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A Population-based Study of Familial Alzheimer Disease: Linkage to Chromosomes 14, 19, and 21

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Summary

Linkage of Alzheimer disease (AD) to DNA markers on chromosomes 14, 19, and 21 was studied in 10 families in which the disease was apparently inherited as an autosomal dominant trait. Families were derived from a Dutch population-based epidemiologic study of early-onset AD. Although in all probands the onset of AD was at or before age 65 years, the mean age at onset was after age 65 years in four families (referred to as "LOAD"). Among the six families with early-onset AD (referred to as "EOAD," i.e., mean age of onset of AD of relatives was at or before age 65 years), conclusive linkage to 14q24.3 was found in one family with a very early onset (around 47 years), while linkage to the same region was excluded in two other families. For the LOAD families, predominantly negative lod scores were obtained, and the overall lod score excluded linkage to chromosome 14. The results with markers on chromosome 19 and chromosome 21 were not conclusive for EOAD and LOAD. The findings of our study confirm genetic heterogeneity within familial EOAD.

Introduction

Alzheimer disease (AD) is a neurodegenerative disorder with a complex genetic etiology. Familial aggregation has been reported for the rare form of early-onset AD (EOAD; onset at or before age 65 years), as well as for the more common, late-onset form (LOAD; onset age after 65 years) (Van Duijn et al. 1991*a*). In a considerable number of families there is evidence for autosomal dominant inheritance of AD (Farrer et al. 1990). However, complex segregation analysis suggests that it is unlikely that one dominant allele can explain a large proportion of all cases with AD (Farrer et al. 1991; Van Duijn et al. 1993). More complex interactions involving two or more genetic loci or perhaps nongenetic factors may be implicated in the etiology of AD.

Molecular genetic studies of familial AD have evidenced genetic heterogeneity between EOAD and LOAD, as well as among EOAD families. Linkage studies have suggested genetic loci for familial AD on three different chromosomes, i.e., chromosomes 14, 19, and 21. Mutations in exon 16 and exon 17 of the β amyloid precursor protein (APP) gene on chromosome 21 (q21.2) were shown to cosegregate with AD (Chartier-Harlin et al. 1991; Goate et al. 1991; Murrell et al. 1991; Mullan et al. 1992*a*). Exon 16 and exon 17 encode for β amyloid, which is found in the parenchymal senile plaques and cerebral vessel walls of AD patients. However, mutations in the APP gene were found only in families with EOAD and have shown to be rare even among these families (Van Duijn et al. 1991*b*; Kamino et al. 1992; Tanzi et al. 1992*a*). Several studies have reported linkage of EOAD to chromosome 14 (q24.3) (Mullan et al. 1992*b*; St George-Hyslop et al. 1992; Schellenberg et al. 1992; Van Broeckhoven et al. 1992). Some families that show significant evidence for linkage to the 14q24.3 region are, in addition, suggestive for linkage to chromosome 21 markers located centromeric of APP (St George-Hyslop et al. 1992; Van Broeckhoven et al. 1992). Although evidence for linkage to chromosome 21 may be the result of a statistical artifact related to the large number of untyped individuals included in linkage studies (Ott 1992), it cannot be excluded that the expression of the chromosome 14 gene is modified by another currently unknown gene on chromosome 21. Linkage to chromosome 19 was initially reported only for LOAD families (Pericak-Vance et al. 1991), suggesting that there may be locus heterogeneity for familial EOAD and LOAD. Yet, a metanalysis of linkage data showed that EOAD in some families may be linked to chromosome 19 also (Farrer and Stice 1993). These findings are supported by the recent observations of an association of the E-4 allele of the apolipoprotein E (APOE) gene on chromosome 19 (q13.2) with EOAD and LOAD (Corder et al. 1993; Poirier et al. 1993; Saunders et al. 1993*a*, 1993*b*; Strittmatter et al. 1993; Van Duijn et al. 1994).

Here we report the findings of a study of 10 pedigrees in which AD was apparently inherited as an autosomal domi-

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nant disorder. Families were derived from a population-based epidemiologic study of EOAD. We have studied linkage of AD with DNA markers on chromosomes 14, 19, and 21. The emphasis of this study was to investigate heterogeneity in AD within these families.

Subjects, Material, and Methods

Family Data

The epidemiologic study from which these patients were derived included all patients diagnosed with EOAD during the period 1980–87 in two areas of The Netherlands (Hofman et al. 1989). For this study, all nursing homes, psychiatric institutions, social/geriatric services, neurologists, and facilities for computed tomography in the specified areas were asked for patients with dementia. The clinical diagnosis of probable AD was subsequently verified by one of the investigators according to a protocol similar to the NINCDS-ADRDA criteria for AD (McKhann et al. 1984). In total, 201 patients were eligible for this study, and 198 (98%) participated.

For all patients, detailed data on family history of dementia in first-, second-, and third-degree relatives were collected by interviewing a next of kin of the patient. To increase the validity, the family data were always verified by a second informant who was a first-degree relative of the patient. Of the 198 patients, 96 had at least one first-degree relative with dementia. The pedigree structure of 17 patients was consistent with autosomal dominant inheritance of AD. The criteria for autosomal dominant inheritance were (1) at least three individuals with clinically diagnosed AD in two or more generations and (2) detailed medical records available on the clinical diagnosis of AD in at least two affected relatives (McKhann et al. 1984).

Affected and unaffected relatives of these 17 families were visited at home, where blood was drawn for DNA extraction and where a standardized interview was taken that included questions on family history of dementia and putative risk factors for AD. All relatives were screened for dementia by using the Mini Mental State Examination (Folstein et al. 1975). Relatives were considered affected if a clinical diagnosis of probable AD had been made or, in the case of relatives deceased before the availability of standardized clinical diagnosis of AD, if the course of disease was compatible with AD (McKhann et al. 1984). For all patients, the age at onset was estimated as either the age at memory loss or the age when a change in behavior was first noted.

The pedigree structure of 11 of the 17 families was considered to be informative for linkage analysis. In one family (1302), a mutation in exon 17 of the APP gene was found changing an amino acid at codon 692. The chromosome 21 linkage findings for this family have been reported elsewhere (Hendriks et al. 1992). The 10 pedigrees that we report here are shown in figure 1.

DNA Analysis

The following probes and restriction enzymes were used to detect RFLPs on chromosomes 21 and 19: ICRFc102B5120 (D21S16) on *Msp*I, pGSE9 (D21S16) on *Xba*I, pGSM21 (D21S13) on *Taq*I and *Eco*RI, pPW511-1H (D21S52) on *Bgl*II, pPW228C (D21S1) on *Msp*I and *Bam*HI, pPW236B (D21S11) on *Taq*I, 9-110 (APP) on *Bgl*II, NJ3.6 (APOCII) on *Taq*I and *Ban*I, pα1.4 (BCL3) on *Ban*I, pMP10 (CYP2B) on *Bam*HI, and pHW60 (D19S13) on *Bgl*II and *Taq*I. The allele sizes of the different RFLPs have been described by Williamson et al. (1991). For the detection of RFLPs, 5 μg of genomic DNA was digested with the corresponding restriction enzyme. DNA was separated on a 0.8% agarose gel at 1 V/cm. Southern blotting and hybridization with a radiolabeled probe were done by using standard methods (Sambrook et al. 1989). For the marker D21S13 the *Eco*RI polymorphism was detected by using PCR amplification and subsequent restriction-enzyme digestion (Stinissen et al. 1990).

The short tandem repeat (STR) polymorphisms used have been described by Cruts et al. (1992) (D21S16), Warren et al. (1993) (D21S120), Kazantsev et al. (1992) (ATP1A3), Sharma et al. (1991) (D14S43), Wang and Weber (1992) (D14S52 and D14S53), Hudson et al. (1992) (D14S57 and D14S59), and the NIH/CEPH Collaborative Mapping Group (1992) (D14S42). For the analysis of the STR markers, 100 ng of genomic DNA was amplified by PCR with one radiolabeled primer. After denaturation, the PCR product was separated on a denaturing 6% polyacrylamide gel.

For SSCP analysis (Orita et al. 1989), radiolabeled PCR products were separated at room temperature on a 5% nondenaturing acryl:bisacrylamide (100:1) gel with or without 10% glycerol. Solid-phase sequencing of exon 17 of the APP gene was done as described by Adroer et al. (1992), by using the primer set of Bakker et al. (1991). Sequencing of exon 16 of the APP gene was done by direct sequencing of the PCR product by using 10% formamide (Zhang et al. 1991). Primers for amplification of exon 16 were as described by Adroer et al. (1992).

Linkage Analysis

Linkage analysis was performed assuming autosomal dominant inheritance of AD, a .001 allele frequency of the familial AD allele, and equal recombination rates for males and females. In the analysis presented here, phenocopy and mutation rates were set to zero. Because of the small number of unrelated individuals in the families, allelic and haplotype frequencies were derived from two extended Belgian families with AD (families AD/A and AD/B) (Van Broeckhoven et al. 1992). Where available, calculated frequencies for the markers were compared with published frequencies; and they were not significantly different. Allele and haplotype frequencies are available via ftp

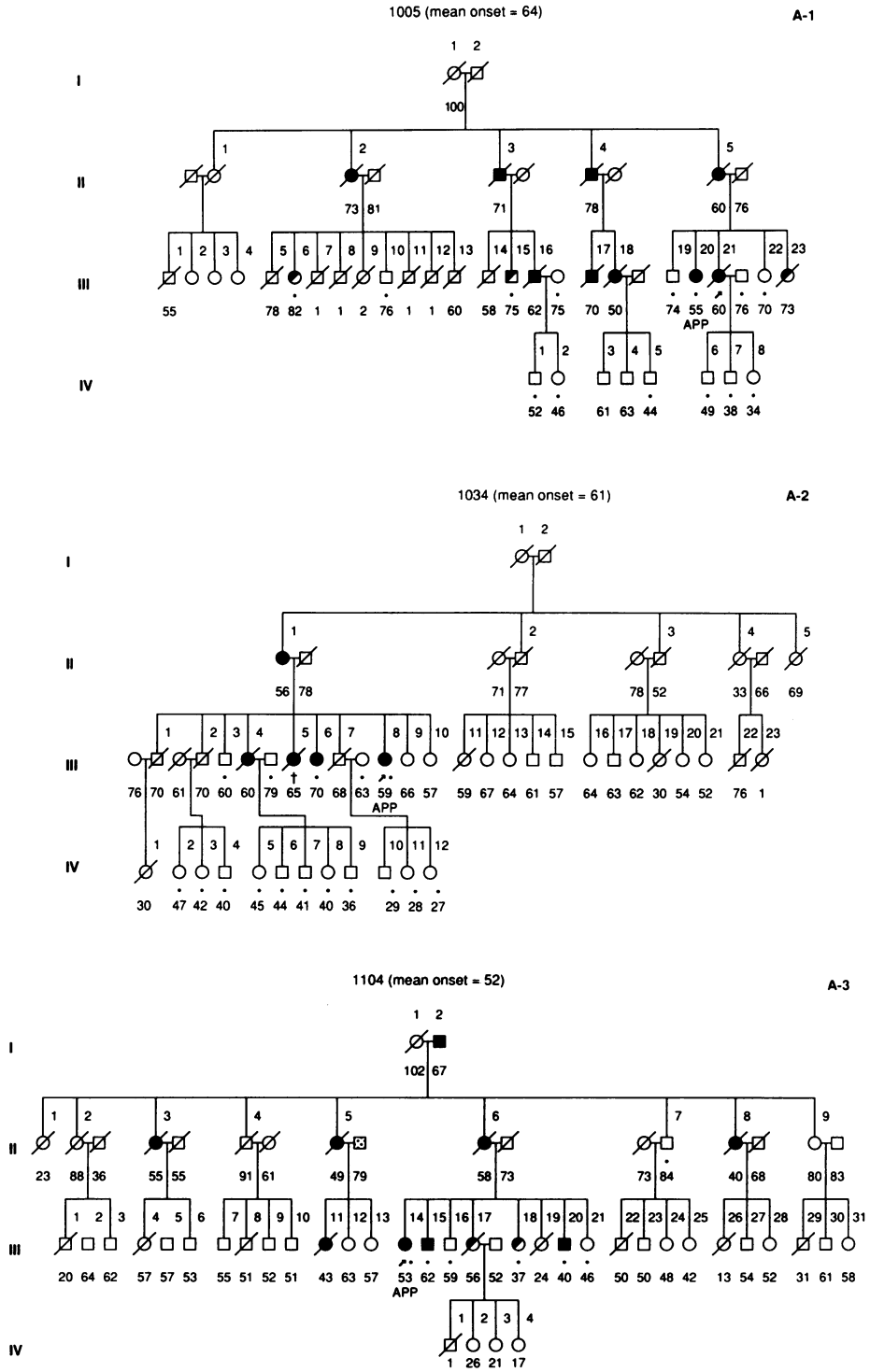

Figure 1 Pedigrees of the six EOAD families (A-1, A-2, A-3, A-4, A-5, and A-6) and four LOAD families (B-1, B-2, B-3, and B-4) included in the linkage analyses. The roman numerals to the left of each pedigree denote generations; the arabic numerals above the symbols denote individuals; and the arabic numerals below the symbols denote either age at examination or age at death, for unaffected subjects, or age at onset in individuals with probable AD, CVA, or Parkinson disease. Squares denote males, and circles denote females. □ and ○ = Unaffected; ■ and ● = diagnosed as having probable AD; ◩ and ◐ = diagnosed as having CVA; ▬ and ⊖ = diagnosed as having Parkinson disease; and ⊙ = reported to be forgetful but did not fulfill criteria for dementia or AD; APP = screened for mutations in exons 16 and 17 of APP gene; and PC = diagnosis pathologically confirmed. A dot (•) denotes that the individual was included in the linkage analyses; an arrow (→) indicates that the individual is the proband in the pedigree; and a cross (†) denotes that the age shown is the age at death for an affected person whose age at onset is unknown.

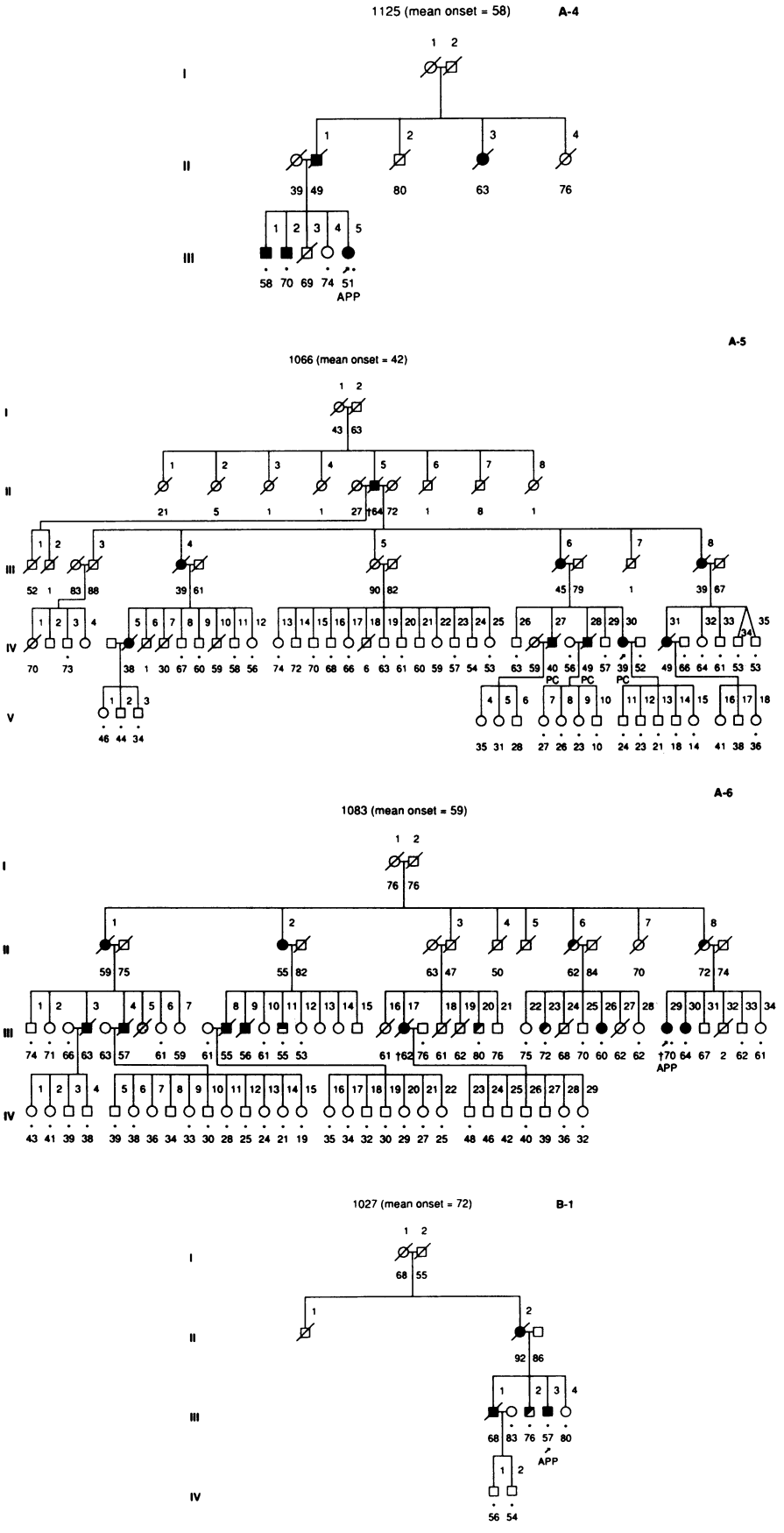

Figure I (continued)

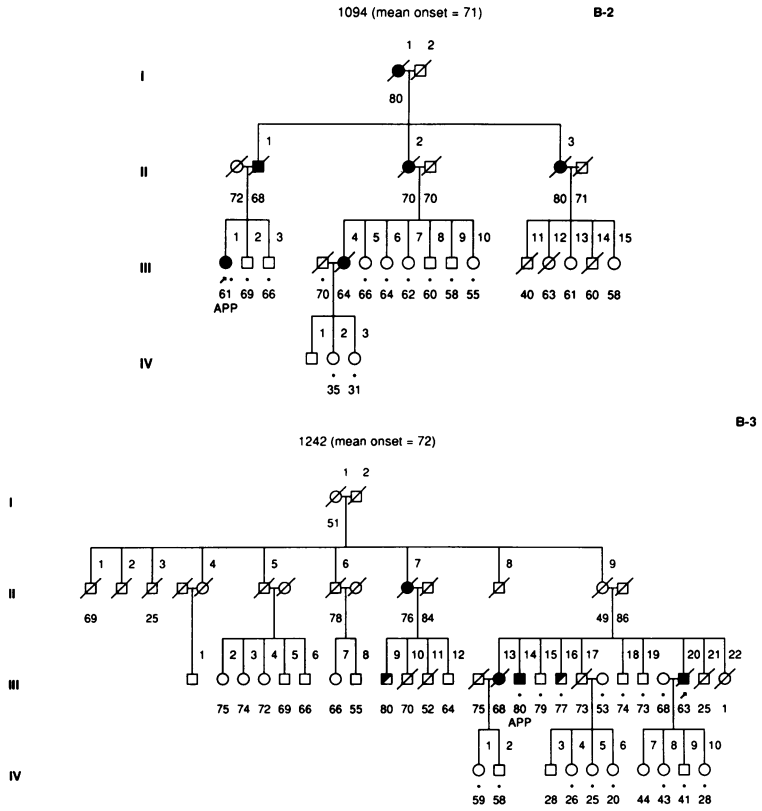

Figure 1 (continued)

(neurogen-ftp.uia.ac.be, directory /departments/neurogenetics, file: frequencies.inf).

We have calculated two-point lod score (Z) values with the program LIPED (Ott 1976), using an age-at-onset correction function that assumed a pedigree-specific normal distribution (Hodge et al. 1979). The mean onset age and SD were calculated from the observed onset ages. As unaffected relatives were censored at the time of the study and may express the disease later in life, the observed mean age at onset is likely to be an underestimate of the true mean age at onset. An earlier analysis of EOAD families showed that the observed age at onset was, on average, 5 years younger than the onset age adjusted for censoring (Farrer et al. 1990). Therefore we added 5 years to the observed mean age at onset, to correct for censored observations (Farrer et al. 1990).

The multipoint linkage analyses were performed using the LINKMAP program of LINKAGE (version 4.9; Lathrop et al. 1984). We have used separate curves for the age-at-onset correction for EOAD and LOAD families (Farrer et al. 1990). We have recoded the information by hand, reducing the number of alleles of STR markers to three or four and constructing haplotypes for RFLP markers with more than one enzyme polymorphism, in such a way that all or nearly all meiotic information was preserved. In one family (1083), only STR markers were tested; RFLP markers on chromosome 21 and chromosome 19 were not determined. In case of significant evidence for linkage of AD to a chromosome, linkage heterogeneity among families was tested using the HOMOG and BTEST programs (Smith 1963; Risch 1988).

The genetic map used in the chromosome 21 multipoint analysis was derived from Tanzi et al (1992*b*). For the chromosome 19 analysis, we used the map from Pericak-Vance et al. (1991). In the analysis of chromosome 19, APOC2 and BCL3 were haplotyped as a single locus, as they are located within 200 kb of each other (Shaw et al. 1989) and did not show recombinants in our families. The order and genetic distances of the chromosome 14 markers were based on data reported by the NIH/CEPH Collaborative Mapping Group (1992) and Van Broeckhoven et al. (1992) and on physical mapping data (authors' unpublished data). The markers used in the multipoint analysis were chosen prior to the analysis.

All analyses were repeated with the disease penetrance set to zero in all unaffected relatives, in order to minimize false evidence for linkage and recombination. The results of the linkage analyses were also analyzed after being stratified for family history of cerebrovascular accidents (CVAs). Finally, we have reanalyzed pedigrees showing

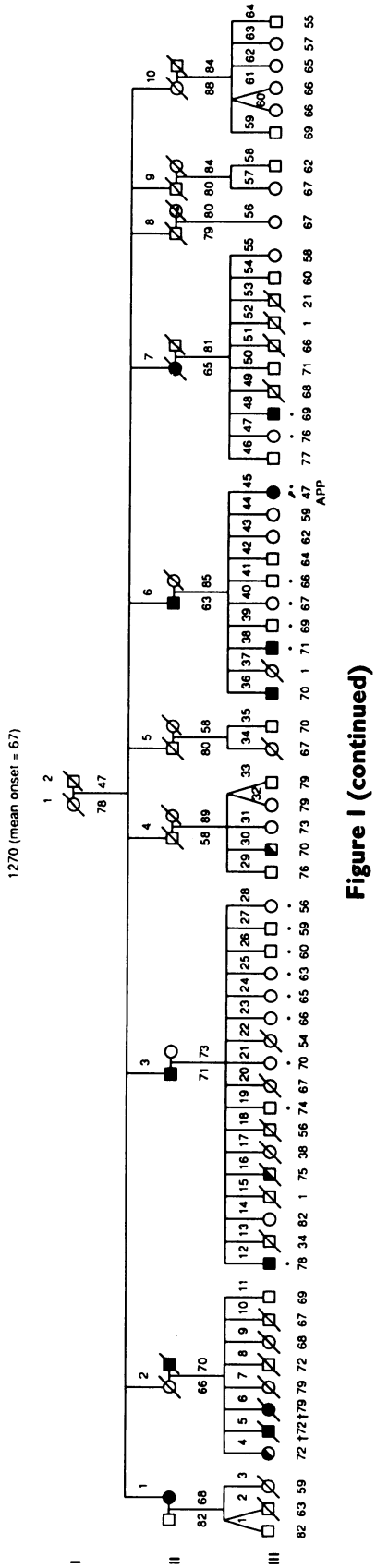

Figure 1 (continued)

strong evidence against linkage to a chromosome, allowing for a phenocopy rate of 10%. None of these analyses changed any of our conclusions.

Results

Family Data

In all probands the onset of AD was at or before age 65 years. The mean age at onset in the families was 62 years when all families were pooled. In four families (1027, 1094, 1242, and 1270), the mean of the onset ages of the relatives was >65 years (fig. 1). These families were therefore classified as LOAD. A wide spread in onset age of AD within the families was observed; the differences between relatives within a family varied from 7 to 35 years. The clinical diagnosis of AD was pathologically confirmed in three patients in family 1066. In six families (1005, 1027, 1083, 1104, 1242, and 1270) there were relatives of patients with probable AD who suffered from a CVA. In none of the patients with CVA was the etiology—i.e., ischemic stroke versus cerebral hemorrhage—known. Particular in family 1104, the CVAs occurred at early ages (37 and 56 years). In each family, a patient was screened for mutations in exon 16 and exon 17 of APP, by SSCP and PCR sequencing (fig. 1). No mutations were detected in any of these patients (data not shown).

Two-Point Linkage Analyses

In table 1, two-point Z values are summarized for all families and for the EOAD and LOAD families separately. None of the pooled Z values were conclusive for linkage of AD to the markers included in this study; Z values were either inconclusive or excluded linkage ($Z < -2$) when the families were pooled. For chromosome 21, a positive Z value was observed with D21S1/S11 ($Z_{max} = .93$ at $\theta = .0$) among EOAD families. Positive Z values were also observed for chromosome 19 and were highest at APOCII for the EOAD families ($Z_{max} = 1.46$ at $\theta = .0$) and at D19S13 for LOAD families ($Z_{max} = 1.06$ at $\theta = .0$).

For chromosome 14, $Z_{max} = 2.26$ at $\theta = .0$ was found at D14S43 in family 1066 (table 2). In none of the other families was there strong evidence for linkage of the disease to one chromosome. Two-point Z values for individual families for chromosomes 19 and 21 are available via ftp (neurogen-ftp.uia.ac.be, directory /departments/neurogenetics, files 2point19.lod and 2point21.lod).

Multipoint Linkage Analyses of Chromosome 14

A multipoint analysis including the D14S57, D14S43, and D14S53 loci was performed. Although initial linkage studies showed that D14S42 and D14S53 were the closest flanking markers of the EOAD gene (Van Broeckhoven et al. 1992), we included D14S57 instead of D14S42 in the multipoint analysis, since physical mapping data provided evidence that D14S42 is not located centromeric of

Table 1

Two-Point Z Values of AD with Chromosome 14, 19, and 21 Polymorphisms

Locus and Group	Z at θ = .00	.01	.05	.10	.20	.30	.40
Chromosome 14:							
D14S52:							
Total	−10.39	−6.25	−3.40	−1.95	−.62	−.12	−.01
EOAD	−7.90	−5.36	−3.39	−2.29	−1.07	−.43	−.11
LOAD	−2.49	−.89	−.01	.34	.45	.31	.10
D14S57:							
Total	−6.51	−3.97	−2.73	−1.90	−.90	−.35	−.09
DOAD	−1.68	−1.58	−1.23	−.88	−.40	−.15	−.04
LOAD	−4.83	−2.39	−1.50	−1.02	−.50	−.20	−.05
D14S43:							
Total	−5.42	−2.97	−1.04	−.13	.52	.48	.17
EOAD	−4.82	−2.44	−.72	.03	.51	.43	.15
LOAD	−.60	−.53	−.32	−.16	.01	.05	.02
D14S59:							
Total	−5.57	−4.07	−1.93	−.84	.03	.15	.08
EOAD	−2.93	−2.48	−1.08	−.34	.21	.21	.08
LOAD	−2.64	−1.59	−.85	−.50	−.18	−.06	.00
D14S53:							
Total	−11.53	−7.89	−4.68	−2.80	−1.00	−.29	−.08
EOAD	−9.35	−5.89	−3.16	−1.70	−.43	−.06	−.02
LOAD	−2.18	−2.00	−1.52	−1.10	−.57	−.23	−.06
D14S42:							
Total	−9.07	−5.65	−3.25	−1.93	−.64	−.15	−.01
EOAD	−6.88	−4.27	−2.52	−1.51	−.50	−.12	.00
LOAD	−2.19	−1.38	−.73	−.42	−.14	−.03	−.01
Chromosome 19:							
APOCII:							
Total	−2.25	−1.46	−.17	.33	.54	.39	.12
EOAD	1.46	1.45	1.37	1.21	.86	.48	.14
LOAD	−3.71	−2.91	−1.54	−.88	−.32	−.09	−.02
BCL3:							
Total	−2.95	−1.86	−.95	−.47	−.05	.04	.02
EOAD	−.03	.02	.16	.26	.28	.17	.05
LOAD	−2.92	−1.88	−1.11	−.73	−.33	−.13	−.03
ATP1A3:							
Total	−10.72	−5.30	−2.25	−.66	.39	.46	.18
EOAD	−4.93	−2.40	−.84	.09	.60	.49	.17
LOAD	−5.79	−2.90	−1.41	−.75	−.21	−.03	.01
CYP2B:							
Total	−2.78	−1.43	−.70	−.39	−.12	−.04	.00
EOAD	.21	.20	.21	.18	.13	.07	.02
LOAD	−2.99	−1.63	−.91	−.57	−.25	−.11	−.02
D19S13:							
Total	.41	.66	1.06	1.18	1.00	.58	.19
EOAD	−.65	−.39	.10	.35	.43	.27	.08
LOAD	1.06	1.05	.96	.83	.57	.31	.11
Chromosome 21:							
D21S16:[a]							
Total	−.60	−1.75	−.44	.05	.30	.20	.05
EOAD	−.91	−.38	.21	.39	.37	.20	.04
LOAD	.31	−1.37	−.65	−.34	−.07	.00	.01
D21S16:[b]							
Total	−6.34	−5.10	−3.04	−1.86	−.68	−.18	−.02
EOAD	−.99	−.87	−.48	−.22	.03	.09	.04
LOAD	−5.35	−4.23	−2.56	−1.64	−.71	−.27	−.06

(*continued*)

Table 1 (continued)

Locus and Group	Z at θ = .00	.01	.05	.10	.20	.30	.40
D21S13:							
Total	−2.67	−.91	.23	.80	.98	.62	.17
EOAD	−.55	−.39	.09	.44	.58	.38	.12
LOAD	−2.12	−.52	.14	.36	.40	.24	.05
D21S52:							
Total	−.56	−.53	−.43	−.33	−.18	−.08	−.02
EOAD	−.41	−.39	−.33	−.26	−.14	−.06	−.01
LOAD	−.15	−.14	−.10	−.07	−.04	−.02	−.01
D21S1S11:							
Total	−5.83	−3.13	−1.50	−.75	−.17	.00	.02
EOAD	.93	.91	.83	.74	.50	.27	.08
LOAD	−6.76	−4.04	−2.33	−1.49	−.67	−.27	−.06
APP:							
Total	−3.72	−2.66	−1.68	−1.09	−.43	−.13	−.02
EOAD	−1.26	−1.18	−.89	−.61	−.24	−.06	−.01
LOAD	−2.46	−1.48	−.79	−.48	−.19	−.07	−.01
D21S120:							
Total	−9.01	−5.90	−2.43	−1.39	−.37	−.01	.05
EOAD	−3.77	−2.16	−.37	−.25	−.07	.01	.02
LOAD	−5.24	−3.74	−2.06	−1.14	−.30	−.02	.03

[a] RFLP marker.
[b] STR marker.

D14S43 but telomeric of D14S53 (authors' unpublished results). The multipoint analysis provided evidence for linkage of AD to D14S43 (Z_{max} = 3.71 at θ = .0) in family 1066 (fig. 2*A*). For the other EOAD families, linkage to the region 14q24.3 was excluded for families 1083 and 1104, while families 1005, 1125, and 1034 were basically uninformative. For the LOAD families, predominantly negative *Z* values were obtained, and the overall *Z* values excluded linkage to chromosome 14 (fig. 2*B*).

Multipoint Linkage Analyses of Chromosome 19

The multipoint analysis of chromosome 19 loci showed inconclusive *Z* values for the individual EOAD families, with the exception of family 1066, for which linkage was excluded (fig. 3*A*). When family 1066 was omitted, the results of the linkage analysis remained inconclusive, Z_{max} being 1.9 at D19S13 (θ = .0) when all EOAD families were pooled. Of the LOAD families, family 1242 showed positive *Z* values that reached a maximum at D19S13 (Z_{max} = 1.2 at θ = .0; fig. 3*B*).

Multipoint Linkage Analyses of Chromosome 21

The findings of the multipoint analysis of the chromosome 21 loci are presented in figure 4*A* and *B*. Family 1066, for which there was strong evidence for linkage of EOAD to chromosome 14, showed negative *Z* values for chromosome 21 (fig. 4*A*). When this family was omitted from the overall analysis of the EOAD families, positive *Z* values were obtained, although they were not conclusive (fig. 4*A*). Among the LOAD families, the overall *Z* values suggested absence of linkage of LOAD to chromosome 21.

Heterogeneity

Statistical testing yielded significant evidence for heterogeneity for the chromosome 14 analysis when all families were pooled (χ^2 = 3.6; P <.03); that is, some families appeared to be linked to chromosome 14, but others did not. However, no significant difference could be shown between EOAD and LOAD families. Finally, all multipoint results for chromosomes 14, 19, and 21 were analyzed after being stratified for family history of CVA. No evidence for linkage to one specific chromosome was found for those with a positive family history of CVA (data not shown).

Discussion

In our population-based study, a wide spread in onset age within the families was observed, varying from 7 to 35 years. Although families were ascertained through probands who showed the first symptoms of disease before age 65 years, the mean age at onset was older than 65 years in 4 of 10 families. There were six EOAD families. In one of these families (1083), multiple markers were tested only

Table 2

Two-Point *Z* Values of AD with Chromosome 14 Polymorphisms

	Z AT θ =						
LOCUS AND FAMILY	.00	.01	.05	.10	.20	.30	.40
D14S52:							
1005	−.32	−.31	−.25	−.19	−.10	−.04	−.01
1027	−.27	−.26	−.23	−.18	−.10	−.05	−.01
1034	−2.56	−1.25	−.61	−.35	−.14	−.05	−.01
1066	−1.66	−1.50	−1.07	−.74	−.35	−.14	−.04
1083	−2.69	−1.66	−.95	−.62	−.29	−.12	−.03
1094	−2.84	−1.38	−.71	−.43	−.19	−.07	−.02
1104	−.32	−.32	−.30	−.26	−.15	−.07	−.02
1125	−.35	−.32	−.21	−.13	−.04	−.01	.00
1242	−.45	−.30	−.02	.12	.17	.12	.04
1270	1.07	1.05	.95	.83	.57	.31	.09
Total	−10.39	−6.25	−3.40	−1.95	−.62	−.12	−.01
EOAD	−7.90	−5.36	−3.39	−2.29	−1.07	−.43	−.11
LOAD	−2.49	−.89	−.01	.34	.45	.31	.10
D14S57:							
1005	−.48	−.46	−.38	−.29	−.15	−.06	−.02
1027	−4.32	−1.91	−1.10	−.71	−.32	−.13	−.03
1034	−.31	−.28	−.21	−.15	−.07	−.03	−.01
1066	−.47	−.43	−.27	−.13	−.01	.01	.01
1083							
1094	−.43	−.40	−.31	−.23	−.12	−.05	−.01
1104	−.38	−.38	−.35	−.30	−.17	−.07	−.02
1125	−.04	−.03	−.02	−.01	.00	.00	.00
1242	−.15	−.15	−.14	−.12	−.07	−.03	−.01
1270	.07	.07	.05	.04	.01	.01	.00
Total	−6.51	−3.97	−2.73	−1.90	−.90	−.35	−.09
EOAD	−1.68	−1.58	−1.23	−.88	−.40	−.15	−.04
LOAD	−4.83	−2.39	−1.50	−1.02	−.50	−.20	−.05
D14S43:							
1005	.04	.04	.03	.03	.02	.01	.00
1027	−.47	−.45	−.38	−.30	−.16	−.07	−.02
1034	.17	.17	.16	.14	.09	.05	.01
1066	2.26	2.21	2.01	1.75	1.22	.67	.20
1083	−3.66	−3.28	−2.15	−1.48	−.72	−.29	−.06
1094	−.75	−.69	−.51	−.36	−.18	−.07	−.02
1104	−4.21	−2.15	−1.28	−.84	−.39	−.16	−.04
1125	.58	.57	.51	.43	.29	.15	.04
1242	−.06	−.06	−.05	−.04	−.02	−.01	.00
1270	.68	.67	.62	.54	.37	.20	.06
Total	−5.42	−2.97	−1.04	−.13	.52	.48	.17
EOAD	−4.82	−2.44	−.72	.03	.51	.43	.15
LOAD	−.60	−.53	−.32	−.16	.01	.05	.02
D14S59:							
1005	.53	.53	.53	.51	.38	.21	.06
1027	−.16	−.15	−.12	−.09	−.04	−.02	.00
1034	−.02	−.01	.02	.04	.05	.03	.01
1066	−.48	−.47	−.38	−.26	−.08	−.01	.00
1083	−2.38	−1.96	−.76	−.23	.08	.08	.03
1094	−.11	−.10	−.08	−.06	−.03	−.01	.00
1104	−.26	−.25	−.21	−.17	−.09	−.04	−.01
1125	−.32	−.32	−.28	−.23	−.13	−.06	−.01
1242	−.03	−.03	−.02	−.01	.00	.00	.00
1270	−2.34	−1.31	−.63	−.34	−.11	−.03	.00
Total	−5.57	−4.07	−1.93	−.84	.03	.15	.08
EOAD	−2.93	−2.48	−1.08	−.34	.21	.21	.08
LOAD	−2.64	−1.59	−.85	−.50	−.18	−.06	.00

(*continued*)

Table 2 (continued)

Locus and Family	Z at θ = .00	.01	.05	.10	.20	.30	.40
D14S53:							
1005	−1.10	−1.03	−.78	−.56	−.27	−.11	−.03
1027	−.20	−.20	−.18	−.15	−.10	−.04	−.01
1034	−.23	−.22	−.19	−.15	−.08	−.04	−.01
1066	.32	.43	.65	.72	.57	.30	.07
1083	−4.81	−3.58	−2.16	−1.39	−.61	−.24	−.06
1094	−.38	−.37	−.32	−.25	−.14	−.06	−.02
1104	−4.21	−2.15	−1.28	−.84	−.39	−.16	−.04
1125	.68	.66	.60	.52	.35	.19	.05
1242	−.72	−.69	−.58	−.45	−.25	−.11	−.03
1270	−.88	−.74	−.44	−.25	−.08	−.02	.00
Total	−11.53	−7.89	−4.68	−2.80	−1.00	−.29	−.08
EOAD	−9.35	−5.89	−3.16	−1.70	−.43	−.06	−.02
LOAD	−2.18	−2.00	−1.52	−1.10	−.57	−.23	−.06
D14S42:							
1005	−.90	−.81	−.57	−.37	−.15	−.06	−.01
1027	−.65	−.63	−.55	−.43	−.23	−.10	−.02
1034	−.39	−.36	−.27	−.19	−.09	−.04	−.01
1066	.57	.55	.50	.44	.30	.16	.05
1083	−2.59	−2.13	−1.46	−1.04	−.50	−.19	−.04
1094	.02	.02	.03	.03	.02	.01	.00
1104	−4.21	−2.15	−1.28	−.84	−.39	−.16	−.04
1125	.64	.63	.56	.49	.33	.17	.05
1242	−1.66	−.86	−.29	−.08	.04	.04	.01
1270	.10	.09	.08	.06	.03	.02	.00
Total	−9.07	−5.65	−3.25	−1.93	−.64	−.15	−.01
EOAD	−6.88	−4.27	−2.52	−1.51	−.50	−.12	.00
LOAD	−2.19	−1.38	−.73	−.42	−.14	−.03	−.01

for chromosome 14. Our study showed strong evidence for linkage of EOAD to chromosome 14 in one family (1066) with a very early onset of AD—around age 47 years. Also, this family did not show evidence of linkage of AD to chromosome 19 markers and chromosome 21 markers. In two families, linkage of EOAD to chromosome 14 could be excluded. This finding supports the findings of other studies that excluded linkage of EOAD to both chromosome 14 and chromosome 21 in families (Mullan et al. 1992*b*; St George-Hyslop et al. 1992; Schellenberg et al. 1992; Lannfelt et al. 1993).

Overall *Z* values from the multipoint analysis for the other EOAD families were not conclusive for linkage to chromosome 19 and/or chromosome 21. For the LOAD families, overall negative *Z* values were observed in the multipoint analysis for chromosomes 14, 19, and 21. Only one family (1242) was suggestive for linkage of LOAD to chromosome 19, although the *Z* value was not conclusive. Two families, the EOAD family 1005 (mean onset age 63 years) and the LOAD family 1027 (mean onset age 69 years), showed negative *Z* values for markers on each of the three chromosomes.

An important issue in this linkage study of AD is the evaluation of the evidence against linkage, as there may be several explanations other than absence of linkage. Despite the careful clinical diagnosis of AD in probands and relatives by neurologists, misdiagnosis of probable AD may have inflated the evidence against linkage. In particular the diagnosis of patients from the six families in which there were relatives with CVA is liable to misclassification. In the analysis presented here, relatives with a history of CVA are considered to be unaffected. However, *Z* values remained negative in an analysis in which all relatives with CVA were considered affected. In the analysis that we present here, phenocopy rate was set to zero. False recombinations may have also occurred because of the existence of phenocopies, particularly in the old-age category, as the prevalence of AD increases exponentially with age. Visual inspection of our data showed that recombinations did not occur typically in relatives with a very late onset (>75 years) of AD. Furthermore, we have reanalyzed pedigrees that showed strong evidence against linkage, allowing for a phenocopy rate of 10%. In each of these analysis, the evidence against linkage remained significant. Also, our conclusions did not change when we performed the multipoint analyses with the disease penetrance set to zero

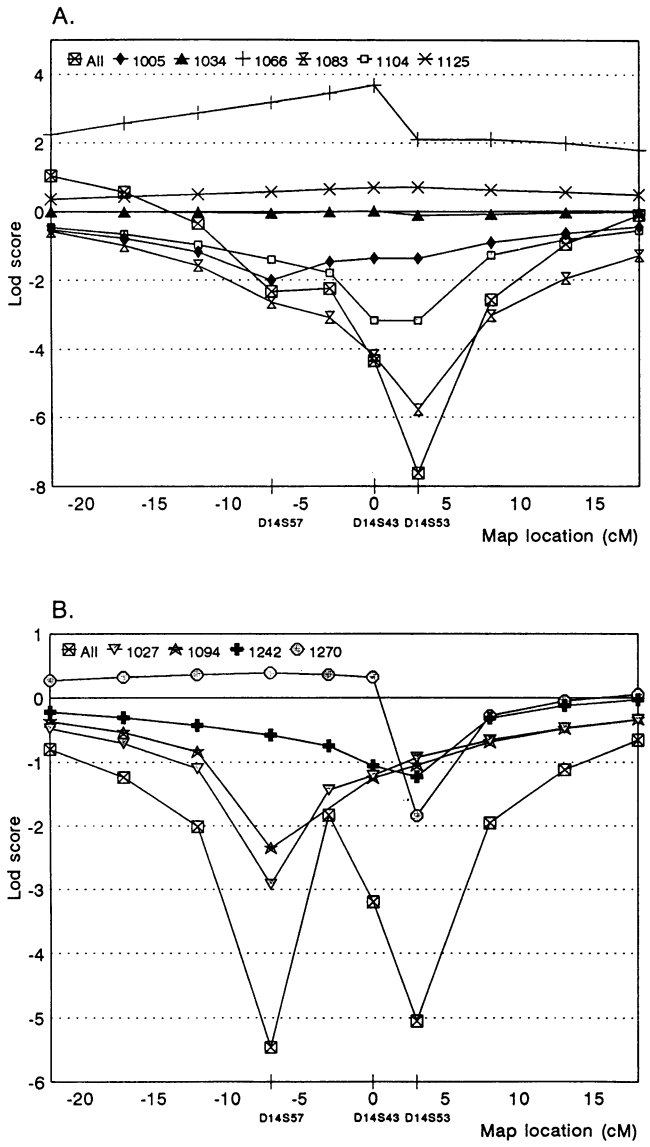

Figure 2 Multipoint linkage analysis of chromosome 14 markers in EOAD families (*A*) and LOAD families (*B*). The map location of D14S43 was arbitrarily set at zero. Map distances are 7 cM between D14S57 and D14S43 and 3.3 cM between D14S43 and D14S53. *Z* values are presented both as summarized for EOAD and LOAD families and for each family separately.

in all unaffected relatives, in order to minimize false evidence for linkage and recombination. Finally, we cannot exclude the possibility that recombinations may have resulted from genotype inferences for individuals who were deceased and from whom no DNA was available. Since allelic and haplotype frequencies were derived from the Belgian population, deductions may have been inaccurate. However, major differences in allele frequencies between the two Caucasian Dutch-speaking populations from neighboring countries are not expected.

The observation that in six families there was familial aggregation of probable AD and CVA is of interest in view of our earlier finding that in one family probable AD and cerebral hemorrhage due to amyloidosis were both linked to a mutation at codon 692 of the APP gene (Hendriks et al. 1992). This suggests that in some families the common factor in the etiology of AD and CVA is cerebral amyloid and perhaps angiopathy. In one of the families (1104) presented here, CVAs occurred very early and at an age similar to the onset age of the dementia in this family. However, no mutations in exon 16 and exon 17 of APP were detected in this family or in the other families. Also, after stratifying for family history of CVA, the present study did

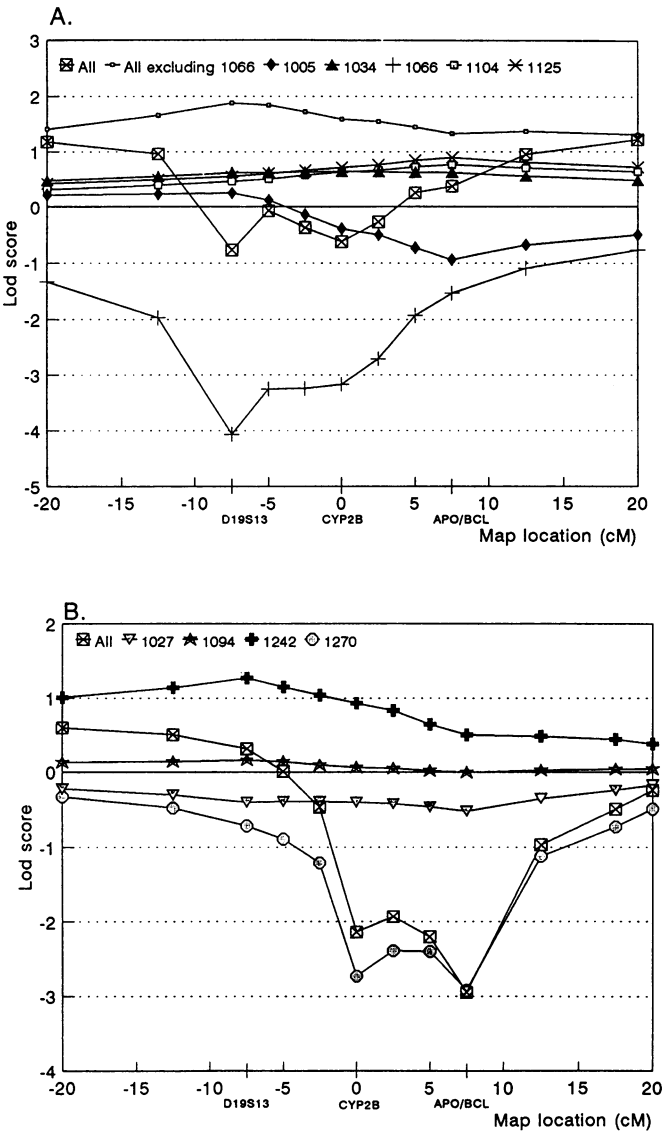

Figure 3 Multipoint linkage analysis of chromosome 19 markers in EOAD families (*A*) and LOAD families (*B*). The map location of D19CYP2B was arbitrarily set at zero. Map distances are 7.5 cM between APOCII/BCL and D19CYP2B and 7.5 cM between D19CYP2B and D19S13. *Z* values are presented both as summarized for EOAD and LOAD families and for each family separately.

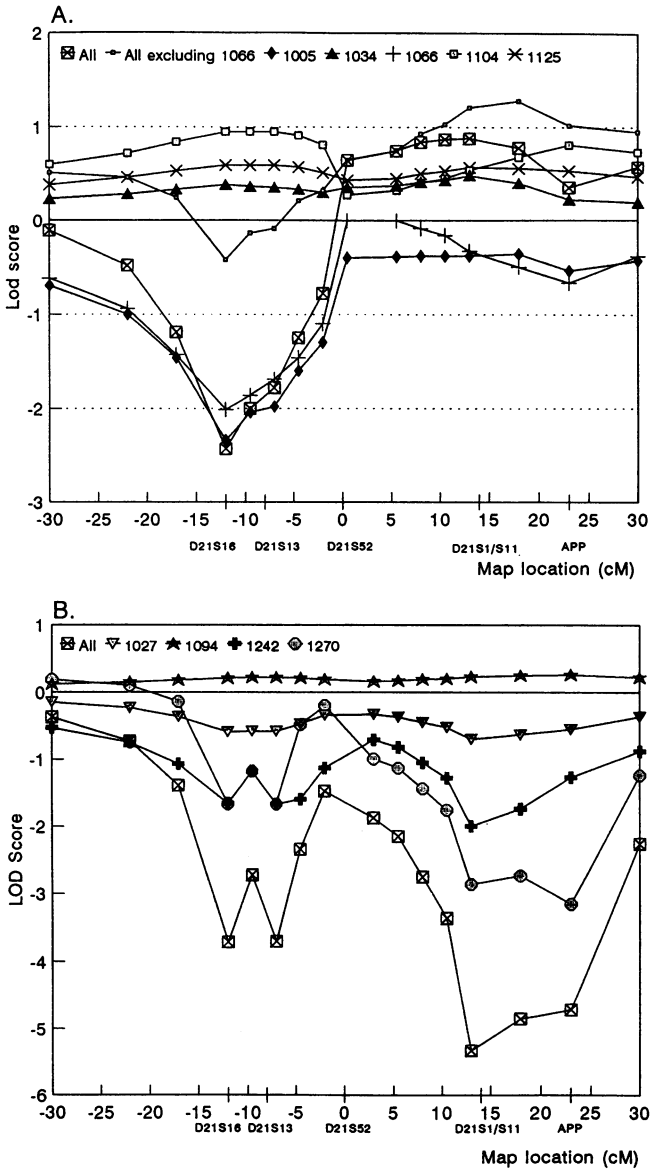

Figure 4 Multipoint linkage analysis of chromosome 21 markers in EOAD families (*A*) and LOAD families (*B*). The map location of D21S52 was arbitrarily set at zero. Map distances are 4 cM between D21S16 and D21S13, 8 cM between D21S13 and D21S52, 14 cM between D21S52 and D21S1/S11, and 9 cM between D21S1/S11 and APP. *Z* values are presented both as summarized for EOAD and LOAD families and for each family separately.

not show evidence for linkage to APP or other chromosome 21 markers, for families in which there was familial aggregation of AD and CVA.

Statistical testing yielded significant evidence of genetic heterogeneity for the chromosome 14 analysis. No significant differences could be shown between EOAD and LOAD families in our study; however, the statistical power to test this hypothesis was low. Indeed, heterogeneity is suggested by the finding that within the EOAD families there was significant evidence for linkage of AD to chromosome 14 (q24.3) for one EOAD family, while linkage to this region was excluded for two other EOAD families. In our population, there was no statistical significant evidence for linkage of EOAD to multiple chromosomes. On the assumption that all AD cases within a family are caused by one underlying genetic factor, the wide spread (up to 35 years) in onset age of AD within the families remains to be explained. As AD is associated with the second commonest allele (E4) of the APOE gene on chromosome 19, onset of AD may be determined by the presence and the number of APOE4 alleles (Corder et al. 1993; Strittmatter et al. 1993; Van Duijn et al. 1994). However, it is also conceivable that other environmental or genetic factors play a role in the onset of AD.

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