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12. Supplementary data are available at *Science* Online at www.sciencemag.org/cgi/content/full/290/5500/2302/DC1.
13. Intermarker distances are according to marker locations from Marshfield, except for interval D10S566 to D10S1671, where 0.7 cM was used according to the MAP-O-MAT program (http://linkage.rockefeller.edu:1802/mapomat/mapomat_menu.html).
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Linkage of Plasma Aβ42 to a Quantitative Locus on Chromosome 10 in Late-Onset Alzheimer's Disease Pedigrees

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Plasma Aβ42 (amyloid β42 peptide) is invariably elevated in early-onset familial Alzheimer's disease (AD), and it is also increased in the first-degree relatives of patients with typical late-onset AD (LOAD). To detect LOAD loci that increase Aβ42, we used plasma Aβ42 as a surrogate trait and performed linkage analysis on extended AD pedigrees identified through a LOAD patient with extremely high plasma Aβ. Here, we report linkage to chromosome 10 with a maximal lod score of 3.93 at 81 centimorgans close to D10S1225. Remarkably, linkage to the same region was obtained independently in a genome-wide screen of LOAD sibling pairs. These results provide strong evidence for a novel LOAD locus on chromosome 10 that acts to increase Aβ.

The autosomal dominant mutations that cause early-onset familial AD all increase Aβ42 in plasma and brain (*1–6*). Compared to age-matched controls, plasma Aβ42 is also elevated in the cognitively normal first-degree relatives and extended families of patients with typical LOAD (*7*). To assess the genetic component affecting plasma Aβ42 levels, we collected 10 LOAD pedigrees [see Web table 1 for family description and ascertainment scheme (*8*)], used a sandwich enzyme-linked immunosorbent assay (*5*) to measure plasma Aβ42, estimated the heritability of plasma Aβ42 using the variance component method implemented in SOLAR (*9*), and found it to be 64.8 ± 15.5% ($P < 0.0001$; n = 203).

Given the association of elevated plasma Aβ42 with AD, the substantial heritability of this quantitative trait in our LOAD pedigrees, and the recent successful linkage of genetic loci to quantitative traits associated with complex diseases (*10–12*), we decided to search for LOAD genes by performing linkage analysis in our LOAD families using plasma Aβ42 as a surrogate trait. Using a traditional affected sibling pair approach, Kehoe *et al.* (*13*) performed a genome-wide screen for LOAD loci that identified regions on chromosomes 1, 5, 9, 10, and 19 with multipoint lod (logarithm of odds for "linkage/no linkage") scores (MLSs) >1. Reasoning that these regions might contain genes linked to AD because they elevate Aβ42, we tested each region for linkage to plasma Aβ42.

In previous searches for genes governing quantitative traits, the power to identify gene(s) with strong effect (major genes) has been increased by performing linkage analysis on families ascertained using probands with extreme values for the quantitative trait in question (*10, 12*). For this reason, we focused our analysis on five families that had an AD proband with extremely high plasma Aβ (top 10% of AD patients). When robust MLSs for these five families were calculated using SOLAR (*9, 14*), the region on chromosome 10 gave a maximum MLS of 3.93 (Fig. 1) at 81 centimorgans (cM) between D10S1227 and D10S1211 (empirical P value by simulation = 0.0001). In all other regions, which were tested both in the extreme families and the entire group, the maximum MLS was <0.5 [see Web tables 2 and 3 for details of the analysis (*8*)]. Because we examined only 10 families and deliberately weighted our collection with pedigrees ascertained via an AD proband with high Aβ (top 10%), our results [Web tables 2 and 3 (*8*)] cannot be used to evaluate the contribution of the chromosome 10 locus to AD in general.

Here, we focused on Aβ42 because of its close association with AD, but we also performed linkage analysis on the five "extreme" families using plasma Aβ40 as the quantitative trait. In this analysis, the maximum MLS obtained for the chromosome 10 region was 1.36 (point-wise P value ~0.006). All other regions gave maximum MLSs < 0.3. This result suggests that the locus on chromosome 10 may influence both Aβ40 and Aβ42.

There are no obvious candidate genes in the chomosome 10 region that we identified (1-lod support interval of ~8 cM), but the gene for insulin-degrading enzyme (IDE), which is 30 cM distal to our peak, is considered by Bertram *et al.* in their accompanying

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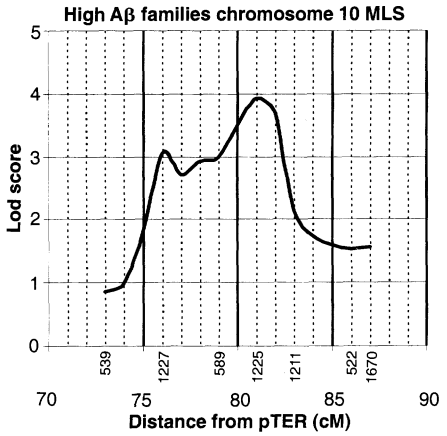

Fig. 1. Chromosome 10 multipoint plot. Robust multipoint lod scores on chromosome 10 were calculated for the five LOAD families that had an AD proband with extremely high plasma Aβ. The markers that were genotyped are shown below the plot. A maximum MLS of 3.93 is at 81 cM between D10S1227 and D10S1211.

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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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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