Evidence for Genetic Linkage of Alzheimer's Disease to Chromosome 10q

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Recent studies suggest that insulin-degrading enzyme (IDE) in neurons and microglia degrades Aβ, the principal component of β-amyloid and one of the neuropathological hallmarks of Alzheimer's disease (AD). We performed parametric and nonparametric linkage analyses of seven genetic markers on chromosome 10q, six of which map near the *IDE* gene, in 435 multiplex AD families. These analyses revealed significant evidence of linkage for adjacent markers (D10S1671, D10S583, D10S1710, and D10S566), which was most pronounced in late-onset families. Furthermore, we found evidence for allele-specific association between the putative disease locus and marker D10S583, which has recently been located within 195 kilobases of the *IDE* gene.

The deposition and aggregation of β-amyloid (Aβ) in various regions of the brain is one of the key neuropathological hallmarks of AD. Consequently, agents that can inhibit and/or reverse these processes are attractive candidate genes for AD. Recent data suggest a principal role for IDE in the degradation and clearance of Aβ secreted by microglial cells and neurons (*1*). We performed genetic linkage analyses with six genetic markers close to the presumed location of the *IDE* gene on chromosome 10q23-q25 in 1426 subjects from 435 multiplex AD families (*2*, *3*). In addition, we genotyped marker D10S1225, which is located 32 to 47 cM proximal to this region and lies closest to a linkage peak identified in a recent whole-genome screen (*4*, *5*) using an overlapping set of families. To test for genetic linkage, we performed parametric [FASTLINK (*6*, *7*)] and nonparametric [GENEHUNTER-PLUS (*8*) and ASM (*9*, *10*)] analyses in the sample as a whole and in subsets stratified by onset age and *APOE* genotype (*11*).

Under a dominant model, we found significant evidence for linkage around marker D10S583 (Z_{max} = 3.3) (Table 1) in the full sample and for *D10S1671* in the late-onset sample (Z_{max} = 3.4). Results were similar under a recessive model, with a maximum lod score (logarithm of the odds ratio for linkage) of 3.8 for marker D10S1671 in the late-onset sample [Web table 1 (*12*)]. Although linkage was generally more pronounced in families without the *APOE* ε4/4 genotype, none of the markers had lod scores >3 in this stratum [Web table 2 (*12*)]. Two-point nonparametric linkage results were consistent with parametric findings, yielding the strongest signals for markers D10S1671, D10S583, and D10S1710 in late-onset families [Web table 3 (*12*)]. Finally, multipoint nonparametric analyses (*13*) generated maximum *Z* scores for the likelihood ratio (Z_{lr}) of 1.9 (P = 0.029, full sample), 2.1 (P = 0.02, late-onset), and 2.15 (P = 0.016, *APOE* ε4/4-negative) at marker D10S1710, which lies between the two markers with the strongest two-point signals [Web table 4 (*12*)].

None of the analyses yielded significant findings for marker D10S1225, located ~40 cM proximal to the linkage peak reported here, in contrast to previous reports in an overlapping sample of National Institute of Mental Health (NIMH) families (*4*, *5*). In an effort to understand this difference, we divided our sample into two groups according to whether or not all sampled affected individuals were included in the previous reports (*14*). Although the distal linkage peak was more pronounced in families included in the previous studies (n = 188), the linkage signal at marker D10S1225 did not increase [Web table 5 (*12*)]. These discrepancies could be due to a number of factors, including sampling issues [sibling pairs (*4*, *5*) versus full families used here], inclusion criteria (diagnostic and age-of-onset cutoffs), stratification procedures, and analytic methods.

During the course of our investigation, public sequence data became available showing that marker D10S583 and *IDE* are located on the same ~195-kb bacterial artificial chromosome (*15*), leading us to test this marker for allelic association with the disease. As determined with the Family-Based Association Test program (FBAT) (*16*, *17*), the multiallelic test on all 11 alleles was not significant (P = 0.15), but the diallelic test revealed significant association of the 211–base pair allele with AD (nominal P = 0.004, Bonferroni corrected P = 0.04). These preliminary findings suggest that there may be linkage disequilibrium between D10S583 and the putative AD locus on chromosome 10q.

Overall, the findings reported here indicate an AD gene on the long arm of chromosome 10. It remains unclear whether the peak reported here between D10S583 (115 cM) and D10S1671 (127 cM) and the more proximal peak at D10S1225 (81 cM) reported previously (*4*, *5*) represent linkage to one or two underlying loci. Recent reports have suggested that chance variation of the location estimates obtained from linkage studies in complex diseases can cover as much as 20 to 30 cM or more, even in relatively large samples (*18*, *19*). The identification of the putative AD gene(s) on chromosome 10q will require more detailed studies of linkage disequilibrium to narrow the region of interest as well as a thorough assessment of candidate genes.

Table 1. Autosomal-dominant model, maximum two-point parametric lod scores (Z_{max}), and recombination fractions (θ). Families were considered "late-onset" if all sampled affected individuals had onset ages ≥65 years. Marker locations are in Kosambi cM according to the Marshfield map. Values in bold indicate significant linkage.

Marker (location, in cM)	All families Z_{max} (θ)	Late-onset Z_{max} (θ)
D10S1225 (80.8)	0.4 (0.32)	0.9 (0.26)
D10S564 (112.6)	0 (0.50)	0 (0.50)
D10S583 (115.3)	**3.3 (0.22)**	2.8 (0.21)
D10S1710 (124.3)	0.7 (0.26)	0.9 (0.25)
D10S566 (127.1)	0.8 (0.28)	0.4 (0.30)
D10S1671 (127.1)	2.3 (0.22)	**3.4 (0.16)**
D10S1741 (128.2)	0.4 (0.29)	0.3 (0.32)

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References and Notes

1. K. Vekrellis *et al.*, *J. Neurosci.* **20**, 1657 (2000).
2. Subjects were collected as part of the NIMH Genetics Initiative following a standardized protocol applying NINCDS/ADRDA (National Institute of Neurological and Communicative Disorders and Stroke/Alzheimer's Disease and Related Disorders Association) criteria for the diagnosis of AD (*3*). Only families in which all sampled affected individuals had onset ages ≥50 years were included (n = 435 families, n = 1426 subjects, mean age of onset = 72.5 ± 7.7 years,

range 50 to 97 years). Additional information on the procedures is available as supplemental material (*12*).
3. D. Blacker *et al.*, *Neurology* **48**, 139 (1997).
4. P. Kehoe *et al.*, *Hum. Mol. Genet.* **8**, 237 (1999).
5. A. J. Myers *et al.*, *Neurobiol. Aging* **21** (suppl.), S103 (2000).
6. R. W. Cottingham Jr., R. M. Idury, A. A. Schaffer, *Am. J. Hum. Genet.* **53**, 252 (1993).
7. A. A. Schaffer, S. K. Gupta, K. Shriram, R. W. Cottingham Jr., *Hum. Hered.* **44**, 225 (1994).
8. L. Kruglyak, M. J. Daly, M. P. Reeve-Daly, E. S. Lander, *Am. J. Hum. Genet.* **58**, 1347 (1996).
9. A. Kong, N. J. Cox, *Am. J. Hum. Genet.* **61**, 1179 (1997).
10. Version 1.0, applying the exponential model.
11. Disease gene frequencies were set to 0.01 in the dominant model and to 0.05 in the recessive model. Penetrance in affected individuals corresponded to phenocopy rates of 5% for definite AD ($n = 278$), 10% for probable AD ($n = 645$), and 14% for possible AD ($n = 65$). Families were classified as "late-onset" when all sampled affected individuals had onset ages ≥65 years, as "*APOE* ε4/4-positive" if at least one affected individual had the ε4/4 genotype, and as "*APOE* ε4/4-negative" otherwise. Parametric analyses were performed on affected individuals only.
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).
14. The list of individuals included in Myers *et al*. (*5*) was supplied by P. Holmans.
15. GenBank accession number AL356128.
16. D. Rabinowitz, N. Laird, *Hum. Hered.* **50**, 211 (2000).
17. Analyses are based on estimated empirical variances (to account for the presence of linkage) (*20*) as implemented in FBAT (version 1.0, 1999) under an additive disease model.
18. S. B. Roberts, C. J. MacLean, M. C. Neale, L. J. Eaves, K. S. Kendler, *Am. J. Hum. Genet.* **65**, 876 (1999).
19. E. R. Hauser, M. Boehnke, *Am. J. Hum. Genet.* **61**, A278 (1997).
20. S. L. Lake, D. Blacker, N. M. Laird, *Am. J. Hum. Genet.* **67**, 1515 (2000).
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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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