

Fig. 1. Chromosome 10 multipoint plot. Robust multipoint lod scores on chromosome 10 were calculated for the five LOAD families that had an AD proband with extremely high plasma Aβ. The markers that were genotyped are shown below the plot. A maximum MLS of 3.93 is at 81 cM between D10S1227 and D10S1211.

report (15, 16).

The results we report show that plasma Aβ can be used as a quantitative trait for identifying novel LOAD loci. This approach is a powerful complement to other methods for identifying genetic risk factors for LOAD. It enables the evaluation of candidate genes at a mechanistic level and, because multiple generations can be analyzed in extended pedigrees grouped according to their phenotypic characteristics, the power to detect linkage and to obtain precise localization is increased. Thus, by analyzing 124 subjects in five families identified via a proband with extremely high Aβ42, we obtained highly significant linkage that was well localized to chromosome 10 with a 1-lod support interval of ~8 cM. These results fit well with those obtained in the second stage of the sibling pair study that provided our candidate regions (17). In that study, published jointly with our findings, Myers *et al.* analyzed 429 affected sibling pairs in 342 sibships and obtained significant linkage to the same region of chromosome 10 with a 1-lod support interval of ~16 cM. Together, the results of these two studies, performed on nonoverlapping family series, provide compelling, mutually confirmatory evidence for a novel LOAD locus on chromosome 10. From our results, it appears that this locus increases risk for AD by increasing Aβ. Because we have sought linkage to the high Aβ phenotype in only a small fraction of the human genome, it is likely that additional LOAD loci will be detected by this method as we evaluate the remainder of the genome in our collection of families.

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15. IDE is an interesting candidate, because there are several reports linking Aβ degradation with IDE activity, but this gene is 30 cM distal to the peak identified by our linkage analysis. We have, moreover, found no potential pathogenic variants by sequencing 90% of the IDE coding region in 11 members from our collection of pedigrees.
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Susceptibility Locus for Alzheimer's Disease on Chromosome 10

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The apolipoprotein E (*APOE*) gene is the only genetic risk factor that has so far been linked to risk for late-onset Alzheimer's disease (LOAD). However, 50 percent of Alzheimer's disease cases do not carry an *APOE4* allele, suggesting that other risk factors must exist. We performed a two-stage genome-wide screen in sibling pairs with LOAD to detect other susceptibility loci. Here we report evidence for an Alzheimer's disease locus on chromosome 10. Our stage one multipoint lod score (logarithm of the odds ratio for linkage/no linkage) of 2.48 (266 sibling pairs) increased to 3.83 in stage 2 (429 sibling pairs) close to D10S1225 (79 centimorgans). This locus modifies risk for Alzheimer's disease independent of *APOE* genotype.

Mutations in three genes encoding β-amyloid precursor protein (APP), presenilin 1, and presenilin 2 cause the rare, early-onset autosomal dominant form of Alzheimer's disease (AD) (1). These mutations all affect APP metabolism such that more Aβ42 peptide is

produced (2). In contrast, most AD cases have ages of onset above 65 years and exhibit no clear pattern of inheritance (late-onset Alzheimer's disease or LOAD). The E4 allele of the apolipoprotein E (*APOE*) gene is the only known genetic risk factor for LOAD (3, 4). However, 50% of LOAD cases carry no *APOE4* alleles, indicating that there must be additional risk factors (4).

To look for other susceptibility loci, we performed a two-stage genome-wide screen in affected Caucasian sibling pairs (ASPs). Each sibling used for analysis had an age of onset ≥65 years and a diagnosis of definite or probable AD. If there were more than two affected siblings within a family, all siblings were sampled and genotyped. In stage 1, 292 ASPs were genotyped with markers spaced about 20 centimorgans (cM) apart throughout

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the genome (5, 6). Nonparametric methods, which do not require the mode of inheritance to be specified, were used to analyze these data in the whole sample and in two subsamples stratified by *APOE4* genotype. We observed 16 chromosomal regions with a multipoint logarithm of the odds ratio for linkage/no linkage (lod score) (MLS) ≥ 1 (6). Four of the sixteen peaks, including one on chromosome 10, met the criteria for "suggestive" linkage (7).

For stage 2, markers within the stage 1 peaks were genotyped in an additional 168 ASPs (83 newly ascertained in the United Kingdom, 80 obtained from the Indiana Alzheimer Disease Center National Cell Repository, and 5 from the National Institute of Mental Health Genetics Initiative) (8). On chromosome 10, 15 additional markers were genotyped in all 429 ASPs, reducing the average interval to 5 cM. Two-point analyses were carried out between each marker locus and the disease with the program SPLINK (9), which was also used to estimate marker allele frequencies for the multipoint analyses. The highest two-point lod scores, 3.10 in stage 1 and 4.85 in stage 2, were obtained in the whole sample with marker D10S1211 (at 82.2 cM on the multipoint map). On average, 50%

of the siblings share alleles at any given locus. However, 64% of ASPs share alleles for D10S1211. Elevated allele sharing and positive lod scores were observed in the whole sample and in *APOE4*+ve (pairs where both siblings had at least one *APOE4* allele) and *APOE4*-ve (pairs where neither sibling had an *APOE4* allele) sibling pairs in an extended region around D10S1211 (10).

Multipoint linkage analyses were carried out with the program MAPMAKER/SIBS (11) on the whole sample, on pairs where both siblings had at least one *APOE4* allele, and on pairs where neither sibling had an *APOE4* allele (Fig. 1). These analyses use information from adjacent markers to determine the most likely location of the disease susceptibility allele. The stage 1 maximum MLS of 2.48 at 77.6 cM is slightly different from that previously reported (6) and reflects changes in diagnoses. This lod score increased to 3.83 at 79 cM in stage 2 (between D10S1227 and D10S1225). An MLS of 3.83 would be expected to occur by chance 0.01 times per genome scan, considering the whole-sample analysis alone (or ~ 0.05 per genome allowing for all three analyses) (12). The stage 2 analysis of *APOE4*+ve ASPs gave a maximum MLS of 2.78 at D10S1225, whereas the *APOE4*-ve pairs gave a maximum MLS of 0.66 at 85.4 cM. However, allele sharing was similarly elevated (to 59%) in all three subgroups, indicating that stratification of the sample by *APOE4* did not change the proportion of allelic sharing at the peak.

In conclusion, we have confirmed our preliminary observation of a region of suggestive linkage on chromosome 10 (6), providing strong evidence for a susceptibility locus for LOAD. This locus was robustly detected in both stages of our genome screen and shows a maximum MLS greater than that observed in the same data set on chromosome 19 around the *APOE* locus. This suggests that the chromosome 10 locus is a major risk factor for LOAD. Indeed, we estimate the chromosome 10 locus-specific λ 's (relative risk to siblings) to be about equivalent to that for *APOE*. In the accompanying report by Ertekin-Taner *et al.* (13), there is evidence that a quantitative trait locus for high A β 42 levels maps to the same chromosomal region. This suggests that the AD susceptibility allele identified in our study increases risk for disease by modifying A β 42 metabolism.

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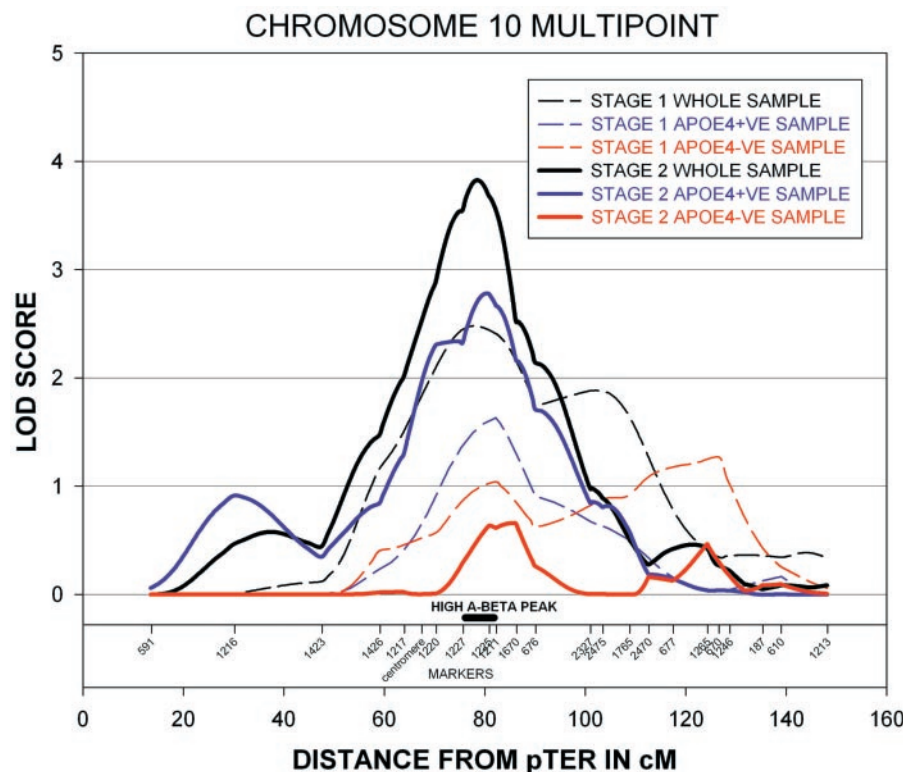


Fig. 1. Chromosome 10 multipoint map for LOAD. Multipoint linkage analyses were carried out with the program MAPMAKER/SIBS (11) on the whole sample (429 pairs), on pairs where both siblings had at least one *APOE4* allele (*APOE4*+ve; 262 pairs), and on pairs where neither sibling had an *APOE4* allele (*APOE4*-ve; 83 pairs). The region of linkage for high plasma A β 42 from the Ertekin-Taner *et al.* (13) report is also shown. Marker distances were taken from the Marshfield database (15).