Absence of Linkage of Chromosome 21q21 Markers to Familial Alzheimer's Disease

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Alzheimer's disease is the most common form of dementia among the elderly population. Although the etiology is unknown, inheritance plays a role in the pathogenesis of the disease. Recent work indicates that an autosomal dominant gene for Alzheimer's disease is located on chromosome 21 at band q21. In the present study of a group of autopsy-documented kindreds, no evidence for linkage was found between familial Alzheimer's disease (FAD) and chromosome 21q21 markers (D21S1/D21S72 and the amyloid β gene). Linkage to the D21S1/D21S72 locus was excluded at recombination fractions (θ) up to 0.17. Linkage to the amyloid gene was excluded at θ = 0.10. Apparent recombinants were noted in two families for the amyloid gene and in five families for the D21S1/D21S72 locus. These data indicate that FAD is genetically heterogeneous.

ALZHEIMER'S DISEASE (AD) IS A progressive neurodegenerative disorder characterized clinically by loss of memory, confusion, and eventual profound disintegration of cognitive function. Although clinical diagnosis of AD is accurate 80 to 90% of the time (*1*), autopsy examination is essential to establish AD as the cause of dementia. Pathologic hallmarks include neurofibrillary tangles, amyloid plaques, congophilic angiopathy, and granulovacuolar change. These same features are also observed in brains from Down syndrome subjects (trisomy 21) over the age of 30 to 40 years (*2*). Deposition of the same amyloid β peptide is seen in the congophilic angiopathy (*3*) and amyloid plaques (*4*) of both AD and Down syndrome, suggesting that a similar or identical disease process is involved.

Epidemiologic evidence (*5, 6*), the occurrence of concordant monozygotic twins (*7*), and the relation between AD and Down syndrome (*2*), strongly suggest that a genetic component is involved in at least some instances of AD. In fact, one genetic model is that all AD is the result of a relatively common autosomal dominant gene (*6, 8*) with an estimated gene frequency of 0.06. Recently, St George-Hyslop *et al.* (*9*) reported that familial Alzheimer's disease (FAD) in four families is linked to two chromosome 21q21 loci, D21S1/D21S11 and D21S16, at a recombination fraction (θ) of 0.08 and 0.00, respectively. D21S1/D21S11 is in turn linked to the amyloid β gene at θ = 0.04 (*10*) and is localized at band q21.15 to q21.2 (*11*). Thus the q21 region of chromosome 21 is of particular interest for FAD. We have assembled a group of pedigrees in which autopsy-documented FAD appears to be inherited as an autosomal dominant trait. In these families, FAD is not closely linked to the chromosome 21q21 markers, D21S1/D21S72 and the amyloid gene.

Probands for FAD pedigrees were identified at the University of Washington Medical Genetics clinic, or were referred by colleagues at other medical centers. Fourteen FAD kindreds were selected (Table 1) by the following criteria: (i) affected subjects having adult onset, progressive memory loss, and dementia consistent with published clinical criteria for AD (*12*); (ii) two or more affected generations; and (iii) at least one autopsy with neuropathologic findings consistent with AD (*13, 14*). A 15th family (HD) for which no autopsy data are available was included because it met clinical criteria for AD and because it is a kindred originating from the same village as the other Volga German families in our study (see below) and shares surnames with them. All autopsied kindreds had neuropathologic findings fully consistent with AD (*15*) including neurofibrillary tangles, amyloid plaques, and granulovacuolar change. Autopsy material from six brains in five families (L, HB, R, H, and W) was stained with antibody directed against the amyloid peptide. In all cases, the senile plaques stained positively. In the E family, one demented individual had clinical symptoms and pathologic features consistent with Creutzfeld-Jacob disease (*16*). This subject was not included in the linkage analysis. Other affected subjects in this kindred had clinical and pathological findings consistent with AD. One demented person in the CSF family with autopsy-documented AD also had signs of anterior horn cell loss in the spinal cord. The clinical and neuropathological findings for all of these kindreds are described in detail elsewhere (*13, 14*).

Seven of the families have a common ethnic and geographic background (Table 1), and are referred to as Volga Germans (*13*). These individuals are descended from Germans who migrated to two small adjacent towns on the Volga river in the Soviet Union and subsequently emigrated to the United States. Affected individuals in these families probably descended from a common genetic founder (whose identity re-

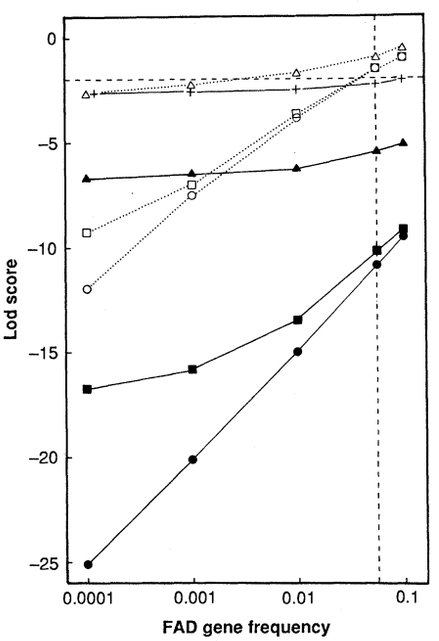

Fig. 1. Lod scores for FAD versus D21S1/D21S72 calculated at different FAD gene frequency values. Lod scores are for the kindreds listed in Table 1. Calculations were performed with either a cumulative normal age-of-onset correction (closed symbols and +) or with penetrance set at 1% (open symbols). Values for θ are: circles, 0; squares, 0.001; triangles, 0.05; and +, 0.15. The horizontal dashed line corresponds to a lod score of −2, the accepted value for exclusion of linkage. The vertical dashed line indicates a gene frequency value of 0.06 (*22*). Exclusion limits based on 1% penetrance calculations were θ ≤ 0.07 (lod = −2.04, gene frequency = 0.0001), θ ≤ 0.06 (lod = −2.05, gene frequency = 0.001), and θ ≤ 0.04 (lod = −2.11, gene frequency = 0.01).

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Table 1. Characteristics of FAD families. The ratio of affected males to affected females is 1/0.94. The pedigrees have been described (*13, 14, 16, 28*). In some families, age-of-onset data were not available for every affected subject.

Family	Ethnic background	Age-of-onset		Number of affected generations	Number of affected individuals	Number sampled*	Number of autopsies
		Mean ± SD	*n*				
L	German	41.1 ± 5.1	12	3	12	19	6†
V	English?	47.4 ± 4.3	6	3	8	2	1
CK	Lithuanian	64.0 ± 11.3	2	2	2	2	1
P	English	67.0 ± 2.5	4	2	7	4	1
T	Norwegian	67.8 ± 6.6	5	2	5	2	2
CSF	Danish	69.5 ± 6.1	4	2	4	7	1
MI	English?	70.3 ± 4.6	4	2	4	3	1
JR	English?	77.7 ± 2.3	3	2	3	5	2
R	Volga German	50.6 ± 7.3	17	4	20	29	4
W	German	54.3 ± 4.5	4	2	4	4	1
HD	German	54.9 ± 6.4	12	4	14	5	0
E	German	56.6 ± 5.0	5	3	6	13	3‡
H	German	57.8 ± 1.1	5	3	7	2	3
HB	German	58.0 ± 6.7	18	3	20	23	4†
KS	German	63.1 ± 5.9	10	3	11	17	1
	Volga Germans				82	93	16
	All families				127	137	31

*DNA samples were obtained from affected and unaffected family members and appropriate spouses. †Includes one autopsy from an unaffected individual. ‡Includes one autopsy suggesting Creutzfeld-Jacob disease.

Table 2. Lod scores for linkage of D21S1 to D21S72. RFLP sites for D21S1 and D21S72 have been described (*29*). Haplotypes were constructed by means of the computer program PATCH (*30*) for D21S1 by means of Msp I and Bam HI RFLP sites and for D21S72 by means of Taq I, Pvu II, and Hinc II RFLP sites. DNA was prepared from peripheral blood leukocytes or Epstein Barr virus–transformed lymphoblastoid cell lines by a modification of previously described procedures (*31*). Restriction enzyme digestion was performed according to manufacturer's instructions and fragments were resolved by agarose gel electrophoresis in circulated TEA buffer (40 m*M* tris-HCl *p*H 8.1, 4 m*M* EDTA, 40 m*M* acetate). DNA was transferred to Zetabind membranes by the NaOH method of Reed and Mann (*32*). Probes were labeled with ^{32}P by hexamer primer labeling (*33*) to a specificity of 1 to 4×10^8 cpm per microgram of DNA. Blots were hybridized and washed as described (*28*). The final wash was in 0.1× SSC, 0.5% SDS at 65°C for 1 hour. The blots were dried and exposed to Kodak XOMAT film at −70°C.

Recombination fraction (θ)					
0.0	0.001	0.1	0.2	0.3	0.4
38.85	38.76	29.89	20.89	12.03	4.11

mains unknown). In this group of families, FAD is likely to be genetically homogeneous.

We determined genotypes for eight restriction fragment length polymorphisms (RFLPs) at three chromosome 21 loci (Tables 2 to 4). Genotypes for the Taq I RFLP site at the D21S11 locus and the Taq I site at the D21S72 locus were identical in all individuals typed for both markers ($n = 124$). Further, polymorphic Taq I restriction fragment lengths apparently were identical when DNA blots of Taq I–digested genomic DNA were sequentially hybridized with the probes for each locus. Therefore D21S11 and D21S72 were considered to be the same locus, consistent with the work of others (*17*). Probes for D21S72 identify both the Eco RI and Taq I sites observed with the D21S11 probes as well as Pvu II and Hinc II RFLP sites. D21S11 and D21S1 are closely linked and in linkage disequilibrium, and no recombinants between these two loci have been observed (*9, 18*). Our FAD pedigree data demonstrate that D21S72 and D21S1 are closely linked (Table 2) and no recombinants were observed.

Two-point linkage analysis of D21S1/D21S72 haplotypes, D21S16, and amyloid β gene haplotypes versus FAD are shown in Tables 3 and 4. The data were analyzed with the computer program LIPED with a cumulative normal age-of-onset correction (*19*) and an FAD gene frequency of 0.001. Use of family-specific versus overall mean age-of-onset correction had negligible effects on the results. In both cases, the overall standard deviation was used. Linkage of FAD to D21S1/D21S72 was excluded up to $\theta = 0.17$, which includes the region previously reported to contain a FAD locus. Although the excluded region corresponds to a large fraction of the known genetic map of chromosome 21 (*9*), additional RFLP markers need to be typed to span the remaining portions of this chromosome. Evidence against linkage derives partly from five families (CSF, HB, KS, R, and V) in which two or more affected individuals did not share common alleles (apparent recombinants). A possible pitfall in studying diseases where penetrance may be incomplete is that most of the evidence against linkage might be due to individuals who have inherited the FAD gene but who are not clinically affected even at an advanced age. To eliminate the possibility that our assumptions about penetrance are inaccurate, the data were reanalyzed under the extreme assumption of 1% penetrance (effectively ignoring unaffected individuals); significant evidence against linkage was still obtained and linkage was excluded to $\theta = 0.06$ (gene frequency = 0.001, Fig. 1). D21S16 is not highly polymorphic (Table 3) and was not sufficiently informative in our families to yield significant results. Linkage between FAD and the amyloid β gene was excluded to $\theta = 0.10$ (Table 4). Apparent recombinants were observed in the HB and the L families. Thus, FAD in our families as in others reported (*20, 21*) does not appear to result from a mutation in the amyloid β gene.

Varying gene frequency can significantly affect the results of linkage analysis (Fig. 1). Using a cumulative normal age-of-onset correction and assuming the FAD gene is very rare (gene frequency = 0.0001) (*9*), we could exclude linkage to $\theta = 0.17$. Similarly, at a high gene frequency value (0.06) (*22*) linkage was excluded to $\theta = 0.16$. The choice of gene frequency values has a greater influence on exclusion when the data are analyzed assuming a penetrance of 1% (Fig. 1).

Our results of linkage exclusion seem to conflict with the findings of St George-Hyslop *et al.* (*9*). Roses *et al.* (*23*), studying late age-of-onset FAD kindreds (average family onset age >60 years), have also excluded linkage of FAD to D21S1/D21S11 in the region of interest. One possible explanation of these conflicting conclusions is genetic heterogeneity. We tested for linkage heterogeneity of FAD and D21S1/D21S72/D21S11 using the large sample test of Morton (*24*). Data corrected for age-of-onset were used. When all our families were compared to the four families of St George-Hyslop *et al.* (*9*), a test statistic $\chi_1^2 = 8.43$ ($P < 0.005$) was obtained. Similarly, comparison of just the Volga German families to the previous study resulted in $\chi_1^2 = 9.03$ ($P < 0.005$). These findings provide strong evidence for genetic heterogeneity.

Several explanations are possible for the difference between our results and the reported linkage of FAD to 21q21 markers. In our study, most of the evidence for exclusion to the D21S1/D21S72-amyloid region

comes from the presenile onset (<65 years) families including the L and V kindreds and three Volga German families (R, HB, and KS) with average ages-of-onset ranging from 41.1 to 63.1 years. Since this age range is somewhat higher than that of the families reported by St George-Hyslop *et al.* (39.9 to 52 years) (*9*) it is possible that a gene in this region of chromosome 21 accounts only for very early onset FAD (*23*). However, as there is no significant neuropathologic difference among these families, age-of-onset may not be a useful indicator of FAD subtypes. The fact that the Volga Germans were genetically isolated might account for a difference in genetic etiology. However, the fact that linkage is also absent in the non–Volga German families (both early and late onset) makes this explanation less likely. Finally the possibility that the positive lod scores obtained by St George-Hyslop *et al.* (*9*) were a chance occurrence cannot be ruled out.

Neuropathologic findings provide critical information for evaluating AD kindreds included in linkage studies. Although the clinical diagnosis of AD in sporadic cases typically is accurate in approximately 80 to 90% of cases by autopsy criteria, little information is available regarding the accuracy of diagnosis in familial dementia. In our studies, one kindred previously described as FAD has subsequently been found to have familial Gerstmann-Straussler syndrome (*25*). Inadvertent inclusion of non-FAD families because of misdiagnosis will have the effect of mimicking genetic heterogeneity. This has two important implications. First, the estimated distance between the disease and the linked marker will be overestimated (*26*). Second, the number of families necessary to detect linkage will be markedly increased even if the fraction of misdiagnosis is low (*27*). Thus autopsy data provide an important means for establishing a reasonable diagnosis of AD for purposes of linkage analysis and for comparing the rapidly expanding number of kindreds with dementia.

Table 3. Lod score for linkage of chromosome 21 markers to FAD. D21S1/D21S72 haplotypes were constructed with Msp I and Bam HI RFLP site data for D21S1, and with Taq I, Pvu II, and Hinc II RFLP site data for D21S72. The values were calculated on the basis of gene frequency = 0.001 for the FAD gene. Lod scores for D21S16 were calculated for the Xba I site (*34*) with allele frequencies of 0.86 for the 6.4-kb allele and 0.14 for the 7.3 kb allele.

Locus	Pedigree	Recombination fraction (θ)						Exclusion limit ($z < -2$)
		0.001	0.05	0.10	0.20	0.30	0.40	
D21S1/D21S72	CK	0.06	0.05	0.04	0.02	0.01	0.00	
	CSF	−2.29	−0.70	−0.42	−0.18	−0.07	−0.01	θ ≤ 0.001
	JR	−0.20	−0.16	−0.13	−0.07	−0.03	−0.01	
	L	0.16	0.27	0.33	0.36	0.27	0.09	
	MI	−0.16	−0.12	−0.10	−0.05	−0.02	−0.01	
	P	−0.03	−0.02	−0.02	−0.01	0.00	0.00	
	T	0.17	0.14	0.12	0.07	0.03	0.01	
	V	−2.07	−0.59	−0.34	−0.14	−0.06	−0.02	θ ≤ 0.001
				Volga Germans				
	E	0.14	0.14	0.13	0.11	0.07	0.03	
	H	0.06	0.05	0.04	0.02	0.01	0.00	
	HB	−4.84	−2.30	−1.52	−0.71	−0.29	−0.07	θ ≤ 0.06
	HD	−0.29	−0.22	−0.16	−0.07	−0.03	0.00	
	KS	−3.38	−1.76	−1.17	−0.55	−0.24	−0.08	θ ≤ 0.03
	R	−3.13	−1.32	−0.83	−0.34	−0.13	−0.04	θ ≤ 0.01
	W	−0.11	−0.10	−0.09	−0.06	−0.03	−0.01	
D21S1/D21S72 totals:								
Volga Germans		−11.56	−5.51	−3.60	−1.61	−0.63	−0.17	θ ≤ 0.17
All families		−15.91	−6.65	−4.12	−1.62	−0.51	−0.12	θ ≤ 0.17
D21S16 totals:								
Volga Germans		−0.20	−0.16	−0.11	−0.05	−0.01	0.00	
All families		−0.42	−0.32	−0.24	−0.11	−0.04	0.00	

Table 4. Lod scores for linkage of the amyloid gene to FAD. The values above were calculated with 0.001 as the FAD gene frequency. Haplotypes for the amyloid locus were constructed from data for an Eco RI RFLP site and a Bgl II RFLP site (*10, 21, 30*). The exclusion limit based on 1% penetrance calculations and gene frequency = 0.001 was θ ≤ 0.001).

Pedigree	Recombination fraction (θ)						Exclusion limit ($z < -2$)
	0.001	0.05	0.10	0.20	0.30	0.40	
CK	−0.03	−0.02	−0.01	−0.01	0.00	0.00	
CSF	−0.17	−0.15	−0.12	−0.07	−0.03	0.00	
JR	0.03	0.02	0.02	0.01	0.00	0.00	
L	−2.79	−1.10	−0.79	−0.42	−0.19	−0.08	θ ≤ 0.001
MI	−0.09	−0.07	−0.05	−0.03	−0.01	0.00	
P	−0.51	−0.33	−0.22	−0.09	−0.03	−0.01	
T	0.14	0.12	0.10	0.06	0.03	0.01	
V	0.00	0.00	0.00	0.00	0.00	0.00	
			Volga Germans				
E	0.01	0.01	0.01	0.00	0.00	0.00	
H	0.11	0.09	0.07	0.04	0.02	0.01	
HB	−2.50	−0.87	−0.58	−0.28	−0.12	−0.04	θ ≤ 0.001
HD	−0.11	−0.07	−0.05	−0.01	0.01	0.01	
KS	−0.23	−0.17	−0.13	−0.07	−0.04	−0.02	
R	−0.24	−0.19	−0.15	−0.09	−0.05	−0.02	
W	−0.32	−0.20	−0.12	−0.04	−0.01	0.00	
Volga Germans	−3.28	−1.41	−0.94	−0.45	−0.20	−0.07	θ ≤ 0.01
All families	−6.69	−2.93	−2.02	−1.01	−0.44	−0.12	θ ≤ 0.10

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