAD gene is excluded ($z < -2$) surrounding each marker is given. (**B**) Lod scores for markers above 21q22. Peak positive lod scores provide a maximum likelihood estimate of the recombination fraction ($\hat{\theta}$) for two markers. Lod scores were calculated by using LIPED with an age-of-onset correction (*21, 22*). A gene frequency of 0.0001 was assumed for the FAD defect. A family-specific curve was used for FAD4 and a general curve was used for the other families, as described in the text. Use of the general curve for FAD4 minimally reduced the peak score with D21S1/D21S11 for pedigree FAD4 by 0.18 at the same $\hat{\theta}$ value, but had no effect on the scores of D21S16.

A. Markers in 21q22

Marker locus	Pedigree	Recombination fraction (θ)						Limit of exclusion ($z < -2$)
		0.00	0.05	0.10	0.20	0.30	0.40	
D21S15	Total	−∞	−1.90	−0.88	−0.19	−0.04	−0.02	θ = 0.04
D21S17	Total	−∞	−0.97	−0.41	−0.03	+0.02	+0.01	θ = 0.02
D21S19	Total	−3.26	−0.49	−0.24	−0.09	−0.03	−0.02	θ = 0.01
D21S53	Total	−∞	−3.86	−2.70	−1.49	−0.90	−0.47	θ = 0.14
D21S55	Total	−∞	−2.68	−1.33	−0.21	+0.10	+0.10	θ = 0.07
D21S58	Total	−∞	−1.33	−0.82	−0.30	+0.05	+0.05	θ = 0.02

B. Markers above 21q22

Marker locus	Pedigree	Recombination fraction (θ)						Peak lod score ($\hat{z}$)
		0.00	0.05	0.10	0.20	0.30	0.40	
D21S8	Total	−∞	−1.33	−0.52	+0.03	+0.10	+0.03	
D21S13	Total	−4.18	−0.64	−0.33	+0.11	−0.04	0.00	
D21S16	FAD1	+0.15	+0.10	+0.06	+0.02	+0.01	+0.01	
	FAD2	+0.13	+0.10	+0.08	+0.04	+0.02	+0.01	
	FAD3	−0.42	−0.29	−0.20	−0.10	−0.04	+0.01	
	FAD4	+2.46	+2.19	+1.91	+1.34	+0.76	+0.23	
	Total	+2.32	+2.10	+1.85	+1.30	+0.75	+0.26	+2.32 ($\hat{\theta}$ = 0.00)
D21S1/ D21S11	FAD1	−∞	+0.59	+0.61	+0.41	+0.18	+0.03	
	FAD2	−0.20	+0.04	+0.12	+0.12	+0.07	+0.02	
	FAD3	+0.62	+0.63	+0.61	+0.49	+0.33	+0.16	
	FAD4	−∞	+1.00	+1.01	+0.79	+0.47	+0.16	
	Total	−∞	+2.26	+2.35	+1.81	+1.05	+0.37	+2.37 ($\hat{\theta}$ = 0.08)

The positive scores obtained with D21S16 and D21S1/D21S11 suggest that the FAD gene is located in the vicinity of these markers on chromosome 21. Because neither lod score exceeds +3, however, they cannot by themselves be considered proof of linkage. We have previously determined by two-point linkage analysis in kindreds without FAD that the two marker loci, D21S16 and D21S1/D21S11, are genetically linked with a maximum likelihood estimate of $\hat{\theta}$ = 0.08 ($\hat{z}$ = 4.26). The relationship of D21S16 to other chromosome 21 markers has not yet been clearly defined; consequently, it could be located either above or below D21S1/D21S11. However, knowledge of the frequency of recombination between D21S16 and D21S1/D21S11 permitted us to maximize the linkage information from our pedigrees with multipoint methods of analysis. We employed LINKMAP, from the LINKAGE package of computer programs (*23*), to perform a three-point analysis of FAD, D21S16, and D21S1/D21S11. The program was used to approximate lod scores in favor of linkage at many locations relative to the two fixed DNA markers (Fig. 4). A peak score of 4.25 was obtained, confirming that the FAD gene does reside on chromosome 21. Although the position of the peak occurs between the two DNA markers, a secondary peak with a score of 4.06 occurs to one side of both DNA loci. Thus, although the multipoint analysis provided conclusive evi-

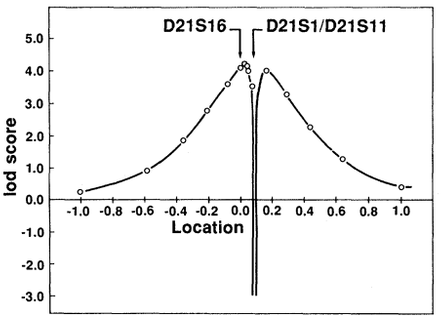

Fig. 4. Multipoint linkage analysis of FAD, D21S16, and D21S1/D21S11. The program LINKMAP from the LINKAGE package (version 3.5) was used to calculate lod scores for various locations of the FAD gene relative to fixed positions for the two DNA loci. D21S16 was arbitrarily placed at 0.0. Based on the previously established recombination frequency between the two loci, D21S1/D21S11 was set at a map distance of 0.087 with Haldane's formula as described in the documentation of LINKAGE. Lod scores at each map location were approximated in LINKMAP by subtracting the log10 of the likelihood for a distant map position (−10, to approximate the "unlimited" state) from the log10 of the likelihood at the given location. Two peaks are observed with lod scores of 4.25 and 4.06, to the left and right, respectively, of D21S1/D21S11. For these calculations, the family-specific age-of-onset curve was used for FAD4, while the general curve was used for the other three pedigrees. If the general curve was used in all cases, the maximum lod score was reduced but remained above the critical value of +3 at its peak.

dence that the FAD gene does map to chromosome 21, it was unable to provide firm support for the relative order of the three loci tested. The curve (Fig. 4) plunges to $-\infty$ at the position of D21S1/D21S11 due to the presence of one obligate crossover with FAD in each of FAD1 and FAD4 (Fig. 3). Multipoint analysis employing either of the other two loci mapped in this region, D21S13 and D21S8, also yielded positive lod scores but did not provide conclusive evidence to support a particular location of FAD relative to D21S1/D21S11.

Contrary to initial expectations based on the association between AD and DS, the FAD gene is located outside the obligate DS region, in the 21q11.2→21q21 region of chromosome 21. The precise localization of the FAD gene relative to the DNA markers D21S1/D21S11 and D21S16, however, has been hampered by the fact that FAD pedigrees are typically less informative than those of other autosomal dominantly inherited diseases of the central nervous system such as Huntington's disease. The limited informativeness of FAD pedigrees stems largely from the late onset of symptoms and the subsequent rapid demise of affected subjects. The consequence of these clinical characteristics is that, within a single family, relatively few informative subjects are available for study at any given time. The examination of additional pedigrees will therefore be necessary, not only to confirm these results, but also to determine the exact position of the FAD gene in the 21q11.2→21q21 region. Linkage studies with other FAD pedigrees will also be required to address the possibility of nonallelic genetic heterogeneity in this disorder. However, the fact that minor differences in clinical presentation were observed among these families indicates that the defect on chromosome 21 is not restricted to a single phenotype or ethnic origin of FAD.

Testing of additional DNA markers from 21q11.2→21q21 region of chromosome 21 should permit a better definition of the location of the FAD defect and facilitate attempts to isolate and characterize the gene affected in FAD. Furthermore, even before the FAD gene is isolated, use of linked markers could establish whether sporadic AD also results from an allelic genetic defect that has a heritability difficult to detect due to either a very late age of onset or a reduced penetrance (*4, 5*). Clarification of this issue will determine the degree to which environmental factors must be sought to explain the high incidence of AD. The similarity between the spectrum of clinical features observed in sporadic cases of AD, and those observed in these FAD pedigrees suggests that even if the majority of AD cases have a nongenetic etiology, knowledge of the function of the FAD gene may provide useful insights into the pathogenesis of sporadic forms of the disorder.

The existence of DNA markers linked to the FAD gene raises the possibility that they may be of use for presymptomatic or prenatal diagnosis of FAD in appropriate families. However before "at-risk" individuals can be offered an informed choice, it will be necessary to confirm our results with additional probes from this region, to define the precise recombination frequencies between these markers and the FAD gene, and to examine the question of nonallelic genetic heterogeneity in other FAD pedigrees. Furthermore, given the current uncertainty about the genetic origin of AD in any individual case, it is likely that the number of families in which genetic counseling could be accurately performed is small. Finally, the ethical, social, and legal issues surrounding predictive testing in a currently untreatable late onset disorder associated with cognitive impairment must also be resolved.

The major significance of our findings is that they provide a basis for attempts to identify the gene causing FAD. The AD-like phenotype of aged DS individuals presumably results from the overexpression of an otherwise normal gene (or genes) on chromosome 21 as a result of increased gene dosage. The mapping of FAD to chromosome 21 suggests that FAD may involve the same gene. FAD, however, could result either from expression of an abnormal protein or from overexpression of a normal protein due to, for instance, gene duplication or amplification, increased transcription, increased RNA processing, or decreased degradation of the gene product. We have recently isolated and mapped to chromosome 21 a gene encoding the amino acid sequence of the amyloid β peptide found in senile plaques of both AD and DS (*24, 25*). The chromosomal localization of FAD and the amyloid β peptide gene to the same region of chromosome 21 makes the latter a strong candidate for the site of the primary FAD defect (*25*). It is, as yet, unknown whether the two genes are identical. In the absence of conclusive proof, it remains possible that the localization of both genes on chromosome 21 is merely coincidental.

The ultimate promise of a linked marker for FAD is that it should permit cloning and characterization of the defect based only on its chromosomal location without any prior knowledge of the nature of the gene. The dramatic success of this approach in identifying and isolating the gene causing Duchenne muscular dystrophy bodes well for similar efforts in FAD (*26*). An understanding of the FAD defect at the molecular level could help to define the biochemical pathways involved in initiation and progression of various clinical and neuropathologic features of the disease. Clues may also be obtained concerning environmental factors that affect similar functions in causing sporadic AD.

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Cytochrome P-450–Catalyzed Formation of Δ^4-VPA, a Toxic Metabolite of Valproic Acid

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Liver damage induced by the antiepileptic drug valproic acid (VPA) is believed to be mediated by an unsaturated metabolite of the drug, Δ^4-VPA. In studies of the biological origin of this hepatotoxic compound, it was found that liver microsomes from phenobarbital-treated rats catalyzed the desaturation of VPA to Δ^4-VPA. Indirect evidence suggested that cytochrome P-450 was the responsible enzyme, a conclusion that was verified by studies with a purified and reconstituted form of the hemoprotein, which catalyzed the oxidation of VPA to 4- and 5-hydroxyvalproic acid and to Δ^4-VPA. Desaturation of a nonactivated alkyl substituent represents a novel metabolic function of cytochrome P-450 and probably proceeds via the conversion of substrate to a transient free radical intermediate, which partitions between recombination (alcohol formation) and elimination (olefin production) pathways. These findings have broad implications with respect to the metabolic generation of olefins and may explain the increased hepatotoxic potential of VPA when it is administered in combination with potent enzyme-inducing anticonvulsants such as phenobarbital.

VALPROIC ACID (VPA, FIG. 1) IS A simple, branched-chain fatty acid that has broad-spectrum anticonvulsant activity and is used in the treatment of multiple forms of epilepsy (*1*). Although VPA was initially considered to be relatively free from serious side effects, there is recent clinical evidence that small numbers of patients develop serious liver damage while maintained on VPA therapy (*2*). Indeed, this hepatic injury may prove fatal, and estimates of the frequency of VPA-related patient deaths have ranged from 1 in 37,000 to 1 in 500, depending on factors such as the age of the recipient and the nature of concomitant therapy (*3*). Although the mechanism of this liver toxicity remains unknown, animal studies have implicated 2-*n*-propyl-4-pentenoic acid (Δ^4-VPA), an unsaturated metabolite of the drug (Fig. 1), as a causative agent (*2*). Thus, Δ^4-VPA was found to be the most toxic metabolite of VPA in rat hepatocytes in vitro (*4*), to inhibit both hepatic cytochrome P-450 (*5*) and fatty acid β-oxidation activity (*6*) in vitro, and to be a potent inducer in vivo of hepatic microvesicular steatosis (the characteristic tissue lesion in VPA-induced liver injury) (*7*).

Typically, Δ^4-VPA is present at low concentrations in biological fluids from humans and animals given VPA (*8*), although very high levels of this metabolite were detected in a child who died from VPA-induced liver failure (*9*). Studies in the rhesus monkey have shown that the fraction of an intravenous dose of VPA that is metabolized to the Δ^4 olefin may be as high as 3 to 4% (*10*), and thus the quantitative significance of the desaturation pathway leading to this toxic product may be greater than suspected previously. Despite the interest in Δ^4-VPA, however, details of the metabolic process by which the parent drug is transformed to this unsaturated product are unclear. One early hypothesis was that Δ^4-VPA is formed by loss of the elements of water from 4-OH-VPA or 5-OH-VPA (*11*). This possibility is unlikely, since control experiments have shown that these alcohols are chemically (*8*) and metabolically (*12*) stable entities that do not undergo dehydration reactions. The metabolic origin of Δ^4-VPA, therefore, remains obscure.

In studies with rats, Lewis *et al.* (*13*) reported that the incidence of VPA-induced liver injury was increased in animals treated with phenobarbital. Significantly, many of the human subjects who developed serious hepatotoxicity during VPA therapy had been treated concomitantly with enzyme-inducing drugs such as phenobarbital or phenytoin (*2*). Since these agents have a profound influence on microsomal enzymes in general, and on the cytochrome P-450 system in particular (*14*), we investigated whether Δ^4-VPA was formed in hepatic microsomes from phenobarbital-treated rats (*15*).

Metabolites of VPA formed in microsomal incubations were isolated, converted to

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