# Genetic evidence for a novel familial Alzheimer's disease locus on chromosome 14

P. St George-Hyslop[1], J. Haines[2], E. Rogaev[1,3], M. Mortilla[1,4], G. Vaula[1,5], M. Pericak-Vance[6], J-F Foncin[7], M. Montesi[8], A. Bruni[8], S. Sorbi[4], I. Rainero[5], L. Pinessi[5], D. Pollen[9], R. Polinsky[10],L. Nee[11], J. Kennedy[12], F. Macciardi[13], E. Rogaeva[1], Y. Liang[1], N. Alexandrova[1], W. Lukiw[1], K. Schlumpf[2], R.Tanzi [2], T. Tsuda[14], L. Farrer[15], J-M Cantu[16], R. Duara[17], L. Amaducci[4], L. Bergamini[5], J. Gusella[2], A. Roses[6] & D. Crapper McLachlan[1]

[1]*Division of Neurology, Depts of Medicine and Physiology, Centre for Research in Neurodegenerative Disease, University of Toronto, Toronto, Ontario, Canada, M5S 1A8*
[2]*Molecular Neurogenetics Laboratory, Massachusetts General Hospital, CNY-6, Charlestown, Massachusetts 02129, USA*
[3]*National Research Center of Mental Health, Laboratory of Molecular Brain Genetics, Academy of Medical Sciences, Moscow, Russia, Zagorodnoe sh. 2/2, 113152*
[4]*Dept of Neurology, University of Florence, viale Morgagni 85, Firenze, Italy*
[5]*Dept of Neurology, University of Turin, via Cherasco 15, 10126-Torino, Italy*
[6]*Bryan Alzheimer's Disease Research Center, Duke University Medical Center, Durham, North Carolina, 27710, USA*
[7]*Laboratoire de Neurohistologie, Ecole Pratique des Hautes Etudes, La Salpétrière, 75651 Paris CEDEX 13, France*
[8]*Servizio di Neurologia, USSL-17, CNR, via Trento, 88046, Lamezia Terme, Italy*
[9]*Dept of Neurology, University of Massachusetts Medical Center, 55 Lake Street, Worcester, Massachusetts, 01655, USA*
[10]*Sandoz Research Institute, Sandoz Pharmaceuticals Corporation, 59 Route 10, East Hanover, New Jersey, 07936, USA*
[11]*Clinical Neuropharmacology Section, NINDS, 9000 Rockville Pike, Bethesda, Maryland 20892, USA*
[12]*Neurogenetics Section, Clarke Institute of Psychiatry, Dept. of Psychiatry, University of Toronto, Toronto, Ontario, Canada M5T 1R8*
[13]*Dept. of Neurosciences, University of Milano School of Medicine, 20127- Milano, Italy*
[14]*Dept of Neurology, Tohoku University School of Medicine, Sendai, Japan*
[15]*Neurogenetics Laboratory, Boston University School of Medicine, Concord Ave, Boston, Massachusetts, USA*
[16]*Dept of Genetics, Istitituo Mexicano del Seguro Social, Guadalajara, Jalisco, Mexico*
[17]*Wein Center, Mt Sinai Medical Center, Miami Beach, Florida 33140, USA*

*Correspondence should be addressed to P.H. St.G-H.*

Familial Alzheimer's disease (FAD) has been shown to be genetically heterogeneous, with a very small proportion of early onset pedigrees being associated with mutations in the amyloid precursor protein (*APP*) gene on chromosome 21, and some late onset pedigrees showing associations with markers on chromosome 19. We now provide evidence for a major early onset FAD locus on the long arm of chromosome 14 near the markers *D14S43* and *D14S53* (multipoint lod score $\hat{z}$ = 23.4) and suggest that the inheritance of FAD may be more complex than had initially been suspected.

Alzheimer's disease (AD) is a common, fatal, degenerative disease of the adult human central nervous system associated with distinctive neuropathologic features which leads to progressive cognitive and intellectual decline during mid to late adult life, and which is inherited as an autosomal dominant trait in some families[1,2]. While initial genetic linkage studies suggested a locus for early onset familial AD (FAD) on chromosome 21 (refs 3,4), subsequent studies have revealed that the disorder is genetically heterogeneous[5]. A small proportion of familial cases are associated with mutations in the amyloid precursor protein (*APP*) gene on chromosome 21 (refs 6–8), while some pedigrees segregating senile onset FAD show linkage and/or association with genetic markers on chromosome 19 (ref. 9). Many FAD pedigrees, however, have not shown strong evidence for linkage to either chromosome, suggesting the existence of additional FAD genes. We report genetic linkage studies which indicate that a major FAD susceptibility gene is located on chromosome 14.

In order to identify the chromosomal location of novel FAD genes, we investigated the segregation of highly polymorphic simple sequence repeat (SSR) markers from chromosomes other than 19 and 21, in a series of 21 FAD pedigrees (Table 1). None of these pedigrees have individually shown significant evidence for linkage to genetic markers on either chromosome 21 or on chromosome 19 (refs 3, 5, 10 and unpublished results). However, some of these pedigrees, when considered cumulatively, do provide evidence for linkage

**Table 1 Phenotypes of FAD pedigrees**

| Symbol | Mean age of onset (yrs) | Ethnic origin | Neuropathologic conformation |
|---|---|---|---|
| FAD1 | 53 | Canadian | + |
| FAD4 | 43 | Italian | + |
| FAD2 | 48 | German | + |
| FAD3 | 48 | Jewish | + |
| M9 | 75 | Jewish | |
| M2 | 67 | American | + |
| M3 | 60 | American | + |
| M4 | 60 | American | |
| NIH2 | 48 | Jewish | + |
| M8 | 61 | American | |
| FLO2 | 61 | Italian | + |
| TOR1 | 62 | Canadian | |
| M11 | 70 | American | |
| R1 | 70 | Russian | |
| Mex1 | 45 | Mexican | |
| FL1 | 74 | Hispanic | |
| Tor1.1 | 43 | Italian | + |
| M13 | 84 | Jewish | + |
| TFL10 | 61 | Italian | + |
| JPN1 | 42 | Japanese | + |
| 603 | 47 | American | + |

Post-mortem histopathologic confirmation of AD has been possible in at least one instance in those pedigrees marked by the (+) symbol.

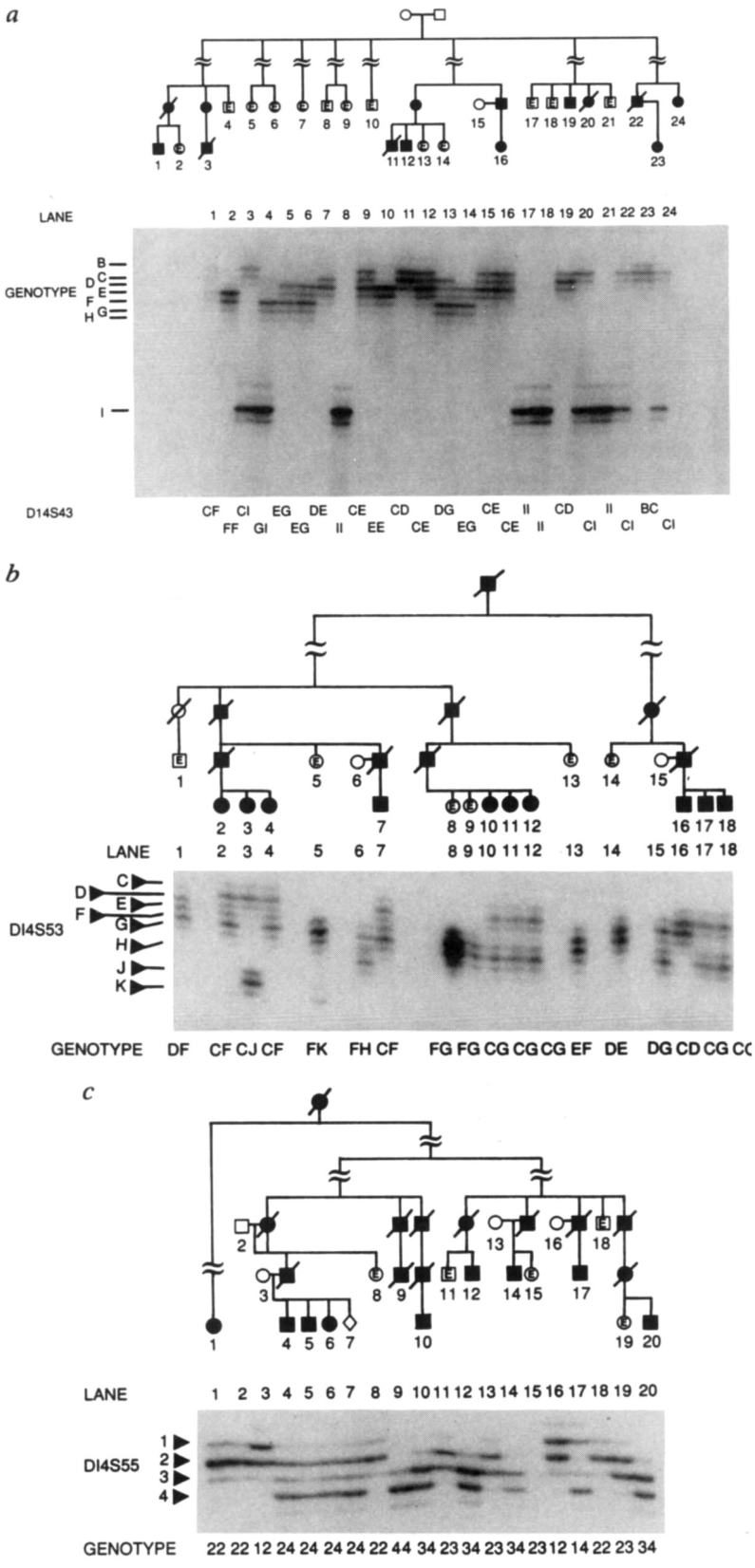


Fig. 1 Abridged pedigree diagrams and genotypes for chromosome 14 markers. *a*, *D14S43* in the FAD3 pedigree with "C" allele segregating with FAD; one possible recombinant individual 9; *b*, *D14S53* in the TOR1.1 pedigree with "C" allele segregating with FAD; and *c*, *D14S55* in the FAD4 pedigree with "4" allele segregating; one recombinant in individual 1. Filled symbols represent FAD affected subjects; open symbols are at-risk subjects or spouses; E are elderly asymptomatic pedigree members (probable escapees).

to chromosome 21 (*D21S1/S11-D21S16* multipoint $\hat{Z}$ = 4.25)[3]. None of the pedigrees have missense mutations in the APP gene[11].

It has been suggested that the primary event in the pathogenesis of AD is the extracellular deposition of the β/A4 fragment of *APP*[12–16]. Although this may occur in a few pedigrees due to mutations within *APP*, it is conceivable that the same sequence of events might more commonly arise from mutations in other genes involved in the metabolism of APP or from mutations in genes involved in other aspects of the neuropathology of AD. This hypothesis argues that a search for additional FAD loci should be focused upon those areas of the genome known to contain proteases, protease inhibitors, molecular chaperons and genes coding for other components of the senile plaques or neurofibrillary tangles.

Several such candidate genes have been mapped to chromosome 14 (for example α-1-anti-chymotrypsin (*AACT*), a cluster of serine proteases (including Cathepsin G), the *FBJ* osteosarcoma viral oncogene homologue (*cFOS*), and the heat shock proteins *HSP70* and *HSP90*)[17]. Further evidence for a putative FAD locus on chromosome 14 is provided by a report of AD associated with a familial Robertsonian translocation involving chromosomes 14 and 21 (ref. 18). To investigate whether these or other genes on chromosome 14 might be the site of an FAD mutation in our pedigrees, we investigated the segregation of a series of eight SSR markers spanning the long arm of chromosome 14 (ref. 19).

## Linkage of FAD locus to chromosome 14q

The genotype at each of these loci was determined for each pedigree member (Fig. 1)[19]. Evidence for co-segregation of each marker locus with FAD was then sought by computing pedigree specific logarithm of the odds (lod) scores using pair-wise (disease versus a single locus) tests of linkage and the same maximum likelihood parameters as previously described[5]. Markers at the extreme telomeric (*D14S48*, *D14S51*) or extreme centromeric regions (*D14S50*, *D14S49*, *D14S52*) of chromosome 14q provided no strong evidence for co-segregation with FAD in either the overall data set (Table 2) or in individual FAD pedigrees (data not shown). However, three markers spanning a 12 centiMorgan (cM) region in the centre of the long arm of chromosome 14 (*D14S43*, *D14S53* and *D14S55*) gave highly significant two point lod scores in the overall data set (Tables 2 and 3). More impressively, six independent pedigrees provided statistically significant lod scores for at least one of these markers (Table 3).

Multipoint analyses of the *D14S53* and *D14S43* data using LINKAGE[20] confirmed these findings, providing a peak lod score of 23.4 at 5 cM distal to *D14S53*. This analysis did not permit definitive placement of FAD relative to these two markers because a similar lod score (23.17) was generated at 5 cM proximal to *D14S43* (odds ratio favouring the former are 2:1 (Fig. 2).

To ensure that these results did not arise from errors in the estimated statistical parameters necessary for the maximum likelihood calculations (that is, marker allele frequencies, and age dependent penetrance functions) we analyzed the data using the following parameter sets: *Set 1:* marker allele frequencies estimated from 66 spouses of affected individuals and one at-risk per family (these observed allele frequencies did not differ from those previously published, and did not differ substantially

**Table 2 Cumulative lod scores for chromosome 14 markers**

| Locus | Recombination fraction | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| ID No. | 0.00 | 0.05 | 0.10 | 0.20 | 0.30 | 0.40 | $\hat{Z}$ | θ |
| *D14S50* | $-\infty$ | -8.07 | -3.45 | -1.34 | -0.47 | -0.19 | – | – |
| *D14S49* | $-\infty$ | -8.69 | -4.13 | -0.91 | 0.01 | 0.15 | 0.15 | 0.40 |
| *D14S52* | $-\infty$ | -2.74 | -1.48 | -0.58 | -0.24 | 0.07 | 0.07 | 0.40 |
| *D14S43* | $-\infty$ | 20.47 | 18.47 | 13.52 | 8.20 | 3.28 | 20.50 | 0.04 |
| *D14S53* | $-\infty$ | 11.64 | 11.69 | 9.24 | 5.83 | 2.48 | 12.10 | 0.07 |
| *D14S55* | $-\infty$ | 6.94 | 6.76 | 5.50 | 3.75 | 1.76 | 7.10 | 0.07 |
| *D14S48* | $-\infty$ | -3.11 | -0.49 | 1.13 | 1.21 | 0.62 | 1.25 | 0.25 |
| *D14S51* | $-\infty$ | -9.82 | -5.35 | -2.61 | -0.62 | -0.14 | – | – |

These markers have been arranged in the following genetic map: cen-*D14S50*-(23 cM)-*D14S49*-(24 cM)-*D14S52*-(25 cM)-*D14S43*-(3 cM)-*D14S53*-(9 cM)-*D14S55*-(2 cM)-*D14S48*-(18 cM)-*D14S51*-tel[19]. Individual pedigree-specific lod scores for markers yielding significant overall lod scores are displayed in Table 3. Genotype data for these loci are available upon request.

across ethnic groups although the sample sizes for some ethnic groups were small) (results from these analyses are shown in Tables 2 and 3); *Set 2:* equal marker allele frequencies (data not shown); and *Set 3:* zero penetrance for the FAD trait in currently asymptomatic pedigree members (data not shown). We observed a 10–50% increase in lod scores with *Set 2*, suggesting that the reported lod scores were not due to segregation of rare alleles — an observation which was confirmed by direct inspection of the genotype data. We observed a 10–20% reduction in lod scores but still overwhelming evidence for linkage with *Set 3*. This minor reduction in lod scores is probably due to the loss of a small amount of information provided by consideration of age-adjusted risk for FAD in asymptomatic members in the initial analysis.

## Non-allelic heterogeneity

A few pedigrees provided negative lod scores for the *D14S43*, *D14S53* or *D14S55* loci (Table 3). To determine whether these negative results reflected additional non-allelic heterogeneity we investigated the pedigree specific lod scores for the three loci showing linkage and the *D14S43–D14S53* multi-point analysis using the admixture test. Although these tests of non-allelic heterogeneity were not significant using either two-point or multi-point data (odds against homogeneity were less than 10:1 in all analyses), it is apparent that most of the evidence for linkage arose from pedigrees with a pre-senile onset (<65 yrs). The few pedigrees with a later onset cumulatively provided negative scores. It seems likely therefore that this region contains a gene which plays a major role in the susceptibility to FAD in a significant proportion of at least early onset FAD pedigrees.

## Discussion

We have produced convincing evidence for a new FAD locus situated on chromosome 14q. As noted earlier, several of the pedigrees showing robust evidence for linkage to chromosome 14 in this study (especially pedigrees FAD4 and 603) also provide noticeably positive lod scores ($0.75 > Z < 3.0$) for markers on chromosome 21 (refs 5,10). The interpretation of the chromosome 21 results in this and other data sets has been the subject of much speculation but remains problematic[3,4,21–24]. The pedigree specific lod scores for the current chromosome 14 loci far exceed those previously achieved for chromosome 21. However, while these new results now suggest that a major gene effect is unlikely on chromosome 21, they do not negate the possibility that important epistatic modifier loci may exist in these pedigrees on chromosome 21. Indeed, while it is still conceivable that the previous results for chromosome 21 may simply represent chance co-segregation of chromosome 21 markers through parts of these pedigrees, it is more probable that the positive lod scores for chromosome 21 markers in these families reflect the co-segregation of certain alleles at one or more chromosome 21 loci (including perhaps *APP*), and that these alleles may play

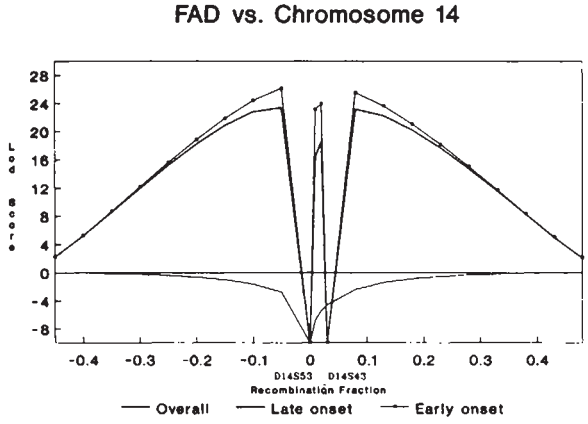


Fig. 2 Multipoint lod scores for *D14S53* (telomeric) and *D14S43* (centromeric) for all pedigrees (——); for late onset pedigrees with mean family specific age of onset greater than 65 years (—); and for pedigree with a presenile onset (—□—).

**Table 3 Pedigree specific lod scores for the *D14S43*, *D14S53*, and *D14S55* loci were calculated as described for Table 2**

| Family | 0.00 | 0.05 | 0.10 | 0.15 | 0.20 | 0.30 | 0.40 |
|---|---|---|---|---|---|---|---|
| | | | ***D14S43* versus FAD** | | | | |
| | | | **Recombination fraction** | | | | |
| FAD1 | 3.99 | 3.55 | 3.11 | 2.66 | 2.21 | 1.31 | 0.52 |
| FAD4 | 2.17 | 1.87 | 1.57 | 1.30 | 1.05 | 0.60 | 0.23 |
| FAD2 | 3.65 | 3.16 | 2.67 | 2.17 | 1.68 | 0.75 | 0.11 |
| FAD3 | 6.99 | 6.45 | 5.86 | 5.23 | 4.55 | 3.05 | 1.40 |
| M9 | -0.22 | -0.15 | -0.10 | -0.07 | -0.04 | -0.02 | 0.00 |
| M2 | 0.27 | 0.24 | 0.20 | 0.17 | 0.14 | 0.08 | 0.03 |
| M3 | 0.14 | 0.12 | 0.09 | 0.07 | 0.06 | 0.03 | 0.01 |
| M4 | 0.31 | 0.25 | 0.18 | 0.13 | 0.09 | 0.03 | 0.00 |
| NIH2 | $-\infty$ | -0.75 | -0.41 | -0.23 | -0.13 | -0.03 | -0.01 |
| M8 | -0.08 | -0.10 | -0.10 | -0.09 | -0.07 | -0.04 | -0.01 |
| FLO2 | 0.64 | 0.54 | 0.44 | 0.35 | 0.26 | 0.12 | 0.04 |
| TOR1 | -0.13 | -0.10 | -0.08 | -0.06 | -0.04 | -0.02 | 0.00 |
| M11 | 0.26 | 0.22 | 0.18 | 0.14 | 0.11 | 0.05 | 0.01 |
| R1 | 0.08 | 0.07 | 0.05 | 0.04 | 0.03 | 0.01 | 0.00 |
| MEX1 | $-\infty$ | 1.79 | 1.85 | 1.75 | 1.56 | 1.05 | 0.48 |
| FL1 | 0.05 | 0.00 | -0.03 | -0.05 | -0.05 | -0.03 | -0.01 |
| TOR1.1 | 1.72 | 1.52 | 1.30 | 1.09 | 0.87 | 0.48 | 0.17 |
| M13 | -0.51 | -0.41 | -0.32 | -0.24 | -0.17 | -0.07 | -0.02 |
| FLO10 | -0.26 | -0.19 | -0.15 | -0.11 | -0.08 | -0.03 | -0.01 |
| JPN1 | 0.75 | 0.67 | 0.58 | 0.49 | 0.40 | 0.22 | 0.06 |
| 603 | 1.94 | 1.75 | 1.55 | 1.34 | 1.11 | 0.66 | 0.27 |
| Total | $-\infty$ | 20.47 | 18.47 | 16.09 | 13.52 | 8.20 | 3.28 |
| | | | ***D14S53* versus FAD** | | | | |
| FAD1 | $-\infty$ | 2.46 | 2.27 | 1.96 | 1.61 | 0.91 | 0.34 |
| FAD4 | 2.52 | 2.21 | 1.90 | 1.59 | 1.30 | 0.76 | 0.32 |
| FAD2 | 0.72 | 0.58 | 0.44 | 0.30 | 0.18 | 0.02 | -0.03 |
| FAD3 | $-\infty$ | 0.66 | 0.84 | 0.86 | 0.81 | 0.56 | 0.26 |
| M9 | $-\infty$ | -0.57 | -0.32 | -0.19 | -0.12 | -0.04 | -0.01 |
| M2 | 0.25 | 0.21 | 0.17 | 0.13 | 0.09 | 0.04 | 0.00 |
| M3 | $-\infty$ | -0.53 | -0.28 | -0.16 | -0.09 | -0.02 | 0.00 |
| M4 | 0.26 | 0.21 | 0.16 | 0.11 | 0.07 | 0.02 | 0.00 |
| NIH2 | $-\infty$ | -0.30 | -0.02 | 0.10 | 0.15 | 0.13 | 0.05 |
| M8 | -0.14 | -0.16 | -0.15 | -0.12 | -0.09 | -0.04 | -0.01 |
| FLO2 | -0.03 | -0.02 | -0.01 | -0.01 | -0.01 | 0.00 | 0.00 |
| TOR1 | 0.07 | 0.06 | 0.05 | 0.04 | 0.03 | 0.01 | 0.00 |
| M11 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| R1 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| MEX1 | 0.94 | 0.84 | 0.74 | 0.63 | 0.53 | 0.33 | 0.16 |
| FL1 | $-\infty$ | -1.44 | -0.89 | -0.58 | -0.39 | -0.15 | -0.04 |
| TOR1.1 | 5.38 | 4.93 | 4.45 | 3.95 | 3.41 | 2.26 | 1.04 |
| M13 | $-\infty$ | -0.79 | -0.52 | -0.36 | -0.25 | -0.11 | -0.03 |
| FLO10 | 0.14 | 0.11 | 0.09 | 0.07 | 0.05 | 0.02 | 0.01 |
| JPN1 | 0.46 | 0.40 | 0.33 | 0.27 | 0.21 | 0.10 | 0.03 |
| 603 | 3.13 | 2.79 | 2.45 | 2.09 | 1.73 | 1.02 | 0.39 |
| Total | $-\infty$ | 11.64 | 11.69 | 10.68 | 9.24 | 5.83 | 2.48 |
| | | | ***D14S55* versus FAD** | | | | |
| FAD1 | 0.35 | 0.24 | 0.16 | 0.10 | 0.06 | 0.02 | 0.00 |
| FAD4 | $-\infty$ | 5.21 | 4.92 | 4.46 | 3.91 | 2.63 | 1.22 |
| FAD2 | -0.74 | -0.62 | -0.49 | -0.36 | -0.25 | -0.10 | -0.03 |
| FAD3 | 0.68 | 0.56 | 0.46 | 0.37 | 0.29 | 0.15 | 0.06 |
| M9 | -0.06 | -0.05 | -0.04 | -0.03 | -0.02 | -0.01 | 0.00 |
| M2 | $-\infty$ | -0.72 | -0.44 | -0.29 | -0.19 | -0.08 | -0.02 |
| M3 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| M4 | 0.08 | 0.06 | 0.05 | 0.04 | 0.03 | 0.01 | 0.00 |
| NIH2 | 0.07 | 0.06 | 0.05 | 0.04 | 0.03 | 0.01 | 0.00 |
| M8 | -0.21 | -0.22 | -0.20 | -0.17 | -0.14 | -0.06 | -0.02 |
| FLO2 | -0.19 | -0.13 | -0.09 | -0.06 | -0.04 | -0.02 | -0.01 |
| TOR1 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| M11 | 0.08 | 0.06 | 0.05 | 0.04 | 0.03 | 0.01 | 0.00 |
| R1 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| MEX1 | 1.82 | 2.30 | 2.23 | 2.04 | 1.79 | 1.18 | 0.53 |
| FL1 | -0.15 | -0.17 | -0.16 | -0.14 | -0.12 | -0.06 | -0.01 |
| TOR1.1 | 0.36 | 0.29 | 0.23 | 0.18 | 0.13 | 0.06 | 0.02 |
| M13 | -0.25 | -0.21 | -0.16 | -0.12 | -0.09 | -0.04 | -0.01 |
| FLO10 | 0.08 | 0.06 | 0.05 | 0.04 | 0.03 | 0.01 | 0.00 |
| JPN1 | 0.28 | 0.21 | 0.15 | 0.10 | 0.06 | 0.02 | 0.00 |
| 603 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Total | $-\infty$ | 6.94 | 6.76 | 6.21 | 5.50 | 3.75 | 1.76 |

a role in generating or modifying the AD phenotype. A strong but epistatic role for one or more chromosome 21 loci would be in keeping with two other observations. First, some of the putatively pathogenic mutations in *APP* (including $APP_{717}$) also do not segregate perfectly with the AD phenotype[25–27]. Second, weakly positive pedigree specific lod scores and highly significant sib pair and affected pedigree member results have been reported for the same chromosome 21 markers in at least two other FAD data sets[9,28].

Several candidate genes have been mapped to chromosome 14, and mutations in any of these genes could be related to some aspect of the known pathology of AD. However, the *AACT* and protease inhibitor genes are tightly linked to *D14S48* and *D14S51* loci[19,29], while the serine protease gene cluster maps to 14q11–q12 near the *D14S50* and *D14S49* loci[17,19]. Although close linkage of FAD to these candidate genes would thus seem unlikely, the genetic evidence against these candidate genes must be interpreted in the context of two caveats. First, the telomeric region of chromosome 14 displays a high frequency of genetic recombination[30]. Second, strictly genetic methods for defining the precise location of the FAD gene on chromosome 14 may be confounded by the inclusion of sporadic AD phenocopies and by the inclusion pedigrees with non-allelic genetic defects. However, the two remaining candidate genes (*cFOS* and *HSP70*) both map to chromosome 14q24 very close to *D14S43* and *D14S53*, and in the context of the "amyloid hypothesis", both could be regarded as strong candidates for the site of an FAD mutation. *cFOS* may regulate *APP* expression by binding with *cJUN* to *AP-1* concensus sequences in the *APP* promoter (perhaps in response to injury)[31,32], while *HSP70* could act as a molecular chaperon for either full length APP or for potentially amyloidogenic proteolytic fragments. It is of course also possible that none of these known genes are the site of the mutation, and that the mutated FAD sequence may have no direct relationship to *APP*.

The overwhelming evidence presented here for a major chromosome 14 locus, in the face of weakly positive lod scores for chromosome 21 (and the absence of *APP* mutations), implies that even in apparently autosomal dominant pedigrees, the underlying genetic aetiology of FAD may be more complex than initially thought. The ultimate resolution of these data will be simplified once the chromosome 14 gene has been cloned, and its function more clearly understood. It will be of particular interest to determine whether or not this function is related to the metabolism of APP.

## Methodology

**FAD pedigrees.** The diagnosis of living affected pedigree members was achieved according to NINCDS-ADRDA criteria[33] by a qualified specialist in neurology or psychiatry using institutionally approved protocols and the informed consent of the study participants[6]. Diagnosis in deceased affected pedigree members was achieved through review of medical and family records. Post-mortem histopathologic confirmation of AD has been possible in at least one instance in 13 pedigrees. Pedigrees 1–19 inclusive have been reported in previous genetic linkage studies, and in some phenotypic studies[3,4]. Genetic studies of pedigree 603 have also been reported in detail elsewhere[10,22].

**Genotyping studies.** Genomic DNA was obtained from informative living family members as described[3], and from formalin fixed tissue using methods to be described elsewhere (M.M. *et al.*, in preparation).

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100 ng of genomic DNA from each individual was amplified by PCR using 10 pmol of the oligonucleotide primers in 10 µl of reaction buffer as described[19] and cycled through 94 °C × 6 s, 54 °C × 20 s, 72°C × 20 seconds for 35 cycles. For *D14S43*, the primer sequences were 5'-TGGAACACTCAGGCGA-3' and 5'-ACTTTCTACTTTGGGT-CACT-3'. For *D14S51* the annealing temperature 60 °C × 20 s. PCR products were resolved according to size by denaturing polyacrylamide gel electrophoresis and visualized by autoradiography. Genotypes were determined relative to those of other pedigree members loaded on the same gel and relative to a sequencing standard. Alleles at each locus were scored in an identical way across all pedigrees.

**Statistical analyses.** Lod scores were calculated with the LINKAGE programs using the same initial maximum likelihood parameters as described[5]. A new mutation rate of 0.001 was used to force the analysis to consider all discordant individuals as obligate recombinants, thereby making it harder to detect linkage. Marker allele frequencies were deduced from spouses of members of these pedigrees and from one at-risk member of these pedigrees to ensure that the allele frequencies used accurately reflect the frequencies existent in the ethnic populations from which these pedigrees were drawn. No significant difference was observed between the observed allele frequencies and those previously reported for these markers[19]. To allow inclusion of information on the segregation of FAD gene to be included from currently asymptomatic pedigree members, we employed an age-of-onset correction as previously described. To ensure that errors in the empiric estimates of age-dependent risk were not misleading our analyses, we also analyzed the data setting the penetrance of FAD to zero in the asymptomatic family members, but still including their genotypes at the chromosome 14 marker loci.

**Multipoint analysis.** For the *D14S43–D14S53* multipoint, the data were re-coded to 4 allele systems using standard procedures to improve computational tractability. Maximum likelihood scores were then calculated assuming *D14S43* and *D14S53* to be at fixed map positions 3 cM apart, using the LINKMAP program and the same maximum likelihood parameter sets described for the two-point analyses.

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