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Linkage Studies in Familial Alzheimer Disease: Evidence for Chromosome 19 Linkage

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Summary

A genetic component in the etiology of Alzheimer disease (AD) has been supported by indirect evidence for several years, with autosomal dominant inheritance with age-dependent penetrance being suggested to explain the familial aggregation of affecteds. St. George Hyslop et al. reported linkage of familial AD (FAD) in four early-onset families (mean age at onset [M] < 50 years). Subsequent studies have been inconsistent in their results; Goate et al. also reported positive lod scores. However, both Pericak-Vance et al.'s study of a series of mainly late-onset FAD families (M > 60 years) and Schellenberg et al.'s study failed to confirm linkage to chromosome 21 (CH21). These various studies suggest the possibility of genetic heterogeneity, with some families linked to CH21 and others unlocalized. Recently, St. George Hyslop et al. extended their analysis to include additional families. The extended analyses supported their earlier finding of linkage to CH21, while showing strong evidence of heterogeneity between early-onset (M < 65 years) and late-onset (M > 60 years) FAD families. Because our families did not show linkage to CH21, we undertook a genomic search for an additional locus for FAD. Because of both the confounding factor of late age at onset of FAD and the lack of clear evidence of Mendelian transmission in some of our families, we employed the affected-pedigree-member (APM) method of linkage analysis as an initial screen for possible linkage. Using this method, we identified two regions suggesting linkage: the proximal long arm of chromosome 19 (CH19) and the CH21 region of FAD linkage reported by St. George Hyslop et al. Application of standard likelihood (LOD score) analysis to these data support the possibility of an FAD gene located on CH19, particularly in the late-onset FAD families. These data further suggest genetic heterogeneity and delineate this region of CH19 as an area needing additional investigation in FAD.

Introduction

Alzheimer disease (AD), the leading cause of dementia in the elderly, is a catastrophic neurodegenerative disorder. Although the etiology of AD is unclear, a genetic component has long been implicated. Autosomal dominant inheritance with age-dependent penetrance has been used to explain the familial aggregation of affected individuals seen in AD. Recent epidemiological studies support this hypothesis (Mohs et al. 1987; Breitner et al. 1988). Chromosome 21 (CH21) has been suspected as a possible location of the AD gene because of the reported associations of AD and Down syndrome (Heyman et al. 1984). St. George Hyslop et al. (1987) reported linkage to two markers (D21S1/D21S11 and D21S16) on CH21 in four early-onset families (mean ages at onset [M] in the families were 52.0, 48.7, 49.8, and 39.4 years). Goate et al. (1989) also reported several early-onset AD families with CH21 linkage. However, familial AD (FAD) families studies by Pericak-Vance et al (1986*b*) and Schellenberg et al. (1988) failed to confirm linkage to CH21. The Schellenberg et al. study contained FAD families

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of Volga German origin (M = 41.1–63.1 years), while the majority of the families in the Pericak-Vance et al. study were late-onset (M > 60 years). Late-onset AD is the most frequent form of the disease. The failure to confirm linkage in the latter two studies suggested the possibility of genetic heterogeneity in AD with (*a*) an early-onset form linked to CH21 in some families, (*b*) a late-onset form, and (*c*) perhaps a subset of early-onset families still not localized. In addition, St. George Hyslop et al. (1990) recently extended their study to include 48 pedigrees, of both early and late onset. Using additional CH21 markers, the updated analyses supported their earlier findings of linkage. They also showed strong evidence for heterogeneity by using the predivided-sample (PDS) test of Morton (1956), by dividing the families into two groups, according to whether they were early or late onset. These linkage findings prompted us to search other chromosomal regions for a location of an additional major gene(s) for FAD.

The fact that our data set represents mainly late-onset FAD complicates the linkage analysis. While autosomal dominant inheritance with age-dependent penetrance was assumed in our previous study in order to simulate the original linkage report (St. George Hyslop et al. 1987; Pericak-Vance et al. 1988*b*), we cannot determine with certainty the mode of inheritance in late-onset FAD. This could be due to a number of factors, including the very late M (66.1 ± 10.3 years), the possibility of environmental influences, the occurrence of sporadic cases, and the involvement of multiple loci. Because of these complications, we elected to use the affected-pedigree-member (APM) method of linkage analysis (Weeks and Lange 1988) to detect the deviations from independent segregation between marker loci and FAD. We applied the APM method to a series of markers from a variety of chromosome genotypes in our FAD families. After the initial screening of these data, those chromosomal areas that gave significant results were tested further by examining additional markers and polymorphisms in those regions. The results of these studies, as well as the use of standard likelihood (LOD score) analysis for confirmation of the APM results, are presented below.

Material and Methods

A. Family Data

We have examined and obtained blood for DNA studies on 293 members (87 affected) in 32 FAD families (fig. 1). Fifteen of the 32 families have autopsy-confirmed FAD, and five families have autopsy results pending. Twenty-eight of the 32 families have three or more affected individuals in the pedigree. The majority of affected individuals were first-degree relatives. All sampled individuals were examined by a neurologist and associated diagnostic personnel of the Joseph and Kathleen Bryan Alzheimer's Disease Research Center (ADRC) at Duke University, and the clinical diagnosis of probable AD was made according to criteria described elsewhere (McKhann et al. 1984). Before a clinical status of AD was assigned, medical records were obtained and examined on deceased individuals included in the linkage analysis. Care was taken to exclude confounding causes of dementia, such as depression and multiinfarct disease. M in these families was 47–83 years, with an overall M for the entire data set of 66.1 ± 10.3 years. Of the 32 families, only three (families 372, 603, and 317) could be classified as early-onset (i.e., M < 60 years) AD families. We chose M = 60 years as the cutoff between the early- and late-onset groups, as this represented the most striking demarcation between the two groups. The family data were processed via the PEDIGENE system (Haynes et al. 1988; Pericak-Vance et al. 1988*a*).

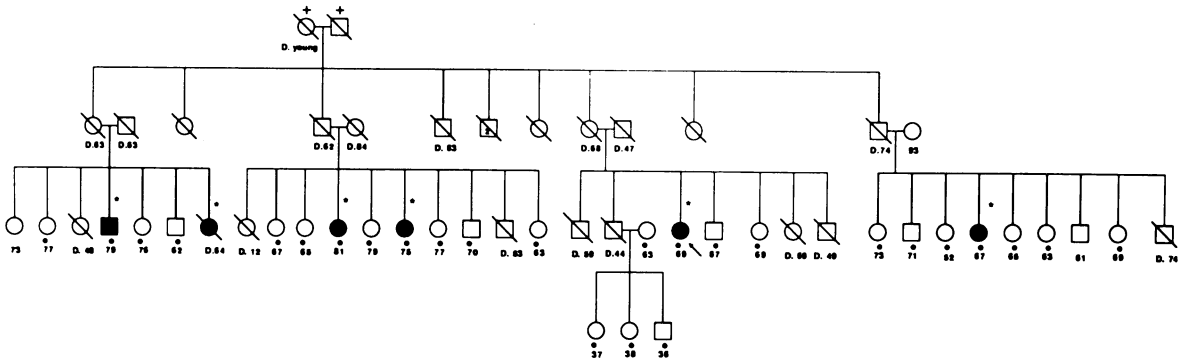
B. DNA Studies

High-molecular-weight genomic DNA was obtained from transformed lymphoblasts according to a method described elsewhere (Pericak-Vance et al. 1986, 1988*b*; Bartlett et al. 1987). The restriction-endonuclease digestion, agarose-gel electrophoresis, Southern blotting, DNA labeling, and hybridization were also done according to methods described elsewhere (Pericak-Vance et al. 1986, 1988*b*; St. George Hyslop 1987). Thirty-six markers from a variety of chromosomes (1, 2, 4–6, 14, 16–19, and 21) were examined for linkage. As with the pedigree data, the marker results were processed via the PEDIGENE system (Haynes et al. 1988; Pericak-Vance et al. 1988*a*) in preparation for analysis.

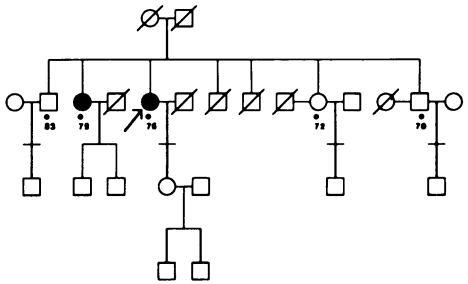
C. Linkage Studies

1. *APM analysis.*—The affected individuals from all the families were incorporated into the APM analysis for the 35 marker loci. The data were analyzed according to the method outlined by Weeks and Lange (1988). We examined the test statistic in each of three situations—$f(p) = 1$, $f(p) = 1/\sqrt{p}$, and $f(p) = 1/p$—where p represents the population allele frequencies of

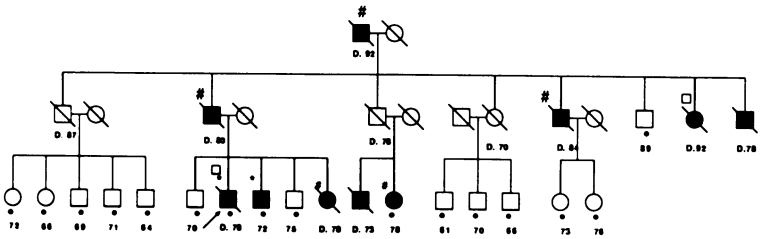
FAMILY 300 (M = 63 years)



FAMILY 976 (M = 66)



FAMILY 794 (M = 71 years)



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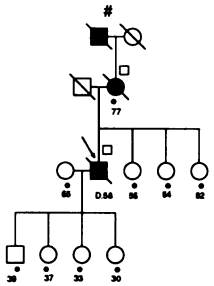
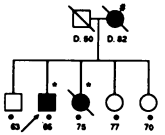


FAMILY 615 (M = 68 years)



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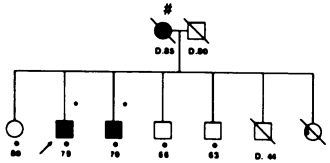

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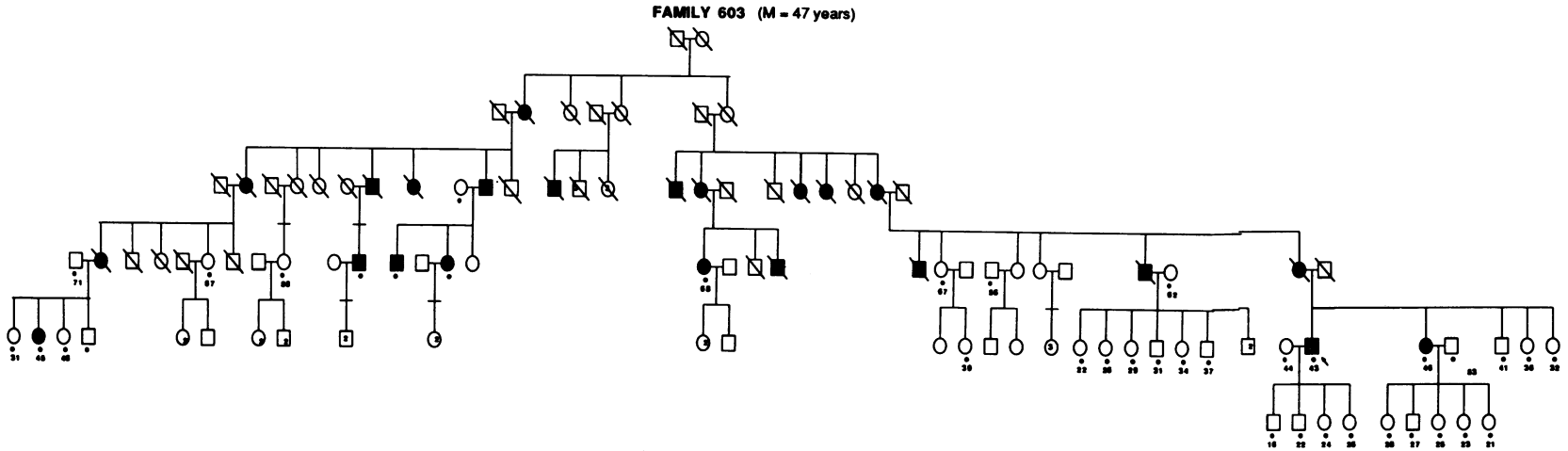
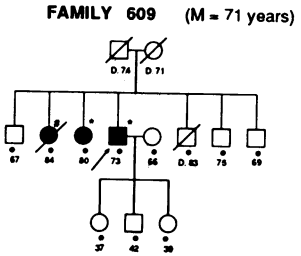

FAMILY 603 (M = 47 years)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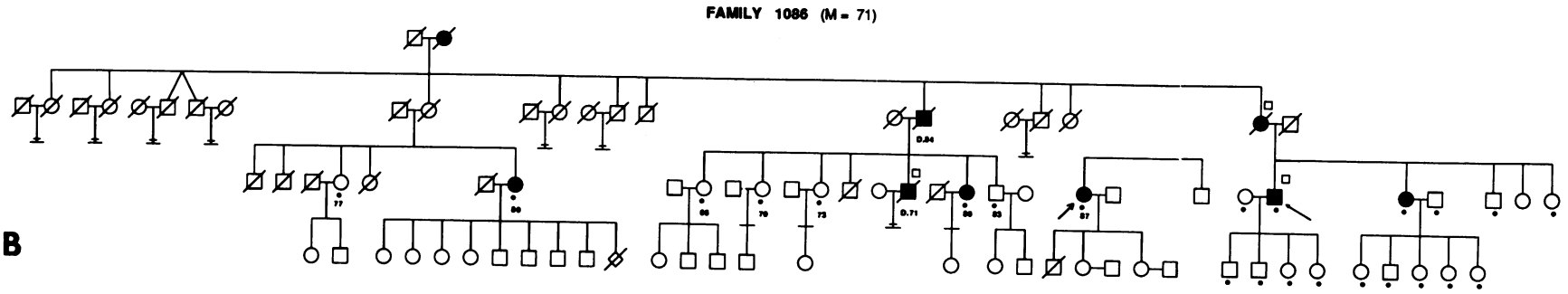
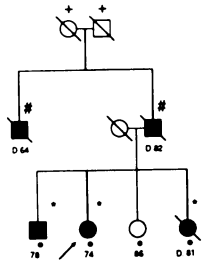

FAMILY 1086 (M = 71)
B

FAMILY 333 (M = 77 years)



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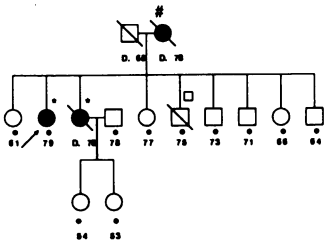
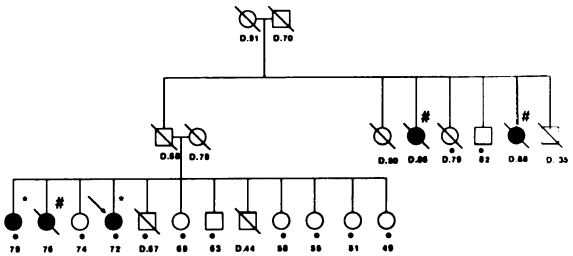
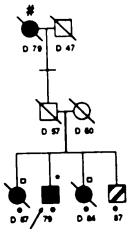


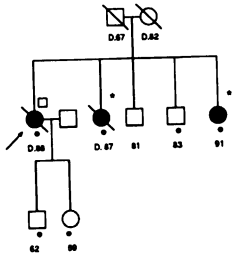
FAMILY 285 (M =68 years)



FAMILY 466 (M = 71 years)



FAMILY 419 (M = 83 years)



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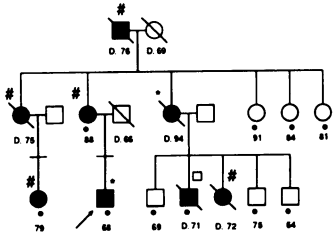
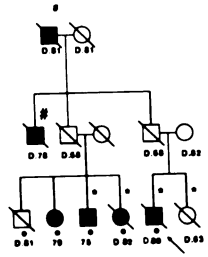
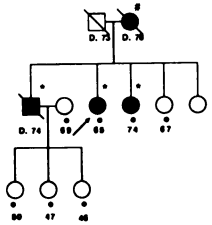


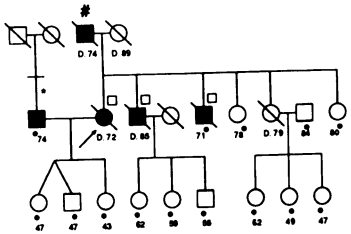
FAMILY 180 (M = 71 years)



FAMILY 820 (M = 65 years)

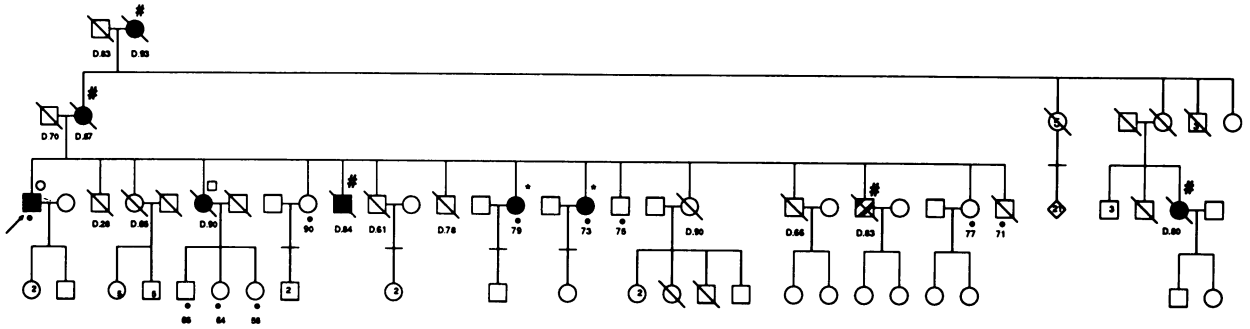


FAMILY 509 (M = 69 years)

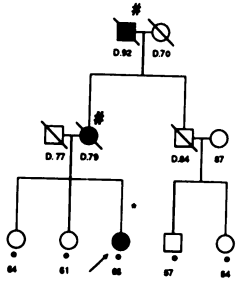


C

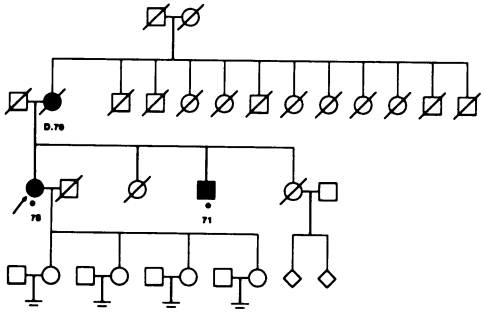
FAMILY 911 (M = 72 years)



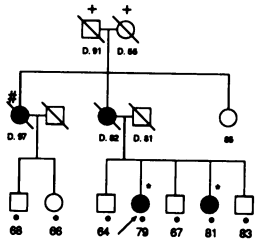
FAMILY 475 (M = 69 years)



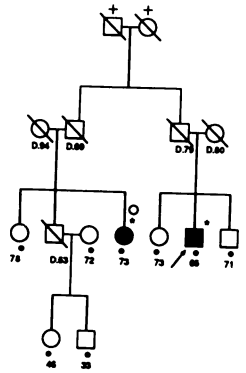
FAMILY 855 (M = 69)



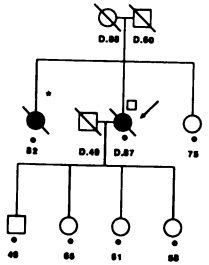
FAMILY 736 (M = 73 years)



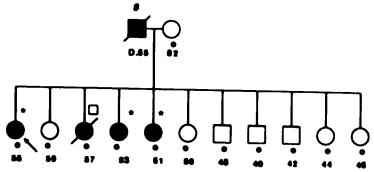
FAMILY 401 (M = 64 years)



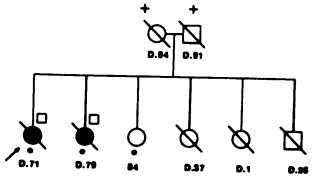
FAMILY 420 (M =78 years)



FAMILY 372 (M = 49 years)



FAMILY 164 (M = 68 years)



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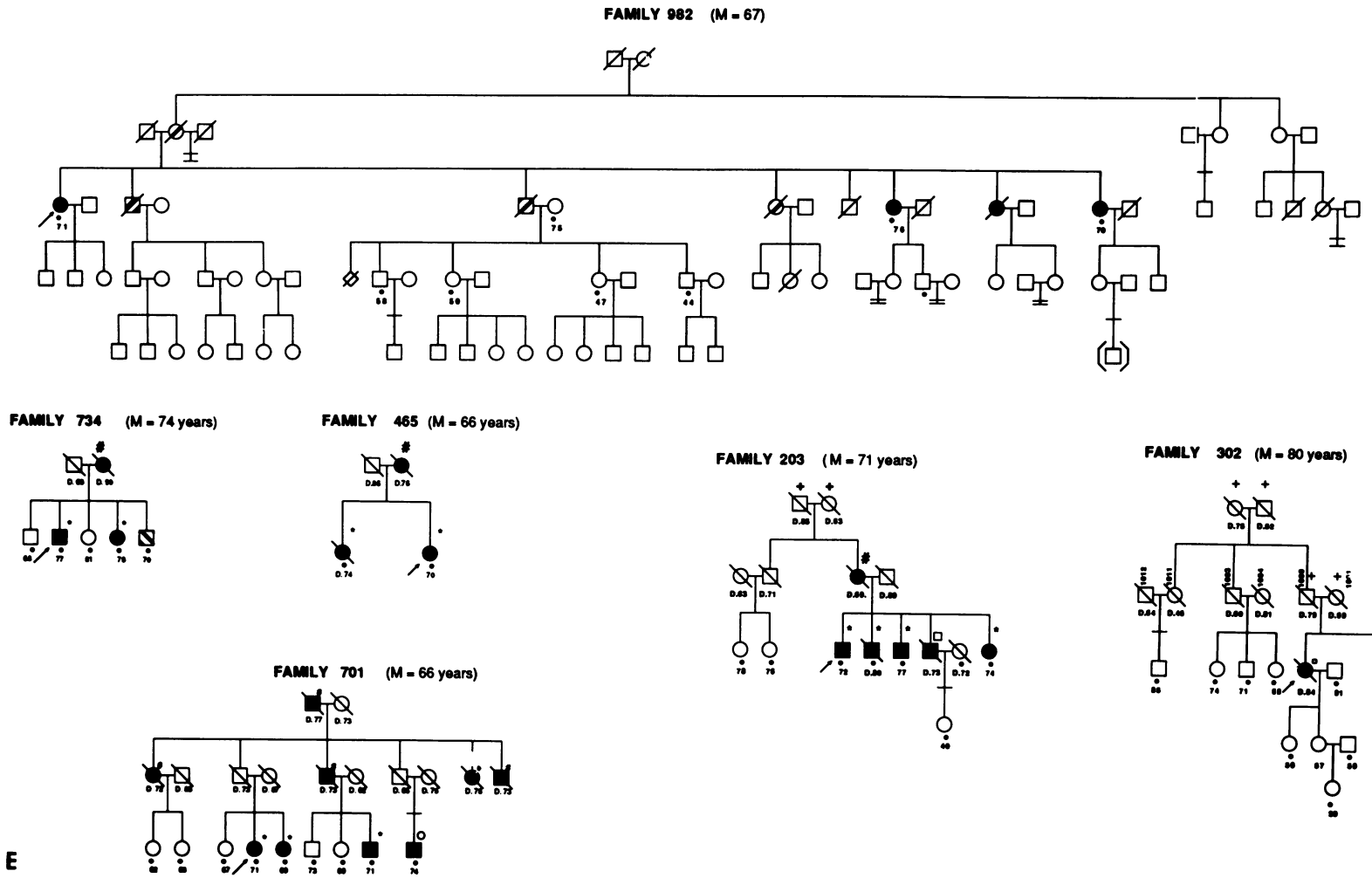

FAMILY 982 (M = 67)
FAMILY 734 (M = 74 years)
FAMILY 465 (M = 66 years)
FAMILY 203 (M = 71 years)
FAMILY 302 (M = 80 years)
FAMILY 701 (M = 66 years)

E

the marker loci. The latter two cases allow the analysis to be weighted on the basis of allele frequency. Their function is to give greater weight to a match of rare alleles in affecteds than to a match of common alleles, since it may be significant when distantly related affecteds share a rare allele rather than a common one (Weeks and Lange 1988). For those markers that exhibited more than one enzyme polymorphism for which significant disequilibrium was established, each enzyme polymorphism was analyzed independently, as it was not possible to incorporate the disequilibrium frequencies into the APM analysis. We also incorporated the recent extension of the method, which enables simultaneous analysis of multiple linked markers (Lange and Weeks 1990). In the case of those markers with multiple enzyme polymorphisms, the most informative polymorphism was incorporated into the multipoint APM. All APM test-statistic results were adjusted for multiple comparisons by using the Bonferroni correction (Elston and Johnson 1987). In addition, because of concern for skewness, we carried out, on a subset of the test statistics, simulation studies (Weeks and Lange 1988) under the null hypothesis of no linkage, in order to evaluate some of the more extreme p values. Markers with significant results were also examined after exclusion of the three early-onset FAD families in our study.

2. *LOD score analysis.*—The data were analyzed for linkage by using both LIPED (Ott 1974) and the MLINK subprogram of the LINKAGE (version 4.6) (Lathrop and Lalouel 1984; Lathrop et al. 1984) computer package. The mode of inheritance in these families is consistent with autosomal dominant inheritance with age-dependent penetrance. Thus, autosomal dominant inheritance was assumed in the LOD score analyses, although other modes of inheritance had been examined previously (Pericak-Vance et al. 1991). The age-at-onset curve used in the analysis was generated from the pedigree data and included 107 individuals ($M = 66.1 \pm 10.3$) (Haynes et al. 1986; Pericak-Vance and Meyers 1986). Age at onset was determined on the basis of the history of the onset of documented memory disorder. A normal distribution was constructed by using the sample M and variance (Haynes et al. 1986; Pericak-Vance and Meyers 1986). On the basis of this age curve, both at-risk individuals and married-in family members were assigned probabilities of being gene carriers. Married-in family members were not unequivocally coded as normal, because of the high gene frequency postulated for FAD (Farrer et al. 1988). The data were also analyzed by using only affected individuals (i.e., at-risk family and married-in family members were coded as unknown with respect for FAD disease status) in the analysis. This eliminated any FAD information contributed by these individuals whose genotype with respect to FAD was uncertain, but it maintained their genetic marker data for reconstructive and phase information. The gene frequency (p) for FAD was set equal to .01 (Farrer et al. 1988). Previous studies varying both p and the age curve (inclusive of family-specific age curves) used in the analysis failed to significantly change the linkage results (Pericak-Vance et al. 1988*b*).

The frequencies used for the marker loci are those published by Kidd et al. (1989). In order to maximize the information for the linkage analysis, those markers that exhibited more than one enzyme polymorphism (i.e., markers D17S71, D17S58, D19S13, D19S16, BCL3, APOC2, CYP2A1/CYP2B1, D21S13, and D21S1/D21S11) had haplotypes constructed. For those markers where the individual polymorphisms within each haplotype exhibited significant disequilibrium, either (*a*) the haplotype frequencies were calculated from all unrelated individuals in the pedigree data or (*b*) the published disequilibrium frequencies (in the case of markers (D17S71, D17S58, APOC2, and D21S1/D21S11) were used (St. George Hyslop et al. 1987; Yamaoka et al. 1990; Vance et al. 1991).

Multipoint analysis was performed by using the LINKMAP subprogram of LINKAGE in order to maximize the linkage information. Multipoint LOD scores were calculated as $\log_{10}[L(x)/L(\infty)]$, where x is the distance of the FAD locus relative to a fixed point

Figure 1 FAD pedigrees. □ and ○ = Unaffected; ⊡ and ⊙ = possibly AD affected; ■ and ● = AD affected; + = no AD information available * = clinical diagnosis of AD; # = diagnosed of AD by history and/or medical records; ● (i.e., small back dot below symbol) = DNA available; □ (i.e., small white square above symbol) = AD confirmed by autopsy; ○ (i.e., small white circle above symbol) = autopsy pending. A diagonal slash through a symbol indicates that the individual is deceased. Numbers below symbols indicate age at examination—or, in the case of deceased individuals, age at death. Arrows indicate probands. A small black dot below a symbol indicates that DNA was available.

Table 1

APM Results

A. Significant Results for APM Single-Locus Analysis				
Marker	Chromosomal Location	f(p) = 1	f(p) = $1/\sqrt{p}$	f(p) = 1/p
BCL3 (*BanI*)	19q13.1	4.05***	3.19*	1.17
ATP1A3	19cen-q13.1	2.50	4.28***	5.84[a],***
D21S1/D21S11 (*BanI*)	21q11.2-q21	−2.76	8.50[a],***	19.27[a],***
D21S1/D21S11 (*MspI*)	21q11.2-q21	.25	2.36	4.45[a],***
D21S1/D21S11 (*EcoRI*)	21q11.2-q21	.09	4.19***	8.23[a],***
D21S16	21q11.2-q21	3.38**	2.52	.49

B. Combined Markers in Multilocus APM Analysis			
Marker	f(p) = 1	f(p) = $1/\sqrt{p}$	f(p) = 1/p
CH19:			
D19S13 (*BglII*), D19S9 and CYP2B1 (*BglII*), and ATP1A3 and BCL3 (*BanI*)	3.91**	3.92**	2.54
D19S13 (*BglII*) and ATP1A3 and BCL3 (*BanI*)	4.32***	4.73***	4.23[a],***
D19S13 (*BglII*), CYP2B1 (*BglII*), and BCL3 (*BanI*)	4.39***	3.43**	1.09
CH21:			
D21S13 (*TaqI*) and D21S16 and D21S1/D21S11 (*BanI*)	−.41	5.29***	11.09[a],***

NOTE.—All *P* values are corrected for multiple comparisons.

[a] *P* values validated by simulations, as described in text.

* $P < .05$.

** $P < .01$.

*** $P < .001$.

on the established map of loci and where ∞ represents an infinite map distance corresponding to a recombination fraction (θ) of 0.5 (Keats et al. 1989). The map used in the CH21 multipoint analysis was as generated by Tanzi et al. (1988), with a genetic distance of 0.15 Morgans being assumed to occur between D21S1/D21S11 and D21S13. The less informative D21S16 was not incorporated in the CH21 multipoint analysis. The map used in the chromosome 19 (CH19) analysis was from Keats et al. (1989). The markers used in the multipoint analysis for CH19 were chosen prior to analysis, on the basis of the accuracy of the genetic map in this region, and they included BCL3, ATP1A3, and D19S13, with a genetic distance of 0.11 Morgans set between D19S13 and ATP1A3 and of 0.04 Morgan between ATP1A3 and BCL3. Haldane's mapping function was used to estimate, the genetic distance between the loci on the basis of established recombination fractions, (Lathrop et al. 1984; St. George Hyslop et al. 1987; Pericak-Vance et al. 1988*b*). Autosomal dominant inheritance with age-dependent penetrance was assumed for both the age-adjusted and affecteds-only multipoint analyses. In addition, the CH19 data were examined by assuming a misdiagnosis frequency of 10% in affected individuals who had not had autopsy-confirmed AD, in order to examine the effect that misclassification had on the linkage results.

With the HOMOG computer package (Ott 1985), three tests of heterogeneity (admixture tests [A-tests]) were examined by using the multipoint LOD scores generated from the LINKMAP analyses. The tests performed were as follows: (1) using the CH19 markers alone (HOMOG), (2) using the CH21 markers alone (HOMOG), and (3) using both the CH21 and CH19 markers and testing for the evidence of two family types (HOMOG2). The tests were done by using both the age-adjusted and affecteds-only data and were repeated by combining our data with published data (St. George Hyslop et al. 1990). In addition, we applied the (PDS) test as outlined by Morton (1956), using M of AD (i.e., early vs. late onset) as the grouping criterion. The testing was done by using several different cutoff points (i.e., M = 60, M = 65, and M = 70 years) for early-onset families. The present data, as well as these data combined with the published data of St. George Hyslop et al., were examined in order to identify heterogeneity between the early- and late-onset groups.

Results

A. APM Results

The results of the APM calculations for the various markers for which a significant test statistic was identified are given in table 1. Preliminary analyses (Pericak-Vance et al. 1989) had previously shown significant results with BCL3 (*Ban*I) on CH19, which led to the subsequent typing both of additional CH19 probes in this region (i.e., markers CYP2A1, CYP2B1, ATP1A3, D19S13, D19S9, D19S19, and D19S7) and of additional families. Two markers, BCL3 (*Ban*I) and ATP1A3, remained significant ($P < .001$) in the subsequent analysis, thus rejecting the null hypothesis of independent segregation between these marker loci and FAD. Similar findings were obtained for the various polymorphisms at the D21S1/D21S11 locus and at D21S16 on CH21. The results of the multipoint APM study (table 1B)—both for CH19, for which various combinations of the markers BCL3 (*Ban*I), D19S13 (*Bgl*I), ATP1A3, D19S9, and CYP2B were used, and for CH21, for which markers D21S13, D21S16, and D21S1/D21S11 (*Ban*I) were used—support the single-locus APM analyses. Results of the simulation studies used to evaluate empirically the more extreme p values (i.e., for f(p) = 1/p, ATP1A3 and D21S1/D21S11 [*Ban*I, *Msp*I, and *Eco*RI], D19S13-ATP1A3-BCL3 and D21S13-D21S16-D21S1/D21-S11, and, for $f(p) = 1/\sqrt{p}$, DS21S1/D21S11 [*Msp*I]) confirmed these markers to be highly significant, as indicated in table 1. All values lay well beyond the 99th percentile obtained for the simulated distributions. The 99th percentile cutoffs were, respectively, 2.40, 3.08, 2.60, 2.62, 2.60, 2.57, and 2.54. Reanalyzing the data with the exclusion of the three early-onset FAD families continued to give significant APM results for both the CH19 and CH21 markers. Analysis of the early-onset families alone only gave significant results for the CH21 markers ($P < .05$).

B. Two-Point LOD Score Analysis

Significant disequilibrium ($P < .01$) was found between the individual polymorphisms of the BCL3 and the D19S13 loci, and thus haplotype frequencies calculated on the basis of the unrelated individuals in the pedigrees were used in the analyses. The haplotype frequencies used in the analyses were as follows: for BCL3, A (3.0-kb *Ban*I, 9-kb *Eco*RI/*Mlu*I = .64), B (3.0-kb *Ban*I, 8-kb *Eco*RI/*Mlu*I = .18), C (2.2-kb *Ban*I, 9-kb *Eco*RI/*Mlu*I = .01), and D (2.2-kb *Ban*I, 8-kb *Eco*RI/*Mlu*I); for D19S13, A (15-kb *Bgl*I, 5.5-kb *Taq*I = .07), B (15-kb *Bgl*I, 2.6-kb *Taq*I = .11), C (6.7-kb *Bgl*I, 5.5-kb *Taq*I = .64), and D (6.7-kb *Bgl*I, 2.6-kb *Taq*I = .17). The two-point LOD score results are shown in table 2, for both the CH19 and CH21 markers analyzed. The highest LOD scores obtained in the age-adjusted analysis were for ATP1A3 ($z[\hat{\theta}] = 1.01$ at $\hat{\theta} = .10$) and BCL3 ($z[\hat{\theta}] = .68$ at $\hat{\theta} = .10$) on CH19 and for D21S13 ($x[\hat{\theta}] = .92$, $\hat{\theta} = .20$) on CH21. Slightly positive LOD scores were also found for the CH19 markers APOC2, D19S13, CKM, D19S19, and INSR and for the CH21 markers D21S17, D21S15, and D21S19.

In the analysis of affecteds only, BCL3 gave $z(\hat{\theta}) = 2.51$ (the highest LOD score) at $\hat{\theta} = .0$, ATP1A3 gave $z(\hat{\theta}) = 1.73$ at $\hat{\theta} = .05$, and D19S13 gave $zIR(\hat{\theta}) = 1.84$ at $\hat{\theta} = .05$. Of the CH21 markers, D21S13 gave the highest scores: with $z(\hat{\theta}) = .69$ at $\hat{\theta} = .20$. Slightly positive LOD scores were also seen both for the CH19 markers D19S19, D19S29, CYP2A3/CYP2B1, APOC2, and CKM and for the CH21 markers D21S15 and D21S110.

C. Multipoint LOD Analysis

The results of the multipoint analysis for the CH19 markers—BCL3, ATP1A3 and D19S13—are depicted in figure 2. With an age-adjusted analysis, a maximum multipoint LOD score of 2.20 was found when FAD was placed distal to BCL3. When FAD was placed at .0 recombination with BCL3, a peak LOD score of 4.38 was found (when only affecteds were used in the analysis). Multipoint analysis of the CH21 markers is depicted in figure 3 and gave no evidence for linkage to this region. These latter results were similar to those reported elsewhere (Pericak-Vance et al. 1988*b*, 1989). Figure 4A and B depicts the multipoint results (for CH21 and CH19, respectively) separated into early- and late-onset families. When only the early-onset families are examined for CH21, the peak multipoint LOD score was 1.21 for age-adjusted analysis and 1.0 for affecteds only, with FAD placed proximal to D21S13. The results for the late-onset families were significantly negative. Examining only the late-onset families for the CH19 multipoint gave a peak multipoint LOD score of 2.48 for age-adjusted analysis and of 4.60 for affecteds only. For the early-onset families the data were slightly negative or noninformative, in both cases. When a 10% misclassification rate was included for affected individuals, the peak multipoint scores were 1.80 in the age-adjusted analysis and 2.75 in affecteds-only analysis.

The multipoint data were also examined by using

Table 2

FAD Two-Point LOD Score Results for CH19 and CH21

Analysis and Marker	Chromosomal Location	θ					
		.001	.05	.10	.20	.30	.40
Age adjusted:							
C3	19p13.3-13.2	−5.57	−2.74	−1.65	−.63	−.20	−.04
INSR	19p13.3-13.2	−3.67	−.09	.46	.61	.36	.10
D19S16	19cen-q13.2	−7.07	−1.88	−.83	−.04	.11	.06
D19S7	19cen-q12	−3.35	−1.70	−1.04	−.44	−.15	−.03
D19S19	19cen-q12	−7.46	−1.66	−.56	.20	.27	.10
D19S29	19q12	−7.13	−2.15	−1.05	.09	.13	.03
D19S13	19q12	−4.71	−.57	.08	.38	.27	.10
ATP1A3	19q	−.76	.82	1.01	.85	.48	.11
BCL3	19q13.1	.34	.61	.68	.57	.32	.03
CYP2A1/CYP2B1	19q13.1	−10.20	−2.77	−1.24	−.13	.12	.06
D19S9	19q12-q13.2	−.91	−.54	−.22	.03	.12	.06
APOC2	19q12-q13.2	−4.21	−.79	.03	.59	.50	.18
CKM	19q13.2	−3.46	−.65	−.13	.17	.15	.05
D21S13	21pter-q21	−6.61	−.57	.45	.92	.69	.28
D21S16	21q11.2-q21	−1.26	−.13	.16	.28	.18	.04
D21S1/S11	21q11.2-q21	−23.88	−7.38	−4.13	−1.43	−.42	−.08
D21S110	21q11.2	−1.46	−1.15	−.87	−.45	−.19	−.04
APP	21q21	−4.88	−1.46	−.86	−.33	−.10	−.01
D21S17	21q21.2-qter	−4.43	−.81	−.05	.45	.37	.14
D21S15	21q22.3	−.24	−.04	.09	.15	.15	.06
BCEI	21q22.3	−3.00	−1.00	−.49	−.07	.03	.02
D21S19	21q22.3-qter	−.27	.01	.17	.23	.15	.05
Affecteds only:							
C3	19p13.3-13.2	−4.44	−2.46	−1.56	−.65	−.23	−.05
INSR	19p13.3-13.2	−3.43	−.83	−.31	.02	.06	.02
D19S16	19cen-q13.2	−4.84	−1.33	−.53	.00	.09	.03
D19S7	19cen-q12	−2.32	−1.17	−.74	−.31	−.12	−.03
D19S19	19cen-q12	−2.27	−.17	.43	.63	.42	.14
D19S29	19q12	−4.38	−.84	−.18	.21	.20	.07
D19S13	19q12	.39	1.84	1.81	1.23	.61	.17
ATP1A3	19q	.90	1.73	1.57	1.01	.50	.13
BCL3	19q13.1	2.51	2.27	1.95	1.24	.60	.16
CYP2A1/CYP2B1	19q13.1	−5.78	−1.25	−.22	.32	.26	.09
D19S9	19q12-q13.2	−.62	−.32	−.15	.07	.11	.05
APOC2	19q12-q13.2	−2.64	.00	.44	.58	.39	.13
CKM	19q12-q13.2	−1.29	−.02	.18	.21	.11	.03
D21S13	21pter-q21	−5.45	−.67	.25	.69	.54	.23
D21S16	21q11.2-q21	−1.06	−.13	.32	.31	.17	.04
D21S1/S11	21q11.2-q21	−20.45	−6.48	−3.55	−1.11	−.26	−.03
D21S110	21q11.2	.46	.43	.39	.30	.17	.05
APP	21q21	−4.12	−1.36	−.82	−.34	−.12	−.03
D21S17	21q21.2-qter	−3.55	−.69	−.21	.08	.09	.03
D21S15	21q22.3	.25	.29	.30	.27	.18	.06
BCEI	21q22.3	−3.04	−1.82	−1.18	−.49	−.17	−.04
D21S19	21q22.3-qter	−.33	−.19	−.10	−.01	.00	.00

Note.—Male θ = female θ.

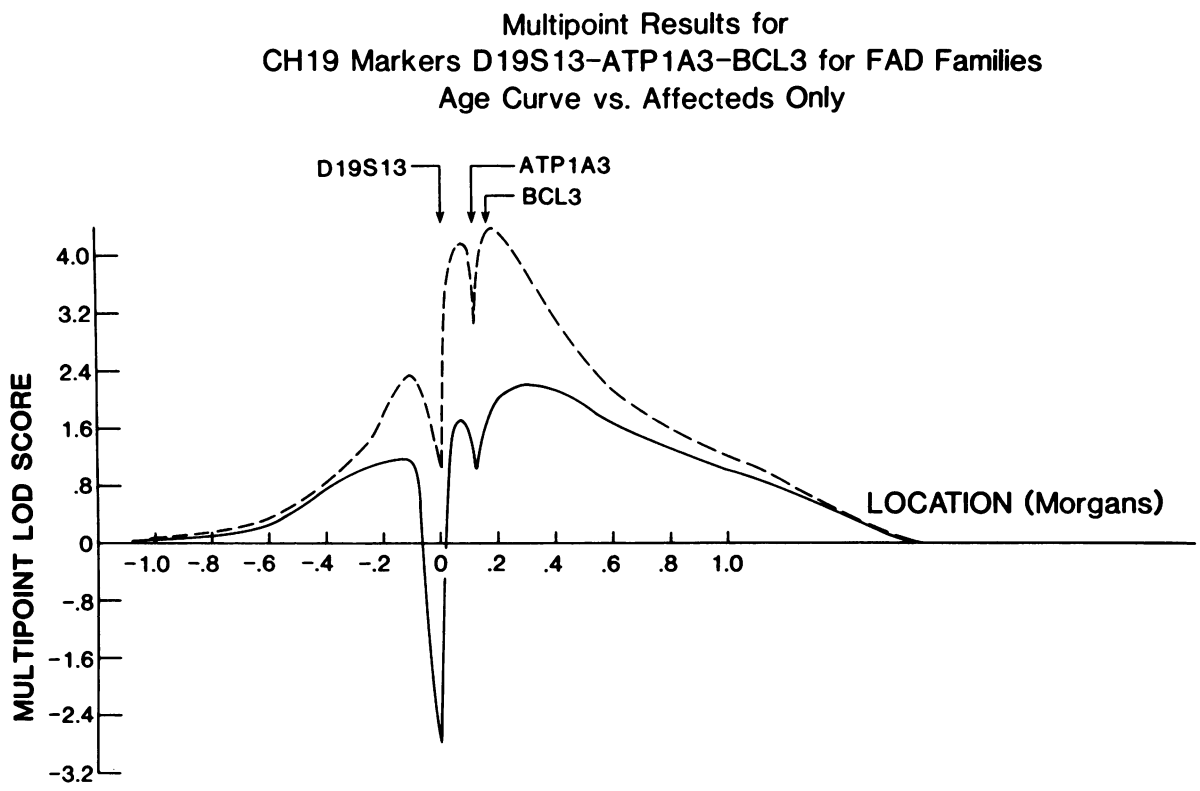


Figure 2 CH19 multipoint analysis in 32 FAD families combining both early- ($M \leq 60$ years) and late-onset ($M > 60$ years) families. The unbroken line (——) represents the age curve (early and late onset), and the broken line (– – –) represents affected only (early and late onset).

only those families that fit the criteria outlined by Haines et al. (1988), who suggested selecting only those families that had both evidence for 3-generation-affected data and at least three tested affecteds (one of which could be an individual whose genotype is inferred from the data on his or her spouse and children), thereby reducing the number of sporadic cases and phenocopies that might be included in the data. When only families meeting these selection criteria were analyzed, a peak LOD score of 1.75 for the age-adjusted data and of 2.04 for affecteds only was obtained (when FAD was located between D19S13 and ATP1AB).

D. Heterogeneity Analysis

The results of the A-tests for heterogeneity using both the two-point and multipoint linkage results for the CH19 markers alone were not significant for either the age-adjusted or affecteds-only analyses. Similar results were found when the CH21 markers were used. When both the CH19 and CH21 multipoint results were examined simultaneously, the results of the affecteds-only analysis were still not significant, but the age-adjusted analyses were nearing significance ($P = .09$) with an estimate of the proportion of CH19-linked families being .65. Combining our two-point data for D21S1/S11 with the published data of St. George Hyslop et al. (1990) also failed to reach statistical significance in the A-test ($P = .27$).

When the PDS test applied to the data in order to detect heterogeneity between the two groups used ≤ 60 years as the cutoff for the early-onset families, the results neared significance ($P = .06$). Similar results were found when the test applied to our data used ≤ 65 and ≤ 70 years as the cutoff for early-onset families. Combining these data with the data of St. George Hyslop et al. (1990) showed significant results ($P < .05$). Significant results ($P < .01$) were also found for the combined data sets when cutoffs of 65 and 70 years were used for the grouping of early- versus late-onset families.

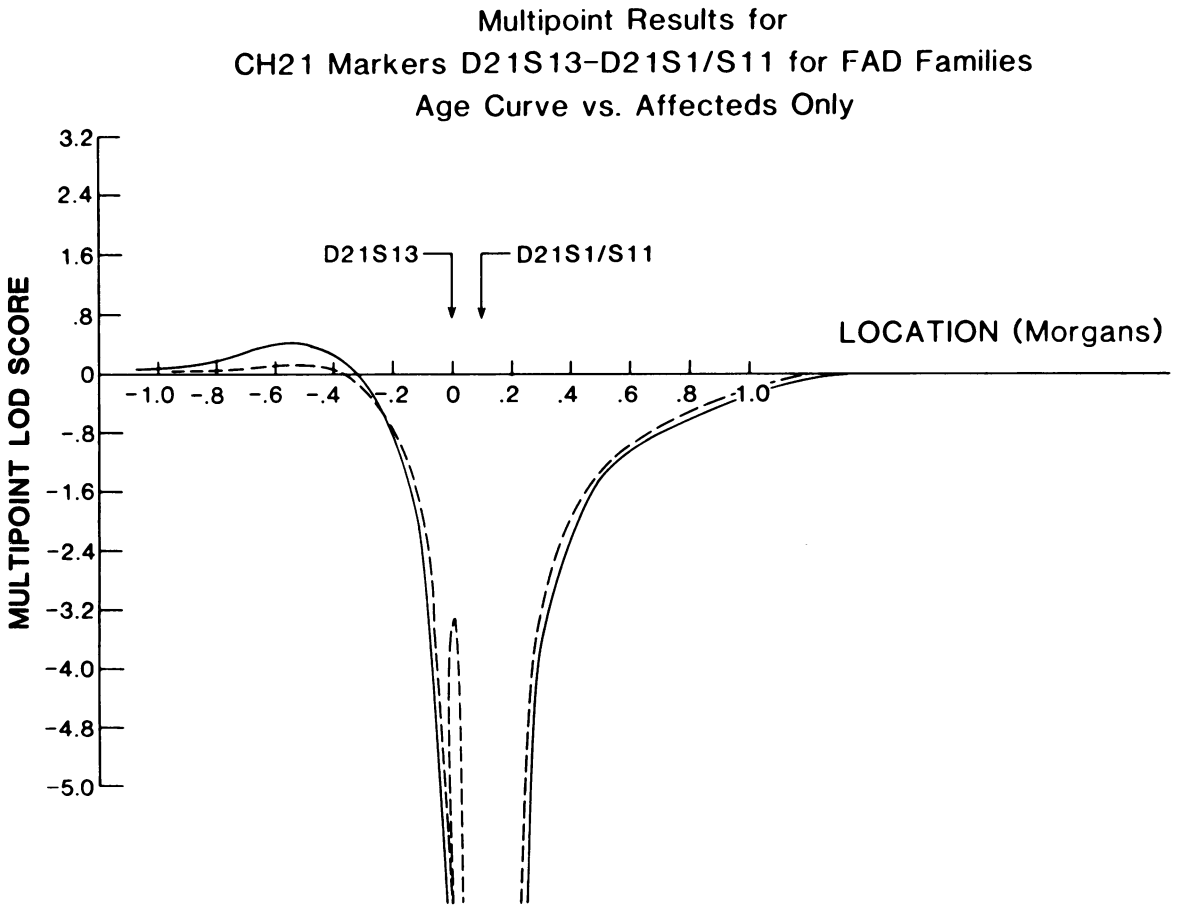


Figure 3 CH21 pedigrees' multipoint LOD score results for 32 FAD pedigrees, combining both early-onset (M ⩽ 60 years) and late-onset (M > 60 years) families. Unbroken and broken lines are as in fig. 2.

Discussion

The failure to find significant evidence for an autosomal dominant form of AD on CH21 in our data set prompted us to begin screening other chromosomal regions for linkage. Our research strategy was to screen for possible linkage(s) in chromosomal regions outside CH21 by using both standard likelihood and nonparametric methods. For the nonparametric approach, we chose the APM method and decided that, once a significant finding was obtained, we would genotype additional probes from those regions, as well as examine the data by using standard likelihood methods of analysis. This would allow us to maximize the information on the individual families before going on to examine new chromosomal areas. The results of our APM screen indicated two regions of significance, the BCL3 region on CH19 and the FAD-linked region on CH21. The multipoint APM CH19 results were significant under each of the three population allele frequency situations, while the CH21 analysis indicated the sharing of a less common allele among affecteds. The significant APM results for CH21 markers in the late-onset-family subset is particularly interesting, in view of the fact that in these data linkage was excluded from this region. These results suggest that there may be (*a*) a subset of late-onset families that are also linked to CH21 or, more likely, (*b*) an association between late-onset FAD and this region. Whether our significant APM results for CH19 could indicate an association—rather than true linkage—cannot be determined at this time. However, preliminary examination of the data does not indicate association, as comparison of the BCL3 gene frequencies in FAD affecteds versus those in controls shows no significant differences. Thus, the APM findings sup-

ported evidence for possible linkage or association on CH21, as well as indicting the need to investigate further the BCL3 region of CH19, for an additional FAD locus.

Application of standard likelihood methods to genetic markers (BCL3-ATP1A3) on the proximal long arm of CH19 gave a peak multipoint LOD score (in the overall data set) of 2.20 for the age-adjusted analysis and of 4.38 for affecteds only. Quite similar scores (2.48 and 4.60, respectively) were obtained when only late-onset families were examined. The use of an affecteds-only analysis eliminates the information contributed by unaffected individuals who may actually carry the gene but who may not yet have expressed it. If the unaffecteds are included, these individuals may appear as potential recombinants in the linkage study, thereby reducing the linkage information. The use of both forms of analysis allows one to differentiate between the contribution made by unaffecteds and those made by affecteds. The higher LOD score in our data for affecteds indicates that there are unaffected family members who appear to be segregating with the linked CH19 genotype. The question remains as to whether these are true recombinants—or individuals who have not lived long enough to express symptoms. Thus, we have examined and report both sets of results. In the age-adjusted case the multipoint LOD score in the late-onset families is approaching the accepted significance level of 3.00, and in the affecteds-only case it is >3.00, leading us to conclude that in these data there is strong support for linkage to CH19.

The discrepancy between age-adjusted and affecteds-only analyses could also be due to phenocopies and nongenetic cases being included in the data set. Thus, it was particularly useful to view the combined multipoint LOD score results for CH19 by using the criteria suggested by Haines et al. (1988), which examined families that represent a group that is homogeneous on the basis of inheritance, structure, and diagnoses. The fact that these findings were similarly positive both in affecteds-only and in age-curve analyses provides a useful observation. In addition, it is particularly encouraging to note that two of our largest late-onset AD families (families 757 and 701) each give a LOD score >1 for the age-adjusted multipoint analysis. These results do not change significantly when only affecteds are examined.

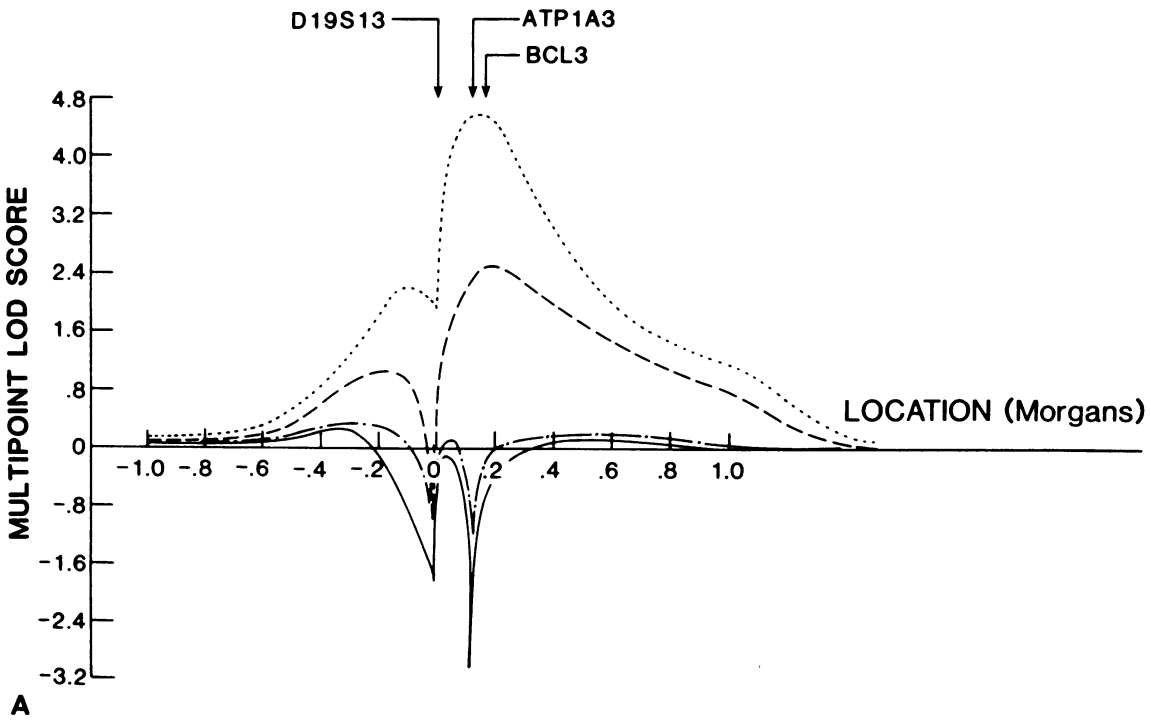
As expected, inclusion of a misdiagnosis parameter for affected individuals decreases the LOD scores, although they are still suggestive of linkage. This is due to the loss of information on affected individuals. The estimate of .10, which was used for the misdiagnosis parameter, represents an overall rate of misdiagnosis for all AD cases. In the case of a known family history of FAD, this figure is probably much lower. Unpublished data from the Bryan ADRC show confirmation, by pathological findings at autopsy, of the clinical diagnosis of AD in 22 of 22 independent individuals in families with multiple affected family members, when the initial autopsy in the family was diagnostic of AD. The use of a less conservative misclassification rate would increase the LOD scores.

Although the evidence for linkage on CH19 is highly suggestive, this region needs further investigation with additional families and with additional markers. Confounding factors such as misdiagnosis, phenocopies, and heterogeneity can have an effect on the linkage results. The significant results found with CH19 markers are interesting, especially in view of an earlier suggestion of an association between APOC2 and FAD, an association reported by Schellenberg et al. (1987) and subsequently confirmed with a larger data set (Schellenberg et al. 1990). Although the overall LOD score analysis of the data excluded close linkage, yet, as the authors point out, the negative scores could represent genetic heterogeneity in the kindreds tested. Our APOC2 LOD scores were slightly positive for both the age-curve and affecteds-only analyses.

All markers tested from the proximal long arm pericentric region of CH19 were slightly positive in the LOD score analyses, for both the affecteds-only and the age-adjusted data (table 2). Application of multipoint analysis to the data significantly increased the overall LOD score in this region, as it increased the information available in the individual pedigrees, because of the virtual haplotyping of the markers used. The multipoint approach is particularly important in these studies, because of both the late age at onset and the structure of the pedigrees with data usually available from only one living diagnosed generation.

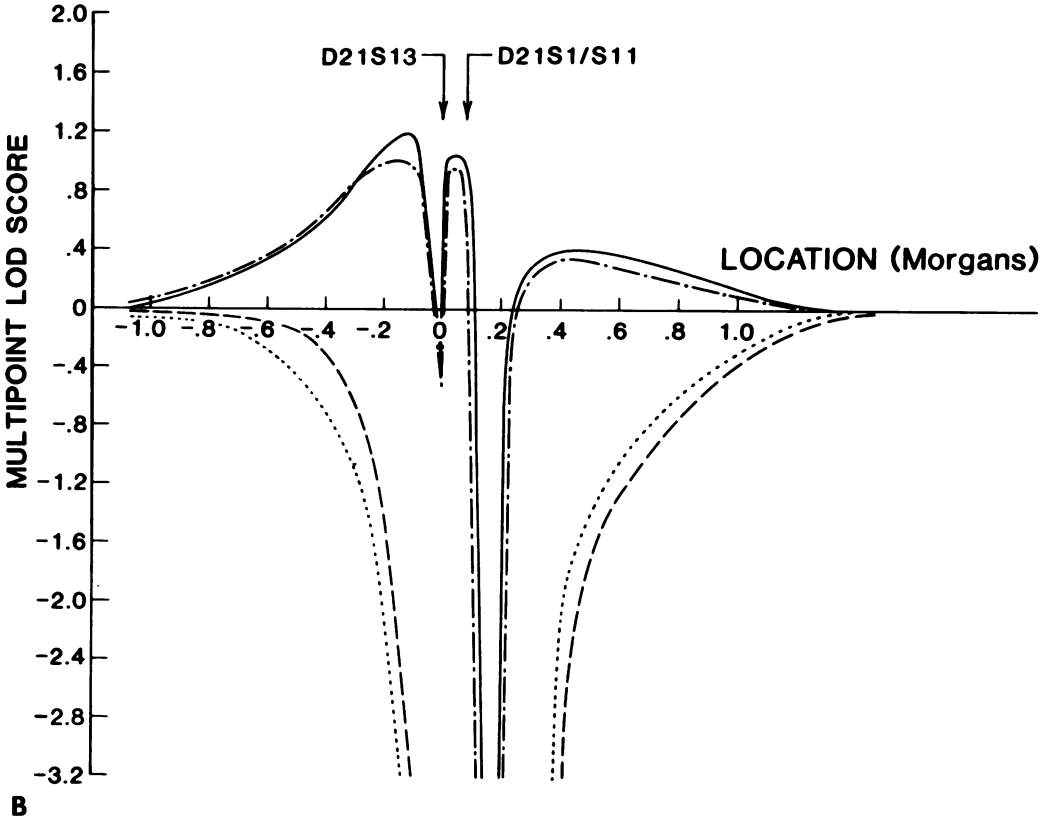
It was interesting to note that the early-onset families accounted for the positive LOD score found with CH21, while the late-onset families were more positive with CH19 markers when the early-onset families were omitted. Testing for heterogeneity by using the A-test in our data was not significant and thus indicated no evidence for genetic heterogeneity in the data. An alternative explanation as to why significant heterogeneity was not found could be based on the lack of power, a lack due to (*a*) limited family size and (*b*)

Multipoint Results for
CH19 Markers D19S13-ATP1A3-BCL3 for FAD Families
Age Curve vs. Affecteds Only



A

Multipoint Results for
CH21 Markers D21S13-D21S1/S11 for FAD Families
Age Curve vs. Affecteds Only



B

the relatively small numbers of early-onset (and thus presumably CH21-linked) families in the data set. Addition of our families to the published data (St. George Hyslop et al.) also failed to show significant heterogeneity. However, the finding of significant heterogeneity between the early- and of late-onset groups when the PDS test was used was encouraging and provides evidence for possible AD heterogeneity between these two groups of families. These results suggest at least two groups: (1) a group comprising early-onset families in which FAD is linked to a region on CH21 and (2) a group of late-onset families in which FAD is not linked to CH21.

In conclusion, we have shown evidence for support of both CH19 linkage in our late-onset FAD families and CH21 linkage in our early-onset FAD families. Additional FAD families and markers need to be examined in order to confirm the above results and to further investigate the question of genetic heterogeneity in FAD.

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Figure 4 *A*, Multipoint LOD score results for 32 FAD pedigrees and CH19 markers, separated by early-onset ($M \leq 60$ years; number of families [*N*] = 3) and late-onset ($M > 60$ years; $N = 29$) families. *B*, Multipoint LOD score results for 32 FAD pedigrees and CH21 markers, separated on the basis of early onset ($M \leq 60$ years; $N = 3$) and late-onset ($M \geq 60$ years; $N = 29$). Represented in both panels are the age curves (for early onset [——] and late onset [– – –]) and the affecteds only (early onset [— - — -] and late onset [- - - -]).

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