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Genome Screen for Loci Influencing Age at Onset and Rate of Decline in Late Onset Alzheimer's Disease

Peter Holmans,[1]* Marian Hamshere,[2] Paul Hollingworth,[2] Frances Rice,[2] Nigel Tunstall,[3] Sue Jones,[2] Pamela Moore,[2] Fabienne Wavrant DeVrieze,[4] Amanda Myers,[4] Richard Crook,[6] Danielle Compton,[6] Helen Marshall,[5] David Meyer,[5] Shantia Shears,[5] Jeremy Booth,[5] Dzanan Ramic,[5] Nigel Williams,[2] Nadine Norton,[2] Richard Abraham,[2] Pat Kehoe,[2] Hywel Williams,[2] Varuni Rudrasingham,[2] Mick O'Donovan,[2] Lesley Jones,[2] John Hardy,[4] Alison Goate,[5] Simon Lovestone,[3] Michael Owen,[2] and Julie Williams[1,2]

[1]*Biostatistics and Bioinformatics Unit, Wales College of Medicine, Heath Park, Cardiff, United Kingdom*
[2]*Department of Psychological Medicine, University of Wales College of Medicine, Heath Park, Cardiff, United Kingdom*
[3]*Institute of Psychiatry, De Crespigny Park, Denmark Hill, London, United Kingdom*
[4]*Laboratory of Neurogenetics, National Institute on Aging, National Institutes of Health, Bethesda, Maryland*
[5]*Department of Psychiatry, Washington University School of Medicine, St. Louis, Missouri*
[6]*Laboratory of Neurogenetics, Birdsall Building, Mayo Clinic Jacksonville, Jacksonville, Florida*

We performed an affected sib-pair (ASP) linkage analysis to test for the effects of age at onset (AAO), rate of decline (ROD), and Apolipoprotein E (APOE) genotype on linkage to late-onset Alzheimer's disease (AD) in a sample comprising 428 sib-pairs. We observed linkage of mean AAO to chromosome 21 in the whole sample (max LOD = 2.57). This came entirely from the NIMH sample (max LOD = 3.62), and was strongest in pairs with high mean AAO (>80). A similar effect was observed on chromosome 2q in the NIMH sample (max LOD = 2.73); this region was not typed in the IADC/UK sample. Suggestive evidence was observed in the combined sample of linkage of AAO difference to chromosome 19q (max LOD = 2.33) in the vicinity of APOE and 12p (max LOD = 2.22), with linkage strongest in sib-pairs with similar AAO. Mean ROD showed suggestive evidence of linkage to chromosome 9q in the whole sample (max LOD = 2.29), with the effect strongest in the NIMH sample (max LOD = 3.58), and in pairs with high mean ROD. Additional suggestive evidence was also observed in the NIMH sample with AAO difference on chromosome 6p (max LOD = 2.44) and 15p (max LOD = 1.87), with linkage strongest in pairs with similar AAO, and in the UK sample with mean ROD on chromosome 1p (max LOD = 2.73, linkage strongest in pairs with high mean ROD). We also observed suggestive evidence of increased identical by descent (IBD) in APOE ε4 homozygotes on chromosome 1 (max LOD = 3.08) and chromosome 9 (max LOD = 3.34). The previously reported genome-wide linkage of AD to chromosome 10 was not influenced by any of the covariates studied.

KEY WORDS: Alzheimer's Disease; age at onset; rate of decline; genome screen; linkage

INTRODUCTION

Alzheimer's disease (AD) is a common debilitating disorder with a prevalence that rises steeply with age from below 1% at 65 years to as high as 40% after the age of 90 [Bachman et al., 1992]. Genes are known to play a role in the development of AD. Twin studies show heritabilities of around 60% [Bergem et al., 1997; Gatz et al., 1997]. Indeed, variation in four genes has already been shown to cause rare forms of early-onset AD [the Amyloid Precursor Protein Gene (APP); Goate et al., 1991; Presenilin 1 (PS1); Sherrington et al., 1995; Presenilin 2 (PS2); Levy Lahad et al., 1995, Rogaev et al., 1995] or increase the general risk of disease development [Apolipoprotein E (APOE), Corder et al., 1993]. As well as increasing disease susceptibility, APOE ε4 alleles are associated with reduced age at onset (AAO) and appear to show their strongest effect below 70 years [Farrer et al., 1997]. There is also evidence from both twin [Pedersen et al., 2001] and family studies [Tunstall et al., 2000; Li et al., 2002] that AAO in AD is heritable. Daw et al. [2000] have estimated that in addition to *APOE*, there are at least four loci with similar effect sizes, which contribute to AAO in AD.

Recently, a number of studies [Olson et al., 2001, 2002; Li et al., 2002] have attempted to map loci contributing to AAO in AD. Using genotypes generated by the UK/US AD Consortium [Kehoe et al., 1999] on affected sibling pairs (ASPs) collectedas part of the National Institute of Mental Health (NIMH) AD Genetics Initiative, Olson et al. [2001, 2002] observed suggestive evidence that the mean AAO of an affected sib-pair (ASP) influenced linkage to markers on chromosome 21, in close proximity to the APP gene, and chromosome 14. However, their strongest findings were observed with mean current age (i.e., age at most recent interview or death), with significant evidence of linkage to markers on chromosome 21, again in the APP region, and markers on chromosome 20.

Li et al. treated AAO as a quantitative trait, including AD patients and unaffected family members (where age at entry was used). Variance components analysis on a large sample of AD families showed suggestive linkage (LOD > 2) to *APOE*, and regions on chromosomes 4, 8, and 10. They also observed weaker evidence of linkage (LOD > 1) to chromosomes 6, 13, and 18.

It is noteworthy that relationships with AAO have been characterized in different ways. Olson and colleagues studied

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*Correspondence to: Prof. Peter Holmans, Biostatistics and Bioinformatics Unit, Wales College of Medicine, Heath Park, Cardiff, CF14 4XN, UK. E-mail: holmanspa@cardiff.ac.uk

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the relationship between alleles shared identical by descent (IBD) in ASPs and the total AAO in each sibling pair (i.e., the sum of both AAOs). In contrast, the variance components analysis of Li et al., tends to focus more on the relationship between IBD and similarity of AAO than that of IBD and mean AAO. These are different questions, but both have potential biological significance. Identifying greater allele sharing in siblings identified by higher or lower mean ages at onset could indicate the presence of a gene contributing to AD risk within the relevant AAO range, for example, with APOE [Li et al., 2002]. However, it is also possible that different disease-susceptibility genotypes give different rates of increase of AD risk with age. In this case, one would expect pairs with similar ages at onset to be more likely to have the same disease-susceptibility genotype, and hence exhibit linkage. This increase in linkage would be seen in pairs with both high and low ages at onset, and thus one would not necessarily expect to see a correlation between IBD and mean AAO. It is therefore important to analyze both mean AAO and similarity in AAO. This was done here using a logistic regression analysis. This differs from the variance-components method use by Li et al. in that it can test for overall linkage in addition to relationship between IBD sharing and AAO.

In addition to AAO, other features of disease development may be influenced by genes. Olson et al. observed stronger linkage to chromosome 21 when both AAO and duration of illness were taken into account, thus indicating that the rate of decline (ROD) may be important. However, 'duration of illness' is not an appropriate measure of decline, since it changes with time, and is thus dependent on when the patient was interviewed. We therefore sought to provide a better estimate of the rate at which AD progresses, and to use this to test for linkage. Differences in AAO and ROD between members of an ASP were used as covariates, in addition to pairwise means, for the reasons outlined above.

MATERIALS AND METHODS

Sample Description

The data presented in this study have come from three samples of affected sibling pairs, all of Caucasian origin, with late-onset AD (LOAD) [Kehoe et al., 1999; Myers et al., 2000, 2002], defined as AAO $\geq$65. In all samples, AAO was defined as the age at which the first symptoms of AD were observed. The samples comprised 277 ASPs selected from those collected by the NIMH-AD Genetic Consortium [Blacker et al., 1997] all with AAO and ROD information, 96 affected sibling pairs from a sample collected in the UK (72 with precise AAO and 68 with ROD information), and a sample of 80 ASPs from the Indiana Alzheimer's Disease Centre (IADC) [Pericak-Vance et al., 2000], of which 79 had AAO data but none had ROD information. This sample is also referred to in this study as the "NIA" sample. In all datasets, affected individuals were diagnosed with either probable or definite AD in accordance with the National Institute of Neurological and Communicative Disorders and Stroke and the AD and Related Disorders Associations (NINCDS-ADRDA) clinical diagnostic criteria for AD [McKhann et al., 1984]. In the NIMH and UK samples, diagnosis was based on a semi-structured interview, while in the IADRC sample diagnosis was based on clinical diagnosis. Descriptions of the structured interview and ascertainment procedures for the NIMH sample can be found at http://zork.wustl.edu/nimh/ad.html. Individuals in the UK sample were assessed using a structured interview with known validity for AD pathology [i.e., positive predictive value of 92%, Holmes et al., 1999, which comprises: (1) The Mini Mental State Examination [Folstein et al., 1975]; (2) The Cambridge Mental Disorders of the Elderly Examination (CAMDEX; informant interview, physical examination sections, and CAMCOG) [Roth et al., 1986]; (3) The Blessed Dementia Scale [Blessed et al., 1968]; (4) TheBristol Activities of Daily Living Scale [Bucks et al., 1996]; (5) The Behavioural Pathology in Alzheimer's Disease Rating Scale [Reisberg et al., 1987]; (6) Webster Rating Scale 9 [Webster, 1968]; (7) Global Deterioration Scale [Reisberg et al., 1982]; (8) Cornell Scale for Depression in Dementia [Alexopoulos et al., 1988, 1989] Manchester and Oxford Universities Scale for Psychological Assessment of Dementia (MOUSEPAD) [Allen et al., 1996].

The three samples have previously been used to perform a two-stage ASP genome scan for linkage to late-onset AD [Kehoe et al., 1999; Myers et al., 2002]. The NIMH sample was genotyped on 237 marker loci throughout the genome, with an average spacing of about 15 cM. The 16 regions giving a maximum LOD score >1 were followed-up by genotyping a further 91 marker loci in all three samples, with an average spacing of about 5 cM, resulting in 10 regions with a LOD >1 and a region on chromosome 10 showing genome-wide significance.

Rate of Decline

ROD was only measured in the NIMH and UK samples. A severity of dementia rating was recorded using the Global Deterioration Scale (GDS) [Reisberg et al., 1982] in the UK sample and the Clinical Dementia Rating [CDR, Hughes et al., 1997] in the NIMH sample. Scores on the CDR were recorded on a 7-point scale, ranging from "unaffected" to "terminal." Scores on the GDS were also recorded on a 7-point scale, ranging from "no cognitive decline" to "very severe cognitive decline." For the purposes of the analysis, the GDR and CDS scores were recoded to make them compatible. Table I shows how the scores were recoded to produce an overall severity scale for the NIMH and UK samples. The recoded severity scores were divided by the number of years between onset of AD and the time of the interview at which severity was assessed, to give a *ROD* score. This can be simply defined as the average increase in severity per year, with higher scores representing an increased ROD.

Details of the distribution of AAO and ROD in the three samples, together with *APOE* genotypes, are found in Table II.

Covariates Tested

Four quantitative variables (all centered around their sample means) were used as covariates in the analyses: (1) mean age-at-onset of the affected pair, (2) difference in age-at-onset of the affected pair, (3) mean ROD of the affected pair, and (4) difference in ROD of the affected pair.

Since AAO appears to depend on APOE genotype (see Table II), the *APOE* status of the sib-pair was also included in analyses (see below).

TABLE I. Equivalence of CDR, GDS, and Recoded Severity Scores

CDR	GDS	Recoded severity score
0.5 Questionable	2 Very mild cognitive decline	1 Very mild
1 Mild	3 Mild cognitive decline	2 Mild
2 Moderate	4 Moderate cognitive decline	3 Moderate
3 Severe	5 Moderately severe cognitive decline	4 Moderately Severe
4 Profound	6 Severe cognitive decline	5 Severe
5 Terminal	7 Very severe cognitive decline	6 Very severe

TABLE II. Descriptive Statistics of Covariates

	Age at onset				Rate of decline per year			
Sample	*N*	Mean	SD	Range	*N*	Mean	SD	Range
NIMH	468	74.97	5.811	65–97	466	1.00	0.67	0.23–5
NIA	171	73.39	5.29	65–95				
UK	150	76.05	6.61	65–91	134	1.30	0.87	0.32–6
ALL	789	74.84	5.93	65–97	600	1.07	0.73	0.23–6
ApoE genotype								
x/x	218	77.78	6.21	65–95	188	1.11	0.79	0.27–6
4/x	456	74.15	5.32	65–97	328	1.02	0.67	0.23–5
4/4	111	71.91	5.49	65–91	82	1.14	0.77	0.33–4

Likelihood Formulation

Following the suggestion of Rice [1997] and Rice et al. [1999], the likelihood is parameterized in terms of P, the probability of an ASP inheriting a given parental allele IBD, which is then modeled as a logistic regression with the intercept α measuring overall linkage, and the regression coefficient β measuring the relationship between IBD and the quantitative covariate (AAO or ROD). *APOE* effects were modeled by denoting individuals as + if they possess an $\varepsilon 4$ allele and − otherwise. ASPs thus fall into three categories: +/+, +/−, and −/−; and the effects of these on IBD can be tested by including a three-level factor γ in the regression.

Statistical Tests

Testing whether a quantitative variable has an effect on IBD sharing is equivalent to testing for $\beta \neq 0$. This is done by maximizing the likelihood with respect to both α and β and dividing this by the likelihood obtained assuming no linkage (i.e., $\alpha = \beta = 0$) to give a LOD score:

$$\mathrm{LOD} = \log_{10}\left(\frac{\mathrm{L}\left(\hat{\alpha},\ \hat{\beta}\right)}{\mathrm{L}(\alpha = 0,\ \beta = 0)}\right).$$

The maximum value of the LOD score across the chromosome region of interest is calculated, and denoted by $\mathrm{ML}_{\alpha+\beta}$. These are given as 'MLS' in Figures 1 and 2. Maximization was carried out constraining $\alpha \geq 0$. This is equivalent to assuming that the overall IBD sharing probability is ≥ 0.5 for pairs with values of the variable equal to the sample mean. When the variable being tested was the *difference* (in AAO or ROD) between the two members of an ASP, the maximization was carried out with β constrained to be less than or equal to zero.

A chromosome-wide significance level for the influence of the variable on IBD sharing was obtained by creating 1,000 replicate samples, each with the observed values of the variable randomly permuted among the affected pairs. For each replicate, $\mathrm{ML}_{\alpha+\beta}$ was calculated. The P-value was defined as the proportion of replicates for which $\mathrm{ML}_{\alpha+\beta}$ was greater than the observed value.

To test whether the APOE4 status of the affected pair has an effect on IBD sharing, the likelihood is maximized with respect to both α and γ to obtain a maximum LOD score $\mathrm{ML}_{\alpha+\gamma}$. Note that $\gamma_{-/-}$ can be set to zero. Furthermore, $\gamma_{+/+}$ was constrained so that $\alpha + \gamma_{+/+} \geq 0$, that is, affected pairs who are both E4-positive have IBD sharing probabilities ≥ 0.5. No constraint was applied to $\gamma_{+/-}$.

A chromosome-wide significance level for the effect of *APOE* on IBD sharing was obtained in a similar manner to that for the quantitative variables.

To test whether the quantitative variable has a significant effect on IBD sharing *after allowing for APOE effects*, the likelihood is maximized with respect to α, β, and γ, subject to the constraints already described, to produce a chromosome-wide maximum LOD score $\mathrm{ML}_{\alpha+\beta+\gamma}$. Again, a chromosome-wide significance level for the influence of the variable on IBD sharing, allowing for the effects of *APOE*, was obtained by permuting the values of the variable while keeping the *APOE* genotypes fixed. Note that the asymptotic distributions of the LOD scores depend on the covariates included in the model. For this reason, chromosome-wide P-values are also quoted.

RESULTS

Multipoint LOD score graphs are shown in Figure 1 for AAO and Figure 2 for ROD, together with maximum LOD scores and corresponding chromosome-wide P-values for the covariates. Only chromosomes with $P \leq 0.05$ for AAO and $P \leq 0.1$ are shown. Results for other chromosomes are available on request from the corresponding author.

The most significant single result was obtained on chromosome 21 with mean AAO in the NIMH sample (see Fig. 1g), a LOD of 0.12 being increased to 3.62. Pairs with later ages at onset were found to show elevated IBD sharing (chromosome-wide $P = 0.0004$) agreeing with the results of Olson et al. [2001]. However, no such effect was observed in the UK + NIA sample (LOD of 1.03 increased to 1.04). Thus, the effect in the combined sample (Fig. 1h), whilst still statistically significant (chromosome-wide $P = 0.007$), is smaller than that in the NIMH sample alone. A statistically significant effect of mean AAO was also found on chromosome 2q in both the combined and NIMH samples (Fig. 2a). Here, the LOD increases from 0.56 to 2.73 at 260 cM (chromosome-wide $P = 0.014$), and the effect was again strongest in pairs with later AAO. Note that, although part of chromosome 2 was genotyped in the UK + NIA samples, this particular region was not.

The effects of mean AAO on IBD sharing on chromosomes 2 and 21 were investigated further by splitting the sample according to mean AAO and analyzing the resulting subsamples separately. The results are shown in Table III. On both chromosomes, IBD rises steadily with mean AAO, with nearly all the evidence for linkage coming from pairs with mean AAO ≥ 80.

A modest effect of AAO difference in the combined sample was observed at the peak corresponding to *APOE* on chromosome 19 (Fig. 1f), the LOD increasing from 1.44 to 2.33 (chromosome-wide $P = 0.03$), although *APOE* genotypes were not included in this analysis. This increase comes mainly from the UK + NIA sample, where the LOD increased from 1.48 to 2.11 (chromosome-wide $P = 0.07$), and may be a result of the known effect of *APOE* genotype on AAO [Olson et al., 2001, this study], although it should be noted that the NIMH sample showed little AAO effect, a LOD of 0.56 being increased only to 0.68. This is consistent with the finding of Tunstall et al. [2000], that *APOE* accounts for only 4% of variation in AAO.

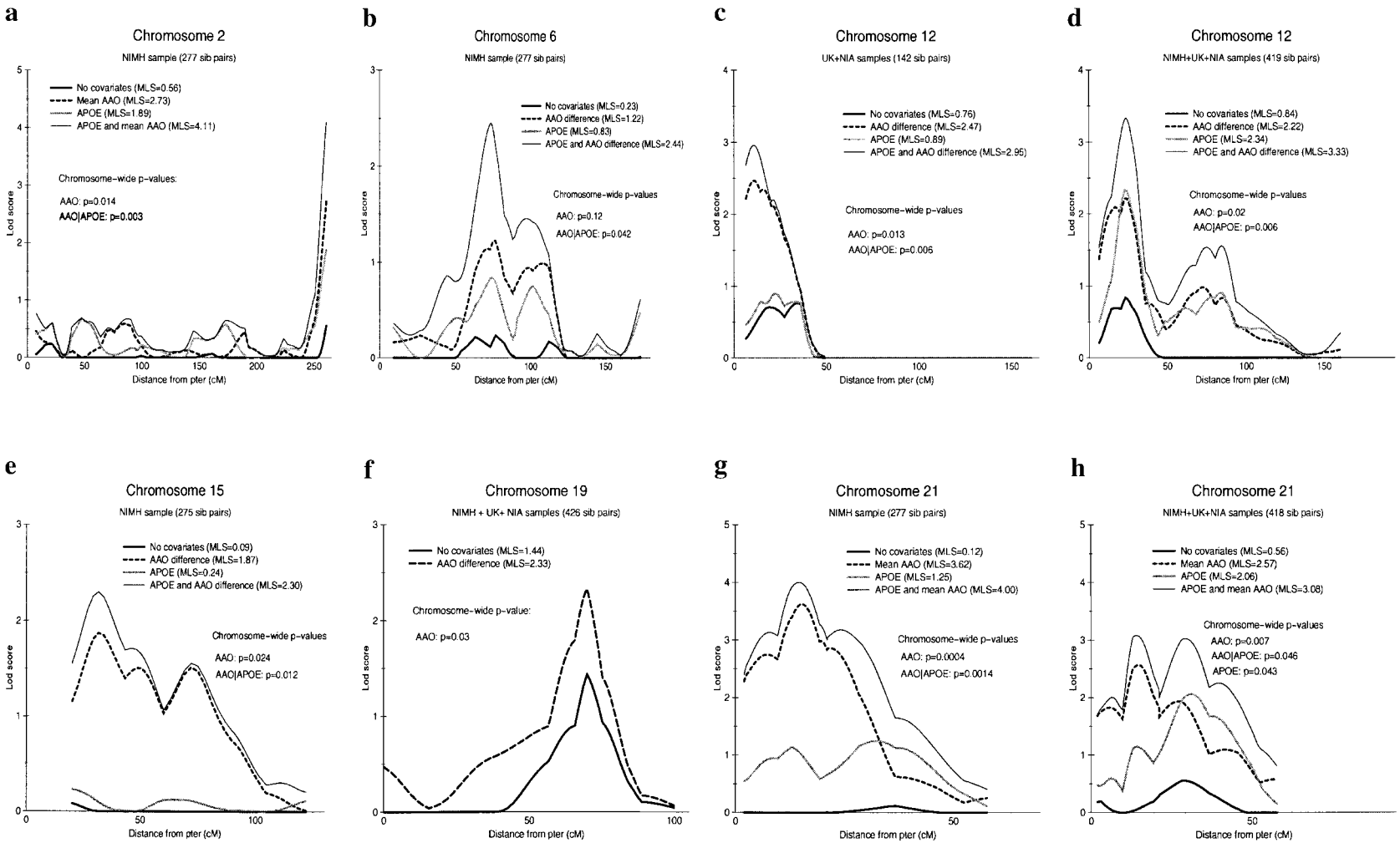

Fig. 1. Multipoint maximum LOD score graphs for chromosomes for which age at onset (AAO) had a significant effect on linkage (chromosome-wide $P < 0.05$).

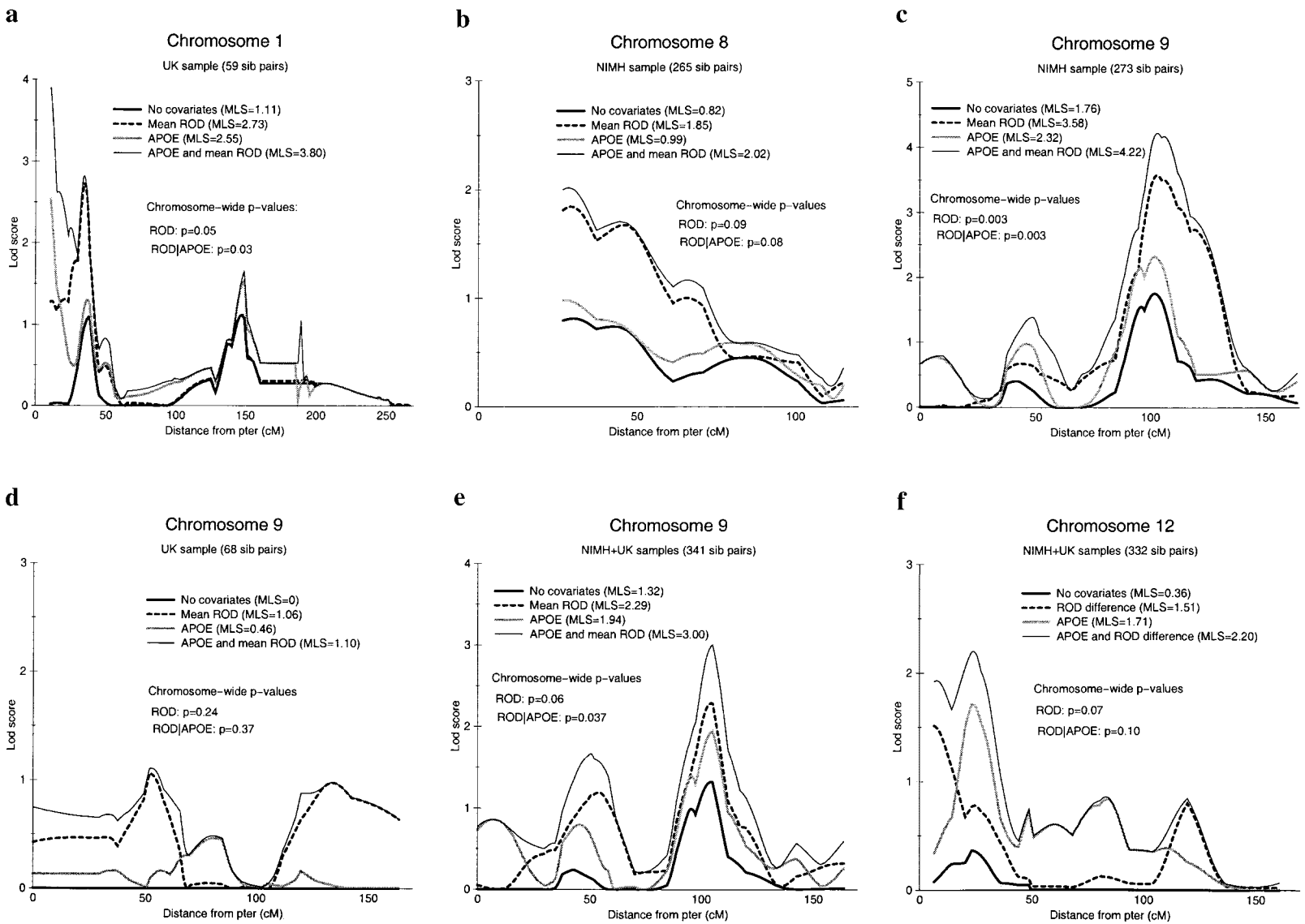

Fig. 2. Multipoint maximum LOD score graphs for chromosomes for which rate of decline (ROD) had a significant effect on linkage (chromosome-wide $P < 0.1$).

TABLE III. Variation of IBD and LOD Score With Mean AAO for Chromosomes With Significant Mean AAO Effects

AAO range	Chr21-Whole sample			Chr21-NIMH			Chr2-NIMH		
	N	IBD	LOD	*N*	IBD	LOD	*N*	IBD	LOD
65–69.9	66	0.42	0	39	0.36	0	38	0.42	0
70–74.9	154	0.51	0.02	104	0.49	0	103	0.51	0.01
75–79.9	128	0.49	0	91	0.46	0	91	0.53	0.09
80–84.9	57	0.61	0.79	35	0.73	2.33	35	0.70	1.89
85+	15	0.81	1.73	8	0.83	1.14	8	0.84	1.34

AAO difference had a statistically significant effect on linkage to chromosome 12 in the combined sample (Fig. 1d), increasing the LOD from 0.84 to 2.22 (chromosome-wide $P = 0.02$). Most of this effect appeared to come from the UK + NIA samples, the LOD increasing from 0.76 to 2.47 (chromosome-wide $P = 0.013$) about 10 cM from the telomere (Fig. 1c). Whilst AAO difference did give a small increase in LOD close to that location in the NIMH sample, the largest effect (LOD increased from 0 to 1.14) was observed at about 60 cM from the p-telomere, and was not statistically significant (chromosome-wide $P = 0.16$). Interestingly, a significant *APOE* effect was observed in the NIMH sample (chromosome-wide $P = 0.036$), due mainly to excess IBD in the ε 4−/−pairs [as noted by Kehoe et al., 1999], but this does not appear in the UK + NIA samples. In any case, the AAO effect is still significant even after allowing for *APOE* in the analysis. A small increase in LOD (from 0.36 to 1.51, chromosome-wide $P = 0.07$) was also observed on chromosome 12p with ROD difference in the combined NIMH + UK sample (Fig. 2f). This effect was greatly reduced when *APOE* was included in the analysis, suggesting that it was at least partially due to *APOE*, rather than ROD.

A significant effect of mean ROD was found on chromosome 9 in the combined NIMH + UK sample (Fig. 2e), a LOD of 1.32 at 104 cM being increased to 2.29 (chromosome-wide $P = 0.06$). This effect was more significant after allowing for APOE effects (chromosome-wide $P = 0.037$). IBD was found to be increased in pairs with high mean ROD. Most of this effect appears to come from the NIMH sample (Fig. 2c), a LOD of 1.76 at 103 cM being increased to 3.58 (chromosome-wide $P = 0.003$). This effect remained significant after allowing for *APOE* (chromosome-wide $P = 0.003$). In the UK sample (Fig. 2d), mean ROD increased the LOD from 0 to 1.06. However, this increase was neither statistically significant (chromosome-wide $P = 0.24$), nor close to the NIMH result. Interestingly, there appears to be a secondary peak with mean ROD in the region implicated by Pericak-Vance et al. [2000].

On chromosome 1, a significant (chromosome-wide $P = 0.05$) increase of IBD with increasing ROD was found in the UK sample at 35 cM (Fig. 2a), a LOD of 1.11 being increased to 2.73. However, this was based on only 59 sib-pairs, and was not observed in the NIMH sample, with the result that the effect of ROD in the combined sample is also non-significant.

AAO difference was found to have a statistically significant effect on linkage to chromosome 6, after allowing for *APOE* effects, in the NIMH sample (Fig. 1b). A LOD of 0.84 was increased to 2.45 at 74 cM (chromosome-wide $P = 0.042$). However, this was not observed in the UK + NIA sample, with the result that the effect is no longer significant in the combined sample.

Statistically significant results were also found in the NIMH sample on chromosome 8 with mean ROD (Fig. 2b) and chromosome 15 with AAO difference (Fig. 1e). However, none of the regions in question was genotyped in the NIA and UK samples.

In our analyses, we chose to code *APOE* genotype as a binary variable: "+" for genotypes containing at least one ε4 allele, and "−" for those without an ε4 allele. It is possible that the effects of AAO in individuals homozygous for ε4 may be different to those in ε4 heterozygotes. To investigate this, *APOE* genotype was coded as a three-level factor representing the number of ε4 alleles in the genotype. This gave six possible categories for the sib-pairs. The analyses involving AAO were repeated on the combined sample, recoding the *APOE* status of the sib-pairs as a six-level factor. The LOD scores for models including just *APOE* as a covariate rose from 1.28 to 3.08 on chromosome 1 (chromosome-wide $P = 0.07$) and from 1.87 to 3.34 on chromosome 9 (chromosome-wide $P = 0.15$), with IBD increased in pairs where both members were ε 4-homozygous. However, there were only 32 such pairs in the combined sample. On no chromosome was the increase in LOD given by AAO allowing for *APOE* effects significantly altered by the recoding of APOE.

DISCUSSION

We have presented an ASP linkage analysis with covariates on the combined NIMH, NIA/IADRC, and UK samples (453 sib-pairs total). The NIMH sample was previously analyzed by Olson et al. [2001, 2002] using a similar analysis method. However, unlike Olson et al., we also investigated the effects of AAO *difference*. This could be important if there are two or more variants of the gene, which influence AAO in different ways, rather than one variant, which acts early or late, but not both. It should be noted that the "NIMH sample" we analyzed was slightly different to that used by Olson et al. They used individuals with AAO ≥60, but stipulated that current age be available, whereas we used individuals with AAO ≥65 without requiring current age. Furthermore, they only used the genotypes from the 15 cM Stage I marker grid [Kehoe et al., 1999], whereas the results presented here also incorporate the genotypes from the 5 cM Stage II grid [Myers et al., 2002]. The NIMH and NIA/IADRC samples formed part of the sample used by Li et al. [2002]. They used a variance-components analysis of AAO as a quantitative trait, including unaffected family members in the analysis (using age at entry). Such an analysis assumes that all individuals would become affected with AD if they lived long enough, and is therefore different to our analysis.

This study, therefore, presents novel analyses, despite some of the sample having been analyzed previously. Firstly, the UK sample has not been previously analyzed. Secondly, ROD has not been previously studied in a linkage analysis. Thirdly, age-at-onset *difference* has not been used as a covariate in analyses of these data. Fourthly, this study is the first to attempt to replicate the significant AAO effects found by Olson et al. [2001] using the same analysis methods.

The effect of increasing mean AAO that increases IBD sharing on chromosome 21 in the NIMH sample, noticed by Olson et al. [2001], is not evident in the NIA (IADRC) and UK

samples, despite these samples having similar AAO distributions to the NIMH sample. However, the NIA and UK samples did show modest evidence of linkage (MLS = 1.03) nearby. This suggests some role for APP, or a gene in its vicinity, in the genetic susceptibility of late onset AD, although its role in influencing AAO is unclear.

A statistically significant effect of AAO difference was found in the combined sample on chromosome 19, close to the *APOE* locus. The effect was small, consistent with findings [Tunstall et al., 2000] that *APOE* accounts for only a small proportion of variation in AAO.

Significant (chromosome-wide $P < 0.05$) effects of covariates on linkage in the combined samples were also found on chromosome 9q (mean ROD) and 12p (AAO difference). As on chromosome 21, the covariate is significant in only one subsample (NIMH on 9q, IADRC + UK on 12p), and it is less significant in the combined sample than in the subsample alone. However, both of these chromosomes are potentially of interest, as each region has been implicated in multiple linkage studies [Rogaeva et al., 1998; Kehoe et al., 1999; Pericak-Vance et al., 2000; Farrer et al., 2002; Mayeux et al., 2002; Myers et al., 2002].

We have also found interesting results in the NIMH sample on chromosomes 1, 2, 8, and 15 in regions, which were not genotyped in the NIA or UK samples. It would be interesting to genotype these regions to see if the results replicate.

Although the results presented here each have chromosome-wide *P*-values of less than 0.05, it should be pointed out that four quantitative measures (mean and difference in AAO, mean and difference in ROD) were each assessed in 22 chromosomes of varying size. Hence, the potential for multiple testing is considerable, and the results presented here need to be replicated in independent studies.

Our method of ASP analysis with covariates is similar to that proposed by Olson [1999] and used by Olson et al. [2001, 2002]. The methods impose different constraints on the IBD sharing probabilities. It is likely that the relative power of the methods depends on the (unknown) true disease model. As expected, the results of our study looking at mean AAO in the NIMH sample are similar to those of Olson et al. [2002]. Both analyses show a large effect on chromosome 21. Our analysis shows an effect close to the qter on chromosome 2, which the Olson et al. analysis does not. Conversely, the Olson et al. analysis reports a LOD increase of 1.89 on chr14, whereas our analysis gives a LOD increase of 1 in the same region.

The other major linkage study of AAO in AD is that of Li et al. [2002]. Their sample included the NIMH and NIA/IADRC samples, together with a number of other samples collected in the US. As mentioned earlier, they treated AAO as a quantitative trait. Their main finding was a LOD of 2.4 on chromosome 10 at D10S1237, 139 cM from the pter. Our analysis of chromosome 10 is shown in Figure 3. It can be seen that we find no significant increase in LOD with either mean or difference in age-at-onset in the region implicated by Li et al. It should be noted that our analysis shows a highly significant LOD score peak for linkage of AD (without the effects of age) to chromosome 10. Since the Li et al. analysis treats IBD between pairs of relatives as the independent variable and AAO as the dependent variable, it is unable to test for the overall IBD proportion in the affected individuals differing from 0.5 (i.e., linkage to AD). Li et al. also found LODs >2 on chromosomes 4 (208 cM from pter), and 8 (150 cM from pter) and LODs >1 on chromosomes 6 (51 cM from pter), 13 (111 cM from pter), and 18 (54 cM from pter). Their chromosome 6 result is in the region in which AAO difference gave a significant result in the NIMH sample in our analyses (Fig. 1b). In our analysis, a LOD of 0.23

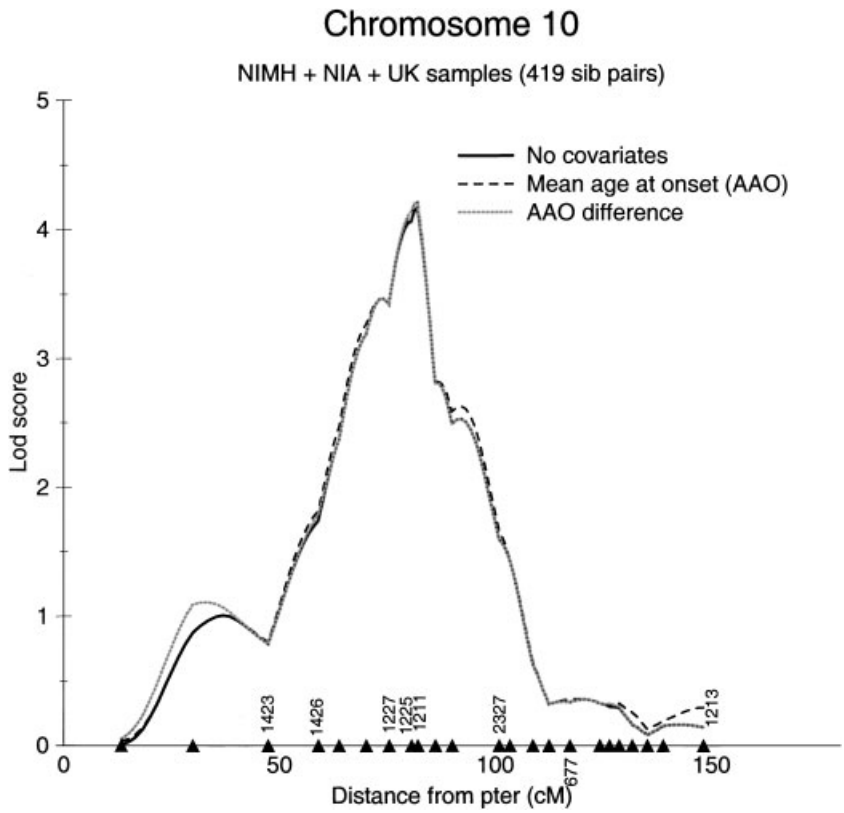

Fig. 3. Multipoint maximum LOD score graph showing the effect of age at onset (AAO) on linkage of AD to chromosome 10.

was increased to 1.22 by adding AAO difference as a covariate, and 2.44 when *APOE* was included. However, in the whole (NIMH + NIA + UK) sample, adding AAO difference merely increased the LOD from 0.82 to 1.24. In the Li et al. chromosome 4 region, we found a modest increase in LOD from 0 to 0.51 with mean AAO (NIMH sample only), on chromosome 8 we observed only a tiny LOD increase, on chromosome 13 we observed a modest increase in LOD from 0 to 0.7 with AAO difference (NIMH sample), and on chromosome 18 we observed an increase in LOD from 0 to 1.18 with AAO difference at 70 cM from the pter (NIMH sample). Thus, there are some similarities between our results and those of Li et al., and the differences may be explained by the different samples and analysis methods.

It is possible that the linkage results of Olson et al. [2002] with high current age may indicate a gene giving rise to a combination of late AAO and slow rate of disease decline, but this is far from clear. Rather than using current age, which is not fixed, but varies with time, we sought to model disease development by using a measure of ROD. Our ROD measure is far from perfect. In particular, it is based on only one assessment of deterioration and two time points. Ideally, such a measure would be based on multiple measures of deterioration spanning the full- time course of the disease, particularly as disease decline in late onset AD may be non-linear. Such data are unavailable for the subjects in the present study but might profitably be collected in future studies. It should be noted that our measure of ROD depends on knowing AAO. Since AAO is assessed retrospectively, based on information given by relatives, it is prone to measurement error. This will affect all studies using AAO.

Finally, it is worth making the point that identification of loci modifying AAO or ROD could potentially allow the discovery of novel treatment targets. However, any linkage finding with complex phenotype variables (such as ROD) will need to be followed-up with linkage disequilibrium studies using samples in which the variable is well characterized, and preferably measured over the full length of disease duration. There is already evidence that genes may contribute to aspects of disease development in addition to disease risk. It is therefore vital that the research field prioritizes the collection of powerful sample sets, suitable for complex genetic association analyses, in which the phenotypic variables are measured longitudinally.

To conclude, the effect of increasing mean AAO that increases IBD sharing on chromosome 21 found by Olson et al. in the NIMH sample did not replicate in the NIA and UK samples, although the latter two samples did show some modest evidence of linkage in the region. Likewise, we did not find any effect of AAO on IBD sharing on chromosome 10, despite finding strong evidence for linkage to late-onset AD itself. We did find suggestive evidence that similarity in AAO influences linkage on chromosome 12p, and that increasing ROD increases linkage on chromosome 9q. These are novel results, although these two regions have previously been implicated in numerous linkage studies of AD.

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