

# A full genome scan for late onset Alzheimer's disease

Patrick Kehoe<sup>+</sup>, Fabienne Wavrant-De Vrieze<sup>1,+</sup>, Richard Crook<sup>1,+</sup>, William S. Wu<sup>2,+</sup>, Peter Holmans<sup>+</sup>, Iain Fenton, Gillian Spurlock, Nadine Norton, Hywel Williams, Nigel Williams, Simon Lovestone<sup>3</sup>, Jordi Perez-Tur<sup>1</sup>, Mike Hutton<sup>1</sup>, Marie-Christine Chartier-Harlin<sup>4</sup>, Shantia Shears<sup>2</sup>, Kimberly Roehi<sup>2</sup>, Jeremy Booth<sup>2</sup>, Wendy Van Voorst<sup>2</sup>, Dzanan Ramic<sup>2</sup>, Julie Williams, Alison Goate<sup>2</sup>, John Hardy<sup>1</sup> and Michael J. Owen\*

Neuropsychiatric Genetics Unit, Tenovus Building, University of Wales College of Medicine, Heath Park, Cardiff CF4 4XN, UK, <sup>1</sup>Birdsall Building, Mayo Clinic, 4500 San Pablo Road, Jacksonville, FL 32084, USA, <sup>2</sup>Departments of Psychiatry and Genetics, Washington University School of Medicine, 4940 Children's Place, St Louis, MO 63110, USA, <sup>3</sup>Institute of Psychiatry, De Crespigny Park, Denmark Hill, London SE5 8AF, UK and <sup>4</sup>CJF 95-05 INSERM, Institut Pasteur de Lille, 1 rue du Pr Calmette, 59019 Lille Cedex, France

Received July 17, 1998; Revised and Accepted November 20, 1998

**We have genotyped 292 affected sibling pairs (ASPs) with Alzheimer's disease (AD) according to NINCDS-ADDA diagnostic criteria and with onset ages of  $\geq 65$  years using 237 microsatellite markers separated by an average distance of 16.3 cM. Data were analysed by SPLINK and MAPMAKER/SIBS on the whole sample of 292 ASPs and subsets of 162 ASPs where both members possessed an apolipoprotein E (APOE)  $\epsilon 4$  allele and 63 pairs where neither possessed an  $\epsilon 4$  allele. Sixteen peaks with a multipoint lod score (MLS)  $>1$  either in the whole sample, the  $\epsilon 4$ -positive or -negative subgroups were observed on chromosomes 1 (two peaks), 2, 5, 6, 9 (two peaks), 10 (two peaks), 12, 13, 14, 19, 21 and X (two peaks). Simulation studies revealed that these findings exceeded those expected by chance, although many are likely to be false positives. The highest lod scores on chromosomes 1 (MLS 2.67), 9 (MLS 2.38), 10 (MLS 2.27) and 19 (MLS 1.79) fulfil Lander and Kruglyak's definition of 'suggestive' in that they would be expected to occur by chance once or less per genome scan. Several other peaks were only marginally less significant than this, in particular those on chromosomes 14 (MLS 2.16), 5 (MLS 2.00), 12, close to  $\alpha 2$ -macroglobulin (MLS 1.91), and 21, close to amyloid precursor protein (MLS 1.77). This is the largest genome scan to date in AD and shows for the first time that this is a genetically complex disorder involving several, perhaps many, genes in addition to APOE. Moreover, our data will be of interest to those hoping**

**to identify positional candidate genes using information emerging from neurobiological studies of AD.**

## INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that occurs predominantly in later life. It is the commonest cause of dementia and represents the fourth largest cause of death in the developed world (1).

To date, four genes have been implicated in the aetiology of AD. Mutations in three of these, coding for the amyloid precursor protein (APP) (2), presenilin 1 (PS-1) (3,4) and presenilin 2 (5) account for most cases of autosomal dominant familial AD (FAD) (6). However, FAD accounts for  $<1\%$  of all cases of AD. The inheritance of common forms of the disorder appears considerably more complex and probably reflects the co-action or interaction of several or many genes together with environmental factors. One gene that is clearly implicated in this form of the disorder is that encoding apolipoprotein E (APOE). The  $\epsilon 4$  allele of APOE, although neither necessary nor sufficient to cause AD, is associated with increased risk of both early and late onset disease (7). The effect of  $\epsilon 4$  appears additive such that heterozygotes and homozygotes are, respectively, approximately three and eight times more likely to be affected than controls (7). However, variation at the APOE locus accounts for at most  $\sim 50\%$  of the genetic variation in liability (8) to develop the disorder and there must be other genetic variants that account for the remaining risk.

A number of strategies are available for mapping genetically complex traits (9). Traditional lod score analysis in multiplex pedigrees is best suited to forms of a disorder showing obvious Mendelian inheritance. This approach is clearly applicable to FAD, where successes have already been evident. However, the

\*To whom correspondence should be addressed. Tel: +44 1222 743058; Fax: +44 1222 746554; Email: owenmj@cardiff.ac.uk

<sup>+</sup>These authors contributed equally to this work

lod score method is at its weakest when the mode of transmission is complex and the genetic parameters cannot be accurately specified. It is generally agreed that the best way to proceed under such circumstances is by a combination of allele sharing linkage methods in pairs of affected siblings or other relative pairs and association studies (9,10). A number of candidate gene association studies have been performed in AD since the identification of the APOE locus. Some positive findings have been claimed (11–30) but unfortunately none of these has been consistently confirmed. These inconsistencies are likely to be due to a number of factors that bedevil genetic association studies, including heterogeneity, issues of statistical power, multiple testing and population stratification (31). Moreover, at the present time, association studies can only be based on testing of genes whose candidature is suggested by an existing understanding of the pathophysiology. In contrast, a systematic genome screen using allele sharing linkage methods offers the potential to identify novel pathogenic pathways and mechanisms. Consequently we have carried out a two-stage genome scan in 600 affected sibling pairs (ASPs) with AD. In the first phase we have genotyped 292 ASPs with a 20 cM grid of markers and report the results here.

## RESULTS

A multipoint ASP analysis was performed on the entire dataset of 292 ASPs using MAPMAKER/SIBS (32). The sample was also stratified for analysis on the basis of whether both (162 ASPs) or neither (63 ASPs) members of an ASP possessed at least one APOE  $\epsilon 4$  allele in order to maximize power to detect loci acting epistatically or heterogeneously with respect to APOE. APOE genotypes were not included in lod score calculations. This is a computationally simple, model-free approach to two-locus analysis which does not require the specification of unknown parameters such as gene frequencies, penetrances and interactions and which allows the multilocus approach implemented in MAPMAKER/SIBS to be used. The resulting multipoint lod scores (MLS) are shown in Figure 1, together with the regions where exclusion analysis based on a  $\lambda_s$  of 1.4 gave lod scores of  $-2$  or less. A genetic effect of size  $\lambda_s = 1.4$  was chosen since this is approximately equal to that given by APOE (see below). This could be excluded from  $\sim 28\%$  of the genome, whereas a  $\lambda_s$  of 2 could be excluded from  $\sim 80\%$  of the genome (data not shown).

The multipoint results are also summarized in Table 1, which shows data from the 16 peaks with an  $MLS \geq 1$  in either the whole sample, the  $\epsilon 4$ -positive ( $\epsilon 4+ve$ ) or  $\epsilon 4$ -negative ( $\epsilon 4-ve$ ) subgroups. These regions were observed on chromosomes 1 (two peaks), 2, 5, 6, 9 (two peaks), 10 (two peaks), 12, 13, 14, 19, 21 and X (two peaks). Only peaks on chromosomes 1, 5, 9, 10 and 19 gave a  $MLS > 1$  in the whole sample. The remaining peaks were observed in either the  $\epsilon 4-ve$  (chromosomes 1, 10, 12, 21 and X) or the  $\epsilon 4+ve$  (chromosomes 2, 6, 13 and 14) sib pairs only. Pointwise and genome-wide significance levels calculated by simulation for all three samples analysed are shown in Table 2. It can be seen that the number of observed regions (i.e. MLSs) exceeding a given lod score is greater than would be expected by chance. Furthermore, the highest lod scores on chromosomes 1 (MLS 2.67), 9 (MLS 2.38), 10 (MLS 2.27) and 19 (MLS 1.79) fulfil Lander and Kruglyak's definition of 'suggestive' in that they would be expected to occur by chance once or less per genome scan (32). Several other peaks were only marginally less significant than this, in particular those on chromosomes 14

(MLS 2.16), 5 (MLS 2.00), 12 (MLS 1.91) and 21 (MLS 1.77). We did not observe any 'significant' linkages (i.e. genome-wide occurrence probability  $< 0.05$ ) since our simulation results indicate that a lod score of  $\sim 3.6$  would be required for this (Table 2).

**Table 1.** Maximum multipoint lod scores and peak identity by descent (IBD) sharing probabilities

Chromosome	Whole sample	IBD	Both $\epsilon 4+ve$	IBD	Both $\epsilon 4-ve$	IBD
1A	0.02		0.00		1.35	0.67
1B	1.33	0.56	2.67	0.61	0.00	
2	0.49		1.01	0.57	0.36	
5	1.07	0.55	2.00	0.61	0.00	
6	0.48		1.40	0.60	0.85	
9A	0.00		0.00		1.12	0.64
9B	1.15	0.56	2.38	0.62	0.00	
10A	2.27	0.60	2.25	0.61	0.92	
10B	0.2		0.00		1.17	0.63
12	0.89		0.20		1.91	0.63
13	0.20		1.20	0.58	0.00	
14	0.30		2.16	0.60	0.00	
19	1.79	0.57	n/a		n/a	
21	0.25		0.50		1.77	0.65
XA	0.10		0.05		1.45	0.72
XB	0.05		0.20		1.93	0.65

Data from the 16 peaks with an  $MLS \geq 1$  in either the whole sample, the  $\epsilon 4+ve$  or  $\epsilon 4-ve$  subgroups are shown.

**Table 2.** Single point  $P$ -values and genome-wide multipoint significance levels

Lod	Single point $P$ -value	Expected no. per genome scan	Observed
All chromosomes except 19 (three analyses)			
3.6	0.00008	0.05	0
2.6	0.0005	0.5	1
2.3	0.001	0.9	2
2.0	0.002	1.7	5
1.7	0.005	3.1	8
1.3	0.015	6.9	9
1.0	0.025	12.5	16
Chromosome 19 (whole sample analysis only)			
1.79		0.7	

The genome-wide significance levels for chromosomes apart from 19 were based on simulated genome scans in the whole sample,  $\epsilon 4+ve$  and  $\epsilon 4-ve$  subgroups.

## DISCUSSION

In a genome screen of 292 sib pairs with late onset AD, we observed 16 loci (i.e. MLSs) with lod scores  $\geq 1$ , which exceeded the number expected by chance. The regions of interest occur on chromosomes 1 (two peaks), 2, 5, 6, 9 (two peaks), 10 (two

peaks), 12, 13, 14, 19, 21 and X (two peaks). The chromosome 19 result appears attributable to the APOE locus, with marker D19S571 (15 cM distal of APOE) showing a lod score of 1.79. This complies with Lander and Kruglyak's (32) definition of 'suggestive' linkage and indicates that our study would have detected a susceptibility gene for AD in this region had association with APOE  $\epsilon 4$  not already been reported. In addition we obtained a single point lod score of 2.02 (IBD 0.64) with marker D19S412 (2 cM distal of the APOE locus).

Recently Pericak-Vance *et al.* (33,34) reported the results of a two-stage genome screen in late onset FAD in 54 families with multiple affected members. This first stage was carried out in 16 of the largest families, in which DNA was available from 52 affected individuals. Fifteen regions of interest were followed up in a further 38 families, in which DNA was available from 89 affected individuals. Interestingly, no evidence for linkage around the APOE locus was obtained despite the enrichment of markers in this region. On follow-up, four regions were identified showing possible or suggestive linkage on chromosomes 4, 6, 12 and 20, with the strongest evidence being found in the pericentromeric region of chromosome 12 (peak MLS 3.5). We found little evidence to support these findings on chromosome 12 in our dataset (35), although we did find some evidence for linkage on 12p (see below). Unfortunately, Pericak-Vance *et al.* provide no information on the specific locations of the other areas of interest on chromosomes 4, 6 and 20 (33,34). We did not observe evidence of linkage on chromosomes 4 or 20 but did find a lod score of 1.4 in  $\epsilon 4$ -ve ASPs on chromosome 6. However, while we cannot assess whether our chromosome 6 region of interest corresponds to that of Pericak-Vance *et al.* (34), it is of interest that it contains much of the HLA region and that associations between AD and HLA-A2 have been reported (20). From initial findings of a separate genome survey for AD susceptibility genes, Zubenko *et al.* (36) also reported a region of interest on the X chromosome, near DXS1047. No support for linkage to this region was obtained in our study. Indeed, we could exclude a locus of  $\lambda_s \geq 1.4$  from this region.

Figure 1 also shows the positions of a number of 'candidate genes' for which evidence exists implicating their involvement in AD. Only three of these are located within the peaks identified in this study: APOE, APP and  $\alpha 2$ -macroglobulin (A2M). APOE is the only unequivocally established genetic risk factor for late onset AD and it is therefore reassuring that we were able to obtain evidence for linkage at this locus without enriching for markers in the region. It is well known that mutations in APP can cause autosomal dominant early onset familial AD (2). Direct analyses of the coding sequence (37) and the promoter (37,38) have not detected APP polymorphisms that predispose to late onset AD. However, the Duke group, also using non-parametric linkage methods, have provided evidence that a locus predisposing to late onset AD might reside in this region of chromosome 21 (39). Our findings suggest that further attention should now be paid to the possibility of a susceptibility locus either within the regulatory regions of APP or, less parsimoniously, in a gene close by. It is therefore of interest that high levels of  $\beta A$  are sometimes found in typical late onset AD and this might indicate genetic variability in APP expression (40). In addition, recent genetic analysis of a case of Down syndrome (DS) due to non-disjunction has firmly implicated triplication of APP in the pathogenesis of AD in DS (41). Together with our data, these findings suggest that a full genetic analysis of the APP gene should now be a priority. A2M

is a serum pan-protease inhibitor which has been implicated in AD because of its ability to mediate the clearance and degradation of  $\beta A$  (42,43) and because, like ApoE, it is a ligand of low density lipoprotein receptor-related peptide (44). Blacker *et al.* (29) have recently demonstrated an association between a pentanucleotide deletion in the 5' splice site of exon 18 of A2M and AD using a subset of the NIMH sample typed in the present study. This association appeared independent of APOE genotype and this would appear to correlate well with our observation that linkage to the A2M region was seen only in  $\epsilon 4$ -ve families. Our data on chromosome 12 have been reported separately (35), but we are now able to place them in the context of a full genome scan. The maximum MLS of 1.91 in this region is less than the threshold calculated by simulation of 2.25 for suggestive linkage according to Lander and Kruglyak (32), but was the second highest score obtained in the  $\epsilon 4$ -ve families. None of the other candidate genes shown in Figure 1 are located within the lod score peaks in our study. However, the power of linkage methods to detect genes of small effect is limited (45). Indeed only PS-1 and  $\alpha 1$ -antichymotrypsin fall within the areas of exclusion for a gene of an effect size equivalent to or greater than APOE ( $\lambda_s = 1.4$ ).

This study is the first stage of a two-stage genome scan for late onset AD in 600 sibling pairs. The 16 areas identified will form the basis of analysis in stage 2. This will comprise further analysis of these areas using the original and new markers spaced at ~5 cM intervals within each region in a total of 600 sibling pairs with late onset Alzheimer's disease. We have calculated that this two-stage study will have a power of >0.80 to detect a locus of effect size  $\lambda_s \geq 1.5$ . Regions showing evidence of linkage at stage 2 will be tested further using positional cloning and candidate gene approaches.

This is the largest genome scan to date in Alzheimer's disease and shows for the first time that this is a genetically complex disorder involving several, perhaps many, genes in addition to APOE. We have provided evidence consistent with a role of A2M as a susceptibility locus and preliminary data suggesting that variation in the regulatory regions of APP may also influence predisposition to AD. The other areas identified in our study, in particular those on chromosomes 1, 9 and 10, will be of considerable interest to those trying to identify positional candidate genes using information emerging from neurobiological studies of AD.

## MATERIALS AND METHODS

### Families

The families used were selected from those collected by the NIMH-AD Genetic Consortium (46). From within this family series 230 families were selected based on the following criteria: at least two affected siblings with probable or definite AD according to the NINCDS-ADRDA diagnostic criteria (47) with onset ages of  $\geq 65$  years sampled and available for genotyping (diagnosis of definite AD requires neuropathological data which are usually obtained post-mortem). Within these families the probable ( $n = 417$ ) or definitely affected ( $n = 79$ ) siblings were genotyped. In families where there were more than two affected siblings (definite or probable) available, all of them were used. In families where there were just two affected siblings but unaffected individuals were available, the oldest of the unaffected individuals was also sampled so that the genotype data from this unaffected

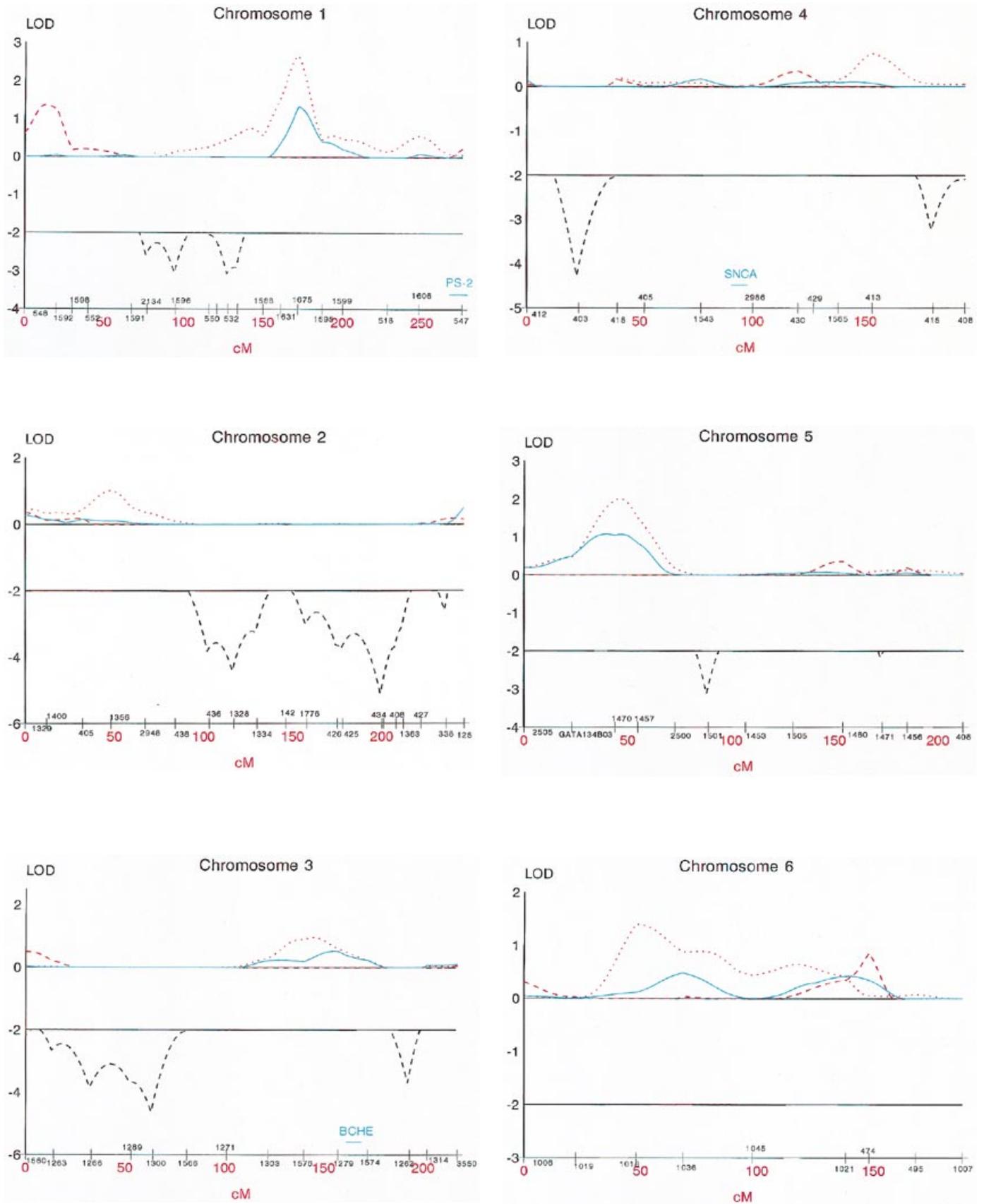


Figure 1. Multipoint lod scores.

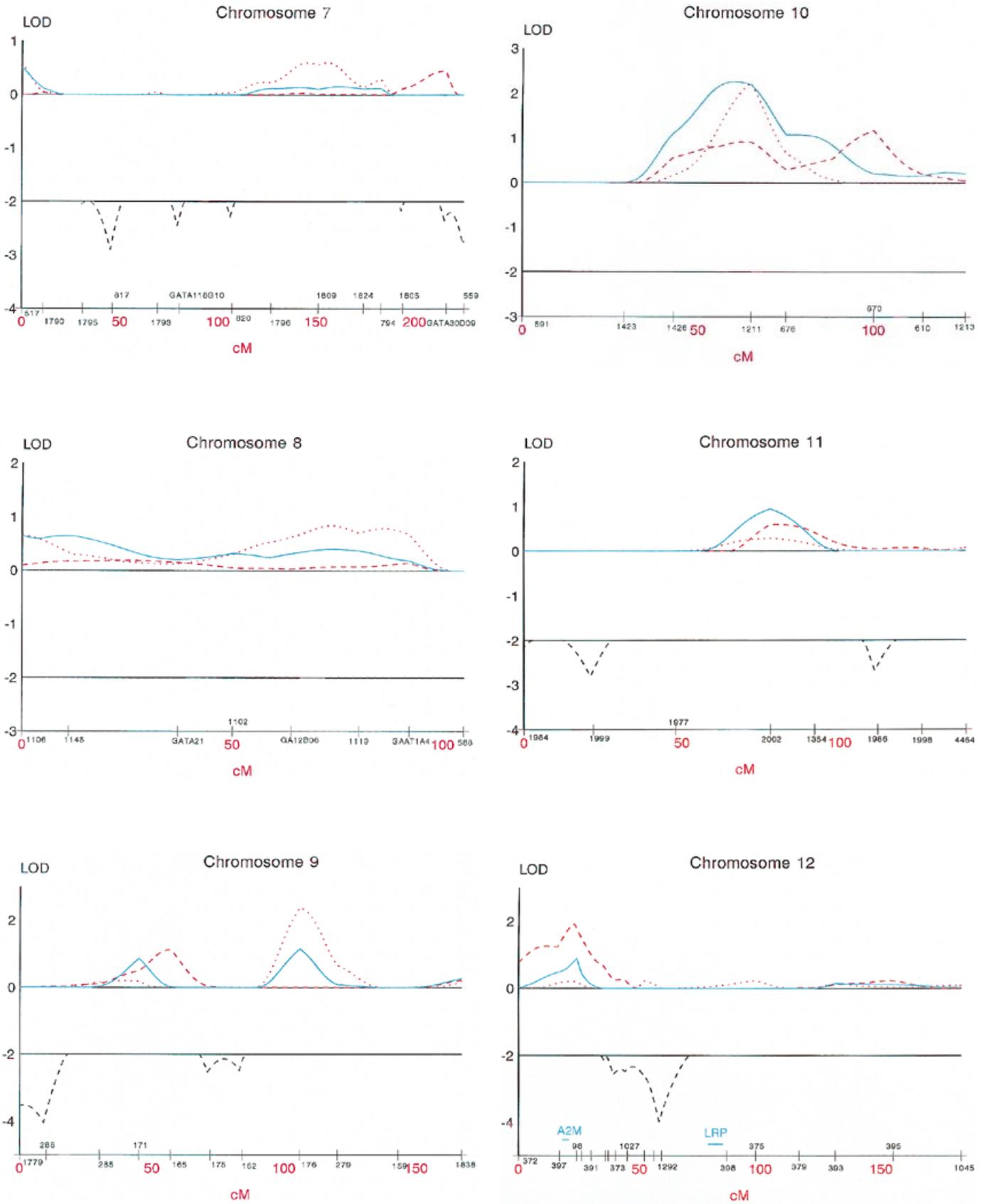


Figure 1. Continued

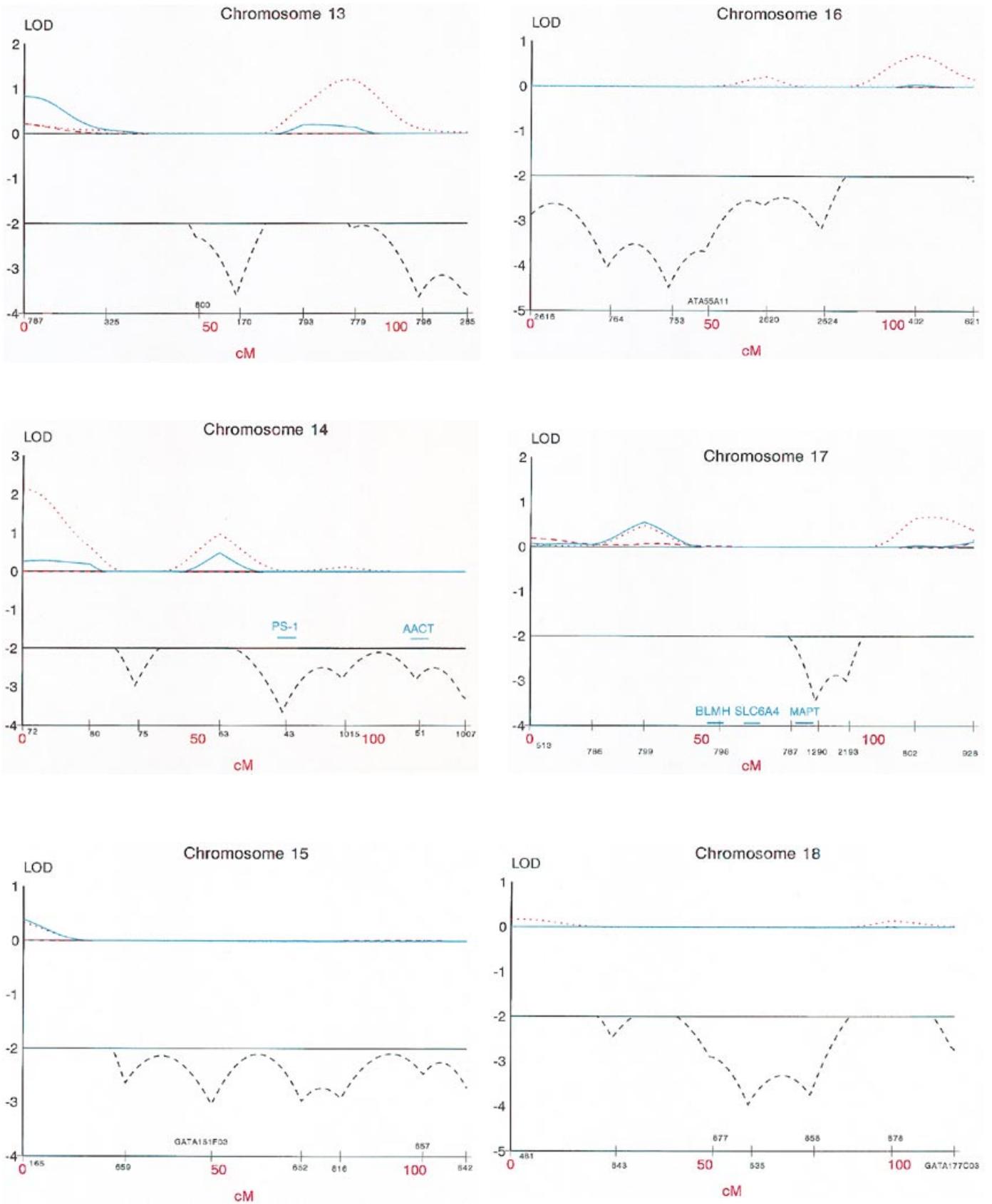


Figure 1. Continued

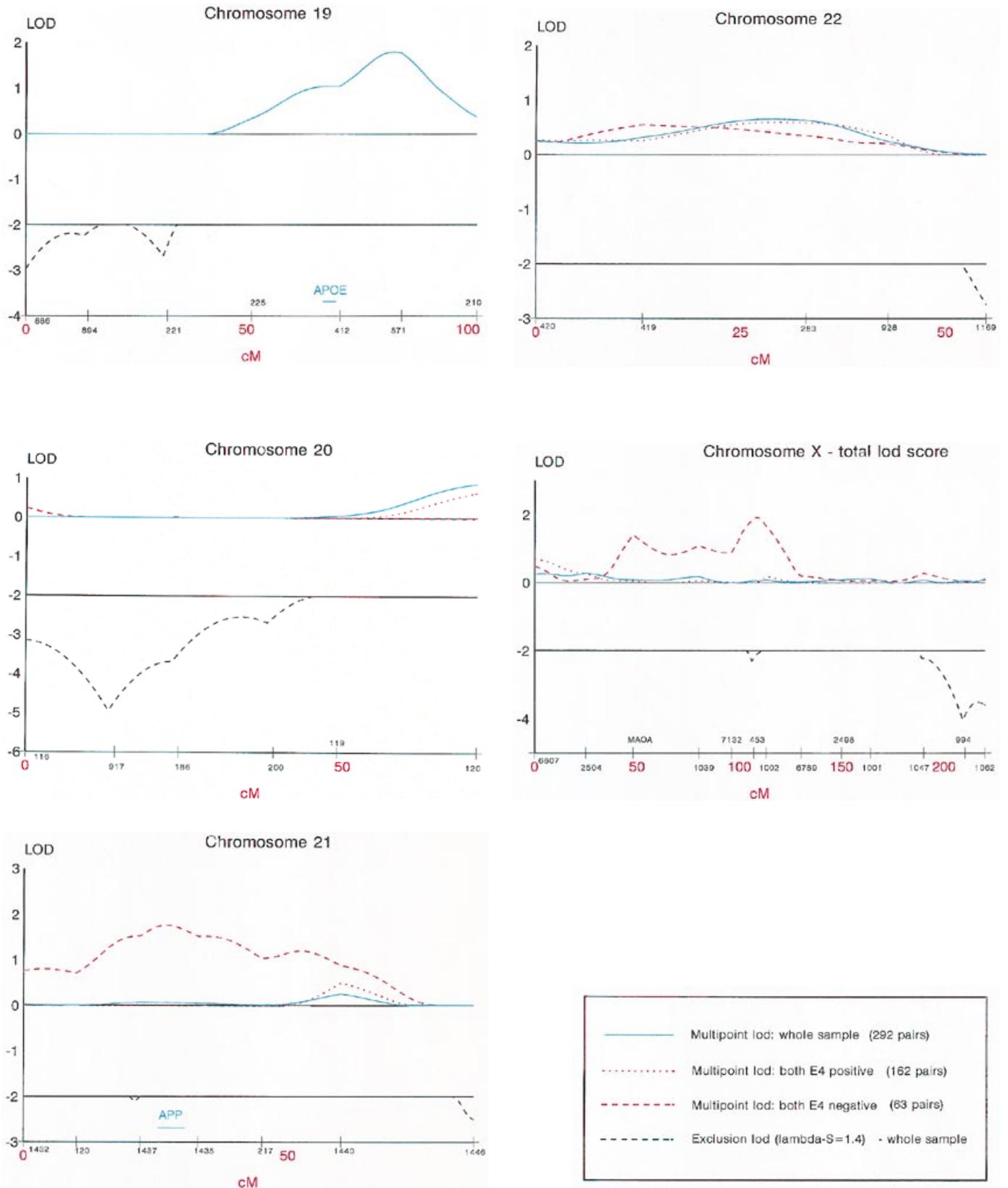


Figure 1. Continued

individual could be used to check for genotyping errors. These 230 families yielded a total of 292 ASPs, in only 17 of which were both definitely affected.

### Genotyping

Lymphoblastoid cell lines were generated from peripheral blood leukocytes and DNA extracted using routine methods. Semi-automated fluorescent genotyping was undertaken using the ABI Genescan/Genotyper system by comparison of the fragment sizes with an internal standard. All genotypes were scored blind as to phenotype. Genotype data from all three participating centres was sent to a centralized database in Cardiff (48). The database (MEGABASE) was used to check the binning of alleles, convert allele sizes to whole numbers and to check for non-Mendelian inheritance where possible. MEGABASE stored all relevant genotypic/phenotypic data and produced all necessary files for statistical analysis.

### Markers

A total of 237 microsatellite markers, obtained from Genethon, CHLC and GDB (106 di-, 21 tri- and 110 tetranucleotides) were typed in an average of 253.8 (159–288) sib pairs. The average distance between adjacent markers was 16.3 cM (1–40 cM) and the average heterozygosity was 0.75 (0.39–0.89).

### Statistical analysis

The program SPLINK (49) was used to compute single point MLSs under the 'possible triangle' restrictions (50) and to calculate allele frequency estimates for each marker. These were used in the multipoint analyses, which were carried out using MAPMAKER/SIBS (32) on the whole sample of 292 sib pairs, the subset of 162 sib pairs where both members possessed an  $\epsilon 4$  allele and the subset of 63 pairs where neither possessed an  $\epsilon 4$  allele. A multipoint exclusion map was also obtained for the whole sample using MAPMAKER/SIBS. For the purposes of this analysis, the disease susceptibility model was parameterized in terms of  $\lambda_s$ , the relative risk to siblings of a case (51). A  $\lambda_s$  of 1.4 was used, since this is approximately equal to that given by APOE, assuming a population gene frequency of 0.15, a relative risk of 4 for  $\epsilon 4$  heterozygotes and a relative risk of 10 for  $\epsilon 4$  homozygotes, similar to observations in a number of Caucasian populations (7). Higher values of  $\lambda_s$  were also tested, although the results are not shown here. Genome-wide significance levels were obtained by simulation in the following way: 500 replicate samples of each chromosome were simulated under the null hypothesis of no linkage, using the observed allele frequencies and ensuring that the individuals typed at each locus were the same as in the original dataset. Stratification into  $\epsilon 4$ +ve and  $\epsilon 4$ -ve subsamples was carried out according to the observed APOE genotypes in the original dataset. Maximum MLS analyses were carried out using MAPMAKER/SIBS for each replicate sample of each chromosome. All three samples (whole,  $\epsilon 4$ +ve and  $\epsilon 4$ -ve) were analysed, except for chromosome 19, which was analysed in the whole sample only. The method for estimating the genome-wide significance of a given lod score was to simulate a genome scan by choosing one replicate of each chromosome at random. The number of peaks exceeding the required level in any of the three analyses (results for chromosome 19 excepted) was

counted and the process repeated 10 000 times. The average number of such peaks per genome scan was used to estimate the true genome-wide occurrence rate and, hence, the significance of the lod score.

### ACKNOWLEDGEMENTS

The samples used in this study were selected from those collected by the NIMH Alzheimer's Genetics Initiative and were banked at the Coriell Cell Repository. Full sample IDs are available from the authors. Genotyping and data analysis were supported by a grant from the MRC (UK), the Alzheimer's Association, the Metropolitan Life Foundation, the Mayo Foundation and the Mayo/USF Program Project Grant. A.G. is the recipient of an NIH career development award (AG00634).

### ABBREVIATIONS

A2M,  $\alpha 2$ -macroglobulin; AD, Alzheimer's disease; ASPs, affected sibling pairs; APP, amyloid precursor protein; APOE, apolipoprotein E; DS, Down syndrome;  $\epsilon 4$ +ve,  $\epsilon 4$ -positive;  $\epsilon 4$ -ve,  $\epsilon 4$ -negative; FAD, familial autosomal dominant AD; IBD, identity by descent; PS-1, presenilin 1.

### REFERENCES

- Clark, R. and Goate, A. (1993) Molecular genetics of Alzheimer's disease. *Arch. Neurol.*, **50**, 1164–1172.
- Goate, A.M., Chartier-Harlin, M.C., Mullan, M.C., Brown, J., Crawford, F., Fidani, L., Giuffra, L., Haynes, A., Irving, N., James, L., Mant, R., Newton, P., Rooke, K., Roques, P., Talbot, C., Pericak-Vance, M.A., Roses, A.D., Williamson, R., Rossor, M.N., Owen M.J. and Hardy, J. (1991) Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. *Nature*, **349**, 704–706.
- Schellenberg, G.D., Bird, T.D., Wijsman, E.M., Orr, H.T., Anderson, L., Nemens, E., White, J.A., Bonnycastle, L., Weber, J.L., Alonso, M.E., Potter, H., Heston, L.L. and Martin, G.M. (1992) Genetic-linkage evidence for a familial Alzheimer's disease locus on chromosome-14. *Science*, **258**, 668–671.
- Sherrington, R., Rogaev, E.I., Liang, Y., Rogaeva, E.A., Levesque, G., Ikeda, M., Chi, H., Lin, C., Li, G., Holman, K., Tsuda, T., Mar, L., Foncin, J.F., Bruni, A.C., Montesi, M.P., Sorbi, S., Rainero, I., Pinessi, L., Nee, L., Chumakov, I., Pollen, D., Brookes, A., Sanseau, P., Polinsky, R.J., Wasco, W. *et al.* (1995) Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. *Nature*, **375**, 754–760.
- Levy-Lehad, E., Wasco, W., Poorkaj, P., Romano, D.M., Oshima, J., Pettingell, W.H., Yu, C.E., Jondro, P.D., Schmidt, S.D., Wang, K., Crowley, A.C., Fu, Y.H., Guenette, S.Y., Galas, D., Nemens, E., Wijsman, E.M., Bird, T.D., Schellenberg, G.D. and Tanzi, R.E. (1995) Candidate gene for the chromosome 1 familial Alzheimer's disease locus. *Science*, **269**, 973–977.
- Hutton, M. and Hardy, J. (1997) The presenilins and Alzheimer's disease. *Hum. Mol. Genet.*, **6**, 1639–1646.
- Corder, E.H., Saunders, A.M., Strittmatter, W.J., Schmechel, D.E., Gaskell, P.C., Small, G.W., Roses, A.D., Haines, J.L. and Pericak-Vance, M.A. (1993) Gene dose of apolipoprotein-e type-4 allele and the risk of Alzheimer's disease in late-onset families. *Science*, **261**, 921–923.
- Pericak-Vance, M.A. and Haines, J.L. (1995) Genetic susceptibility to Alzheimer-disease. *Trends Genet.*, **11**, 504–508.
- Lander, E.S. and Schork, N.J. (1994) Genetic dissection of complex traits. *Science*, **266**, 2037–2048.
- Owen, M.J. and McGuffin, P. (1993) Association and linkage—complementary strategies for complex disorders. *J. Med. Genet.*, **30**, 638–639.
- Wragg, M., Hutton, M., Talbot, C., Busfield, F., Han, S.W., Lendon, C., Clark, R.F., Morris, J.C., Edwards, D., Goate, A., Pfeiffer, E., Crook, R., Prihar, G., Phillips, H., Baker, M., Hardy, J., Rossor, M., Houlden, H., Karran, E., Roberts, G. and Craddock, N. (1995) Genetic association between intronic polymorphism in Presenilin-1 gene and late-onset Alzheimer's disease. *Lancet*, **347**, 509–512.

12. Kehoe, P., Williams, J., Lovestone, S., Wilcock, G., Owen, M.J. and the UK Alzheimer's Disease Collaborative Group (1996) Presenilin-1 polymorphism and Alzheimers disease. *Lancet*, **347**, 1185.
13. Kehoe, P., Williams, J., Holmans, P., Liddell, M., Lovestone, S., Holmes, C., Powell, J., Neal, J., Wilcock, G. and Owen, M.J. (1996) Association between a PS-1 intronic polymorphism and late onset Alzheimer's disease. *Neuroreport*, **7**, 2155–2158.
14. Higuchi, S., Muramatsu, T., Matsushita, S., Arai, H. and Sasaki, H. (1996) Presenilin-1 polymorphism and Alzheimer's disease. *Lancet*, **347**, 1186.
15. Ise, K., Ukrami, K., Ji, Y., Adachi, Y. and Nakashima, K. (1996) Presenilin-1 polymorphism in patients with Alzheimer's disease, vascular dementia and alcohol-associated dementia in Japanese population. *Acta Neurol. Scand.*, **94**, 326–328.
16. Matsushita, S., Arai, H., Muramatsu, T., Makimoto, K., Nakagawa, T., Sasaki, H. and Higuchi, S. (1997) Presenilin-1 polymorphism in Alzheimers disease and vascular dementia. *Exp. Neurol.*, **146**, 567–569.
17. Brookes, A.J., Howell, W.M., Woodburn, K., Johnstone, E.C. and Carothers, A. (1997) Presenilin-I, presenilin-II and VLDL-R associations in early onset Alzheimers disease. *Lancet*, **350**, 336–337.
18. Okuizumi, K., Onodera, O., Namba, Y., Ikeda, K., Yamamoto, T., Seki, K., Ueki, A., Nanko, S., Tanaka, H., Takahashi, H., Oyanagi, K., Mizusana, H., Kanazawa, I. and Tsuji, S. (1995) Genetic association of the very low density lipoprotein (VLDL) receptor gene with sporadic Alzheimers disease. *Nature Genet.*, **11**, 207–209.
19. Lehman, D.J., Johnston, C. and Smith, A.D. (1997) Synergy between the genes for butyrylcholinesterase K variant and apolipoprotein E4 in late-onset confirmed Alzheimer's disease. *Hum. Mol. Genet.*, **6**, 1933–1936.
20. Payami, H., Schellenberg, G.D., Zarepari, S., Kaye, J., Sexton, G.J., Head, M.A., Matsuyama, S.S., Jarvik, L.F., Miller, B., McManus, D.Q., Bird, T.D., Katzman, R., Heston, L., Norman, D. and Small, G.W. (1997) Evidence for association of HLA-A2 allele with onset age of Alzheimer's disease. *Neurology*, **49**, 512–518.
21. Kamboh, M.I., Sanghera, D.K., Ferrel, F.E. and DeKosky, S.T. (1995) APOE\*4-associated Alzheimer's disease risk is modified by alpha-1 antichymotrypsin polymorphism. *Nature Genet.*, **10**, 486–488.
22. Lendon, C., Talbot, C.J., Craddock, N.J., Han, S.W., Wragg, M., Morris, J.C. and Goate, A.M. (1997) Genetic association studies between dementia of the Alzheimer's type and three receptors for apolipoprotein E in a caucasian population. *Neurosci. Lett.*, **222**, 187–190.
23. Wavrant-De Vrieze, F., PerezTur, J., Lambert, J.C., Frigard, B., Pasquier, F., Delacourte, A., Amouyel, P., Hardy, J. and Chartier-Harlin, M.C. (1997) Association between the low density lipoprotein receptor-related protein (LRP) and Alzheimer's disease. *Neurosci. Lett.*, **227**, 68–70.
24. Kang, D.E., Saitoh, T., Chen, X., Xia, Y., Masliah, E., Hansen, L.A., Thomas, R.G., Thal, L.J. and Katzman, R. (1997) Genetic association of the low-density lipoprotein receptor-related protein gene (LRP), an apolipoprotein E receptor, with late-onset Alzheimer's disease. *Neurology*, **49**, 56–61.
25. Kamboh, M.I., Ferrell, R.E. and DeKosky, S.T. (1998) Genetic association studies between Alzheimer's disease and two polymorphisms in the low density lipoprotein receptor-related protein gene. *Neurosci. Lett.*, **244**, 65–68.
26. Baum, L., Chen, L., Ng, H.K., Chan, Y.S., Mak, Y.T., Woo, J., Chiu, H.F.K. and Pang, C.P. (1998) Low density lipoprotein receptor related protein gene exon 3 polymorphism association with Alzheimer's disease in Chinese. *Neurosci. Lett.*, **247**, 33–36.
27. Hollenbach, E., Ackermann, S., Hyman, B.T. and Rebeck, G.W. (1998) Confirmation of an association between a polymorphism in exon 3 of the low density-lipoprotein-related protein gene and Alzheimer's disease. *Neurology*, **50**, 1905–1907.
28. Montoya, S.E., Aston, C.E., DeKosky, S.T., Kamboh, M.I., Lazo, J.S. and Ferrell, R.E. (1998) Bleomycin hydroxylase is associated with risk of sporadic Alzheimer's disease. *Nature Genet.*, **18**, 211–212.
29. Blacker, D., Wilcox, M.A., Laird, N.M., Rodes, L., Horvath, S.M., Go, R.C.P., Perry, R., Watson, B. Jr, Bassett, S.S., McInnis, M.G., Albert, M.S., Hyman, B.T. and Tanzi, R.E. (1998) Alpha-2 macroglobulin is genetically associated with Alzheimer's disease. *Nature Genet.*, **19**, 357–360.
30. Li, T., Holmes, C., Sham, P.C., Vallada, H., Birkett, J., Kirov, G., Lesch, K.P., Powell, J., Lovestone, S. and Collier, D. (1997) Allelic functional variation of serotonin transporter expression is a susceptibility factor for late onset Alzheimer's disease. *Neuroreport*, **8**, 683–686.
31. Owen, M.J., Holmans, P. and McGuffin, P. (1997) Association studies in psychiatric genetics. *Mol. Psychiat.*, **2**, 270–273.
32. Lander, E.S. and Kruglyak, L. (1995) Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nature Genet.*, **11**, 241–247.
33. Pericak-Vance, M.A., Bass, M.P., Yamaoka, L.H., Gaskell, P.C., Scott, W.K., Terwedow, H.A., Menold, M.M., Conneally, P.M., Small, G.W., Vance, J.M., Saunders, A.M., Roses, A.D. and Haines, J.L. (1997) Complete genomic screen in late-onset familial Alzheimer disease—evidence for a new locus on chromosome 12. *J. Am. Med. Assoc.*, **278**, 1237–1241.
34. Pericak-Vance, M.A., Bass, M.L., Yamaoka, L.H., Gaskell, P.C., Scott, W.K., Terwedow, H.A., Menold, M.M., Conneally, P.M., Small, G.W., Saunders, A.M., Roses, A.D. and Haines, J.L. (1998) Complete genomic screen in late-onset familial Alzheimer's disease. *Neurobiol. Aging*, **19**, S39–S42.
35. Wu, B., Holmans, P., Wavrant-De Vrieze, F., Shears, S., Kehoe, P., Crook, R., Booth, J., Williams, N., Perez-Tur, J., Roehl, K., Fenton, I., Chartier-Harlin, M.C., Lovestone, S., Williams, J., Hutton, M., Hardy, J., Owen, M.J. and Goate, A.M. (1998) Genetic studies on chromosome 12p11–12 in late onset AD. *J. Am. Med. Assoc.*, **280**, 619–622.
36. Zubenko, G.S., Stiffler, J.S., Hughes, H.B., Hurtt, M.R. and Kaplan, B.B. (1998) Initial results of a genome survey for a novel Alzheimer's disease risk: association with a locus on the X chromosome. *Am. J. Med. Genet. Neuropsychiat. Genet.*, **81**, 98–107.
37. Liddell, M.B., Bayer, A.J. and Owen, M.J. (1995) No evidence that common allelic variation in the amyloid precursor protein (APP) gene confers susceptibility to Alzheimers-disease. *Hum. Mol. Genet.*, **4**, 853–858.
38. Rooke, K., Goate, A., Fidani, L., Mullan, M., Roques, P., Rossor, M., Hardy, J. and Chartier-Harlin, M.C. (1993) Screening of the promoter and the b-amyloid sequence of the APP gene for polymorphism in families with late onset Alzheimer's disease. *Neurodegeneration*, **1**, 237–240.
39. Pericak-Vance, M., Bebout, J.L., Gaskell, P.C., Yamaoka, L.H., Hung, W.Y., Alberts, M.J., Walker, A.P., Barlett, R.J., Haynes, C.A., Welsh, K.A., Earl, N.L., Heyman, A., Clark, C.M. and Roses, A.D. (1991) Linkage studies in familial Alzheimer's disease. Evidence for chromosome 19 linkage. *Am. J. Hum. Genet.*, **48**, 1034–1050.
40. Scheuner, D., Eckman, C., Jensen, M., Song, X., Citron, M., Suzuki, N., Bird, T.D., Hardy, J., Hutton, M., Kukull, W., Larson, E., Levey-Lahad, E., Viitanen, M., Peskind, E., Poorkaj, P., Schellenberg, G., Tanzi, R., Wasco, W., Lannfelt, L., Selkoe, D. and Younkin, S. (1996) Secreted amyloid b-protein similar to that in the senile plaques of Alzheimer's disease is increased *in vivo* by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease. *Nature Med.*, **2**, 864–870.
41. Prasher, V.P., Farrer, M.J., Kessling, A.M., Fisher, E.M.C., West, R.J., Barber, P.C. and Utler, A.C. (1998) Molecular mapping of Alzheimer's-type dementia in Down's syndrome. *Ann. Neurol.*, **43**, 380–383.
42. Du, Y.S., Ni, B.H., Glinn, M., Dodel, R.C., Bales, K.R., Zhang, Z.Y., Hyslop, P.A. and Paul, S.M. (1997) Alpha(2)-macroglobulin as a beta-amyloid peptide-binding plasma protein. *J. Neurochem.*, **69**, 299–305.
43. Hughes, S.R., Khorkova, O., Goyal, S., Knaeblein, J., Heroux, J., Riedel, N.G. and Sahasrabudhe, S. (1998) Alpha(2)-macroglobulin associates with beta-amyloid peptide and prevents fibril formation. *Proc. Natl Acad. Sci. USA*, **95**, 3275–3280.
44. Rebeck, G.W., Harr, S.D., Strickland, D.K. and Hyman, B.T. (1995) Multiple, diverse senile plaque-associated proteins are ligands of an apolipoprotein E receptor, the a2-macroglobulin receptor/low density lipoprotein receptor related protein. *Ann. Neurol.*, **37**, 211–217.
45. Hauser, E.R., Boehnke, M., Guo, S.W. and Risch, N. (1996) Affected-sib-pair interval mapping and exclusion for complex genetic traits—sampling considerations. *Genet. Epidemiol.*, **13**, 117–137.
46. Blacker, D., Haines, J., Rodes, L., Terwedow, H., Go, R.C.P., Harrell, L.E., Perry, R.T., Bassett, S.S., Chase, G., Meyers, D., Albert, M.S. and Tanzi, R.E. (1997) ApoE 4 and age at onset of Alzheimer's disease: the NIMH Genetics Initiative. *Neurology*, **48**, 139–147.
47. McKhann, G., Drachman, D., Folstein, M., Katzman, R., Price, D. and Stadlan, E.M. (1984) Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA work group under the auspices of Department of Health and Human Services Task Force on Alzheimer's disease. *Neurology*, **34**, 939–944.
48. Fenton, I. and Sandkuijl, L.A. (1992) MEGABASE-PKD: a genetic database for polycystic kidney disease. *Contrib. Nephrol.*, **97**, 118–127.
49. Holmans, P. and Clayton, D. (1995) Efficiency of typing unaffected relatives in an affected-sib-pair linkage study with single-locus and multiple tightly linked markers. *Am. J. Hum. Genet.*, **57**, 1221–1232.
50. Holmans, P. (1993) Asymptotic properties of affected-sib-pair linkage analysis. *Am. J. Hum. Genet.*, **52**, 362–374.
51. Risch, N. (1990) Genetic-linkage and complex diseases, with special reference to psychiatric-disorders. *Genet. Epidemiol.*, **7**, 3–16.