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APOE and Other Loci Affect Age-At-Onset in Alzheimer's Disease Families With PS2 Mutation

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Several kindreds of Volga German (VG) ancestry have a single PS2 mutation that causes an autosomal dominant form of Alzheimer's disease (AD). These families show a wide range in age-at-onset, which suggests the existence of modifying factors other than the PS2 mutation. To examine evidence for a genetic basis of variation in onset age, we performed a Bayesian oligogenic segregation and linkage analysis on nine VG families confirmed to have at least one affected PS2 carrier. This analysis simultaneously estimated the effects of APOE and PS2 and the number and effects of additional loci affecting AD age-at-onset. In addition, a family effect accounted for shared environmental effects. This analysis approach has the advantage of full use of the complete pedigree structure, as well as use of information on unsampled individuals with phenotypic data. These analyses provide evidence that APOE plays a small, but significant, role in modifying the age-at-onset in these VG families. The effects estimated for the APOE ε3 and ε4 genotypes were consistent with those estimated in previous analysis of late-onset AD families, with evidence for a dose-dependent relationship between number of ε4 alleles and age-at-onset. We estimated an ∼83% posterior probability of at least one modifier locus in addition to APOE, and that the fraction of the variance in age-at-onset attributable to PS2, APOE, other loci, and family effects is ∼70, ∼2, ∼6.5, and ∼8.5%, respectively. These results provide evidence that APOE and other loci modify onset in AD caused by PS2 mutation.
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KEY WORDS: modifier gene; Markov chain Monte-Carlo; segregation analysis; complex trait; oligogenic

INTRODUCTION

Alzheimer disease (AD (MIM 104300)) is the most prevalent cause of dementia. The pathophysiologic mechanisms of AD are not fully understood, and the genetic basis of AD is complex: four genes have been conclusively found to affect liability. Three of these—the amyloid precursor protein (APP) and the two presenilins (PS1 and PS2)—have been linked to rare autosomal dominant forms of AD with a typically early age-at-onset [Goate et al., 1991; Schellenberg et al., 1992; Rogaev et al., 1995; Sherrington et al., 1995; Levy-Lahad et al., 1995b,d]. The only confirmed locus contributing to risk for late-onset AD (LOAD) is APOE [Corder et al., 1993; Saunders et al., 1993]. However, the proportion of variance in age-at-onset attributable to APOE is estimated to be only ∼10–20% [Bennett et al., 1995; Slooter et al., 1998], leaving ample room for the involvement of additional loci. While mutations in APP, PS1 and PS2 can be considered to be causative, the APOE locus is more properly modeled as a risk modifier [Corder et al., 1998; Rocchi et al., 2003]: the ε4 allele is associated with reduced age-at-onset in a dose-dependent manner [Corder et al., 1993, 1998; Daw et al., 2000], and the ε2 allele is associated with increased age-at-onset [Corder et al., 1994, 1998; Farrer et al., 1997; Daw et al., 2000], relative to the ε3 allele.

A wide range of age-at-onset is associated with mutations in APP and the presenilins. The many different identified mutations [Campion et al., 1999], which may have different effects on age-at-onset, could explain this. However, there are a few samples consisting of very large families or samples with multiple families bearing the same mutation [Bird et al., 1988; Levy-Lahad et al., 1995b; Lendon et al., 1997], in which there

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also is considerable variability in age-at-onset. For example, AD in the Volga German (VG) families [Bird et al., 1988] is caused by a single PS2 mutation (Asn141Ile) that occurred once in the history of this population [Levy-Lahad et al., 1995b,d]. Age-at-onset among individuals who carry this mutation ranges from 40 to 75 years, with one inferred gene carrier who died at 89 without AD [Bird et al., 1996]. This argues for the existence of additional modifying factors beyond the mutated gene that, if identified, could further the understanding of the mechanisms that underlie AD.

The variability in age-at-onset has lead to a number of attempts to determine whether APOE could also be a risk modifier in the presence of early-onset AD mutations. Weak evidence suggesting that APOE influences age-at-onset of APP mutations in one study [Sorbi et al., 1995], was not confirmed in another [Haan et al., 1994]. Early analyses of interaction with PS1 mutations failed to identify an APOE effect [Van Broeckhoven et al., 1994; Brice et al., 1996; Lendon et al., 1997]. Similarly, a simple analysis of the VG families with a PS2 mutation did not find a detectable effect of APOE on age of AD onset [Levy-Lahad et al., 1995c], although a different analysis based on survival analysis methods gave suggestive evidence that having at least one ε4 allele is a risk factor relative to the ε3/ε3 genotype [Bird et al., 1996]. These studies can all be criticized: some involved a mixture of mutations, which could obscure small APOE effects [Van Broeckhoven et al., 1994; Brice et al., 1996]; stratification by mutation typically results in small numbers of cases, for which power to detect an effect is inherently low; and all use simple statistical methods that are not well-suited to the pedigree-based data sets to which the methods are applied. One exception to two of these three criticisms is a recent study [Pastor et al., 2003] that used a survival analysis approach to provide evidence that age-at-onset in a single, large PS1-mutation pedigree may be influenced by APOE genotype. This study still does not fully use the available data, since the method of analysis used does not account for genetic correlations in the pedigree: use of related individuals in this context could falsely introduce an apparent genotype-specific effect, since individuals with similar APOE genotypes will often be more closely related than other individuals, and thus would share other genetic risk factors for AD. However, this study is based on a larger sample than most other previous studies, and, if confirmed, would provide the first evidence of an interaction between APOE and an early onset AD gene.

Investigation of the role of APOE as a modifier of early-onset AD requires use of both a suitable sample, and use of analysis methods that fully incorporate and exploit the available pedigree data. An ideal sample is large, since based on the available information, APOE effects are likely to be small. Also, such a sample should consist of a single early-onset gene mutation, so that variation among mutations is not a confounding factor. Such a sample is available in the VG families with a PS2 mutation [Bird et al., 1988], as well as in the Columbian families with a PS1 mutation described elsewhere [Lopera et al., 1997]. Analysis methods ideally should be based on four conditions: (1) use of a genetic model that incorporates multiple contributing loci segregating in the pedigrees; (2) a method that is based on survival analysis concepts, since the pedigrees contain both affected and unaffected individuals; (3) ability to incorporate known or suspected covariates; and (4) a method that can be applied to extended pedigrees in a way that maximizes use of the available data. While these demands preclude the use of exact computational methods, recent developments based on Bayesian Markov chain Monte-Carlo (MCMC) methods [Heath, 1997; Daw et al., 1999] coupled with increasing computer speeds now make such complex analyses feasible. Thus, an analysis of datasets such as the VG families, with this powerful and sophisticated approach, is warranted.

Here, we investigate evidence for the existence of genetic modifiers of AD age-at-onset in the VG families. In order to do this, we estimate the effects of PS2, APOE, additional unlocalized quantitative trait loci (QTLs), and the residual family effect on age-at-onset. We also provide an estimate of the number of unlocalized QTLs. All factors are estimated simultaneously and take into account the full pedigree structure. With this novel MCMC analysis approach, we find evidence for the existence of additional loci that affect age-at-onset in the VG families, one of which is APOE.

DATA AND METHODS

Sample and Markers

Nine familial AD pedigrees of VG descent [Bird et al., 1988] were evaluated by the University of Washington Alzheimer's Disease Research center. Each of these pedigrees contained at least one affected individual with the same PS2 Asn141Ile mutation [Levy-Lahad et al., 1995b,d]. Family sizes ranged from 3 to 73 individuals, with a total of 250 individuals. PS2 and APOE genotypes were available for 125 and 124 individuals, respectively, and marker data was available on 119 individuals for flanking markers. Phenotypic information was available for 197 individuals: 123 had a censoring age at which they were last known to be free of AD (unaffected) and 74 had a recorded age of AD onset (affected). Individuals missing affectation status or either censoring or AD onset age were considered to be missing phenotype data for this analysis. In addition, for some analyses, trait phenotypic data were excluded for all individuals who were homozygous for the normal PS2 allele, since such individuals provide no information for detecting modifier loci. Living affected individuals met published criteria for the clinical diagnosis of AD [McKhann et al., 1984]. For deceased individuals without an autopsy, the diagnosis of probable AD was established on the basis of detailed medical records. The mean age-at-onset ranged from 40 to 62 years for the different families used here. The range of onsets among all individuals was 39–85 years (41–75 years among the PS2 sampled carriers), and 8 of the 9 families had at least one autopsy-confirmed member with AD, with a total of 26 autopsy-confirmed cases. Neuropathologic confirmation of diagnosis met published criteria [Mirra et al., 1991; The Ronald and Nancy Reagan Research Institute of the Alzheimer's Association and the National Institute on Aging Working Group, 1998]. The University of Washington institutional review board approved the study, and informed consent was obtained from all participants.

The loci used here were of two types: genes involved in AD and microsatellite markers (STRPs) with no known AD effect. The PS2 gene on chromosome 1, which is the gene responsible for AD in these VG families, was used as a marker in all analyses, as was APOE on chromosome 19, which is a contributor to risk of late onset AD [Corder et al., 1993; Saunders et al., 1993]. In addition, in order to improve imputation of genotypes at the PS2 and APOE loci for individuals without DNA samples, markers in the ~40 cM region on each side of PS2 and APOE were included in the analysis. These markers are standard 10 cM genome scan markers, and thus are not affected by problems with failure of the MCMC process to sample the genotype space properly, as can happen with the very closely linked markers used initially to map and identify the PS2 gene [Levy-Lahad et al., 1995b,d]. All analyses involved a simultaneous multipoint analysis of both chromosomes 1 and 19 (17 markers, total surrounding PS2 and APOE). This maximizes informativeness of the pedigrees, as well as efficiency of parameter estimation, since all critical known components of the model can be included in a single analysis run. Genotypes for PS2, APOE, and STRPs were

obtained as previously described [Hixson and Vernier, 1990; Levy-Lahad et al., 1995d].

Statistical Methods

A Bayesian MCMC approach [Heath, 1997; Daw et al., 1999] was used for analysis. This approach models age-at-onset as a censored quantitative trait in an oligogenic QTL combined segregation and linkage analysis [Daw et al., 1999]. Onset distributions for each multilocus trait genotype, after adjusting for covariates, are assumed to be normally distributed with a common residual variance and different genotype-specific means. An additive model for covariate and genotypic effects is assumed, so the age-at-onset model is

$$y = \mu + X\beta + \sum_{i=1}^{k} Q_i \alpha_i + e,$$

where μ is the baseline age-at-onset, X is the incidence matrix for covariate effects, β is the vector of covariate effects, Q_i is the incidence matrix for the effects of diallelic QTL i, α_i is the vector of effects for QTL i, e is the normally distributed residual effect, and k is the number of QTLs in the model during a particular iteration of the analysis. The MCMC process used here is based on two types of sampling: (1) over trait models, which allows the number of QTLs, k, to be a random variable, and (2) of unobserved marker and multilocus QTL genotypes, which provides a computationally tractable approach for analysis of oligogenic models on large pedigrees. After a sufficiently large number of sampling iterations, the results provide estimates of posterior probability distributions of parameters of interest. Loki, version 2.4, was used for analysis (http://www.stat.washington.edu/thompson/Genepi/pangaea.shtml). In the analyses presented here, the sampler was run for 500,000 iterations, with every 5th iteration retained for estimating the posterior distribution. Multiple runs were performed to check for consistency across analysis runs, which gave similar results. Results presented here are representative of several runs.

Statistical Analyses

PS2 and APOE were included as major gene covariates. PS2 was included as such in some analyses, while APOE was included as a covariate in all analyses. PS2 was not included as a covariate in one analysis to estimate the contribution of PS2 to the total variance in the context of a linkage analysis, in order to compare results to those obtained when PS2 was also treated as a genetic covariate. When a locus was treated as a major gene covariate, unobserved genotypes were imputed by the MCMC sampler in the same fashion as for any other locus, and the resulting imputed complete-data genotypes were then used to estimate genotype-specific effects described by the model above. This approach has two advantages over other approaches: it maximizes the efficiency of the use of the data by using all individuals for whom relevant age data are available, and it takes into account correlations among individuals induced by shared inheritance patterns at all loci that affect age-at-onset. This approach contrasts with attempts to ask similar questions [Pastor et al., 2003] that use analysis methods based on the assumption of unrelated individuals, but are applied to pedigree-based data, and may thus overestimate the effective sample size and consequently the significance of the effects. In our analysis, since ~80% of the individuals had phenotype data while only ~50% had DNA samples and genotypes available, this pedigree-based approach had increased power to detect effects of genetic covariates, compared to earlier, simpler, attempts to carry out such analyses on a small sample of unrelated individuals from the same pedigrees [Levy-Lahad et al., 1995a].

Three different analysis runs were carried out. All included a family-effect covariate, plus APOE as a major gene covariate. The family effect, estimated as a family-specific deviation from the overall baseline mean, was included to account for possible family-specific environmental effects, which may explain a portion of the large variability among families in age-at-onset, despite the shared PS2 mutation in these families [Bird et al., 1996]. In Run 1, PS2 was included only as a marker, but not as a major gene covariate, as described above. Runs 2 and 3 both included PS2 as a major gene covariate in order to explicitly estimate, and thus adjust for, PS2 genotype effects. Run 2 was carried out without, and Run 3 with, exclusion of trait phenotypic data in individuals who were homozygous for the normal allele at PS2. Such individuals provide no information for determining whether there is evidence for the existence of modifier genes for age-at-onset in the presence of the PS2 mutation. In this context the MCMC process cannot estimate the PS2 heterozygous effect as a major gene covariate because of the absence of normal homozygotes, so the results from Run 3 cannot be used to estimate the relative contributions of other components of the model to the variance. Thus analyses were also performed while including such individuals (Run 2). In all analyses, however, the genotypes of all other marker loci for the individuals with the normal homozygous PS2 genotype were retained, so that imputation of genotypes in missing individuals would be as accurate as possible.

Prior distributions necessary for the Bayesian MCMC analyses were specified as follows. Allele frequency distributions for marker loci were estimated from the marker data. The prior distribution on the number of QTLs in the model was Poisson with mean 1; the allele frequency distribution for newly proposed QTLs was uniform on 0–1; and the distribution of the homozygote and heterozygote effects for newly proposed QTLs was Normal with mean 0 and variance, τ_β, $\tau_\beta = 300$ and with this choice of τ_β determined by finding the value that maximized the number of QTLs in the model based on segregation analysis without linked markers [Wijsman and Yu, 2004]. Results were also checked against results obtained with $\tau_\beta = 50$, which may be more suitable for QTLs with small effects. Except where noted, results are presented for the analysis with $\tau_\beta = 300$, since this value was optimal for modeling the PS2 effect. Evaluation of the MCMC process as a final check on the prior distributions was carried out to ensure proper sampling of the underlying missing data values (mixing), as described elsewhere [Daw et al., 1999; Wijsman and Yu, 2004]. Choice of prior distributions to use in the analysis with markers was guided, in part, by evidence that the MCMC process was mixing well.

Parameter summaries were obtained by taking expectations over all iterations in a run. Comparison of the relative position of genotype effects was based on estimated effects within iterations. For parameters based on ratios, the expectation was over the ratio within iterations, because of the inequality between the ratio of the expectations and the expectation of a ratio. The intensity ratio (IR) was used as a measure of the strength of linkage evidence [Wijsman and Yu, 2004] and was estimated from the ratio of the observed to expected rate of acceptance of models with QTL locations, computing the expectation from the total number of QTLs in the model under the assumption of uniformity of location, over 2 cM intervals.

RESULTS

PS2

As expected, the PS2 locus was detected at 247 cM on chromosome 1. The signal was strong, with a maximum IR = 329 (log IR = 2.5) (Run 1, Fig. 1). This location is consistent with previous linkage analyses based on traditional [Levy-Lahad

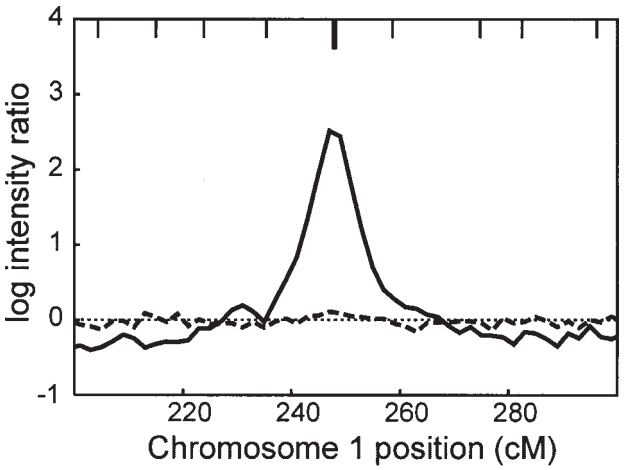

Fig. 1. Linkage analysis of chromosome 1. Log_{10} of intensity ratio for Run 1 (solid line), PS2 not included as a covariate; and Run 2 (dashed line), PS2 included as a covariate. The locations of the 8 markers used in linkage analysis (thin, short tic marks) and PS2 (thick, longer tic mark) are indicated on the top horizontal axis. Markers used were: D1S238, D1S413, D1S249, D1S425, D1S2800, D1S2785, D1S2842, and D1S2836. Map distances were obtained from http://research.marshfieldclinic.org/genetics and converted to a Haldane map.

et al., 1995b,d] and MCMC methods with these pedigrees [Daw et al., 1999]. Once the PS2 effect was accounted for as a major-gene covariate (Run 2), no evidence for linkage ($IR \cong 1$, $\log IR \cong 0$) was obtained for this region (Fig. 1), demonstrating that adjustment for PS2 completely accounts for the chromosome 1 linkage signal.

When PS2 was treated as a major gene covariate (Run 2), the estimated contribution to the variance for PS2 exceeded that obtained in Run 1, which was based only on linkage analysis (Table I). For Run 1, much of the variance in age-at-onset was attributed to a QTL that maps to the location of PS2 and can therefore be attributed to PS2 (Run 1, QTL1 in Table I). This large QTL explained 60.6% of the total variance and 90.9% of the genetic variance in age-at-onset. For Run 2, the estimated contribution of PS2 was 71.1% of the total and 89.6% of the genetic variance. As described under "Data and Methods," for Run 3, which excluded PS2 normal homozygotes, the PS2 contribution cannot be estimated.

APOE

APOE appears to contribute to the genetic variance in age-at-onset of AD in these families. The estimated effect is small, but the 95% CI for APOE does not include 0 (Table I). The estimated contribution of APOE to total variance was 1.4–1.7% under the different analysis conditions. After subtracting off the variance attributable to PS2, the contribution of APOE is 48.7 and 37.0% of the residual genetic variance for Runs 1 and 2, respectively. For Run 3, which excludes the age data of the PS2 normal homozygotes, the variance contributed by APOE was identical to that estimated in Run 2, but because the PS2 effect cannot be estimated, the contribution of APOE to the total variance cannot sensibly be estimated.

The effects estimated for most of the APOE genotypes were similar to those seen in our previous analysis of LOAD families [Daw et al., 2000] (Table II and Fig. 2). The sample size was too small to reliably estimate effects for ε2/ε2 and ε2/ε4, so these genotypes were excluded. Carriers of the ε4 allele had decreased ages-of-onset, compared to individuals with the ε3/ε3 genotype: the posterior probabilities that the ε3/ε3 age-at-onset effect exceeded that for the ε3/ε4 and ε4/ε4 genotypes was 83.42 and 94.5%, respectively. Heterozygotes for ε4 had an age-at-onset that was generally midway between the ε4/ε4 and ε3/ε3, with a posterior probability that the ε3/ε4 effects exceeded the ε4/ε4 effect of 88.8%. The posterior probability that the ε2/ε3 effect was below that of ε3/ε3 was 85.78%, but the overlap in the two posterior distributions was large (Fig. 2). The one discrepancy with previous results was that the posterior distribution estimated here for the ε2/ε3 genotype is lower than in a previous analysis of LOAD [Daw et al., 2000], but may simply reflect the small sample size of the genotype here. In general the estimated genotype effects were insensitive to choice of parameter values of the prior distribution specified in the analysis. However, the difference in age-at-onset for the ε3- and ε4-homozygous genotypes was lower (7.1 years) for $\tau_\beta = 50$ than for $\tau_\beta = 300$ (11.5 years).

Additional QTLs and Environmental Effects

There is evidence for the existence of at least one other trait locus in addition to PS2 and APOE in the VG families (Fig. 3,

TABLE I. Square Root Variance and Percent Variance Contributions

	Run				
	1		2		3
Effects modeled	APOE, QTL, family		PS2, APOE, QTL, family		APOE, QTL, family[a]
Source of variance	Square root variance (SD)	Percent of total variance	Square root variance (SD)	Percent of total variance	Square root variance (SD)
Residual (V_e)	6.9 (1.75)	7.5	7.7 (1.8)	11.8	6.5 (1.8)
Family	13.8 (6.5)	25.8	6.5 (2.4)	8.6	6.9 (1.8)
PS2	—	—	19.3 (1.4)	71.1	–
APOE	3.0 (1.1)	1.4	2.8 (1.0)	1.7	2.8 (1.1)
QTL1[b]	21.0 (2.6)	60.6	4.6 (2.2)	5.5	3.2 (2.1)
QTL2	4.4 (3.0)	4.1	1.4 (1.7)	1.1	0.8 (1.4)
QTL3	0.9 (1.6)	0.4	0.3 (0.9)	0.2	0.2 (0.6)
Total Genetic[c]	22.1 (3.0)	66.7	20.4 (1.6)	79.6	5.1 (2.2)

[a]Phenotypes of PS2 normal homozygotes were excluded from analysis, thus precluding estimation of contribution to variance.
[b]QTLs are listed in rank order of contribution to the variance.
[c]Variance contribution of PS2, APOE, plus all QTLs. Sum of individual percentage contributions does not give the total genetic variance–see "Methods" for details.

TABLE II. Estimated Shift in Mean (SD) Age-at-Onset Relative to Baseline Mean for APOE Genotypes, in an Analysis Model That Included PS2, APOE and Family Effect as Covariates

	APOE genotype			
Run	ε3/ε3 (n = 76)[a]	ε3/ε4 (n = 38)	ε4/ε4 (n = 3)	ε2/ε3 (n = 6)
2	2.13 (2.1)	−2.45 (2.92)	−9.34 (6.14)	−3.90 (4.63)
3	3.27 (2.26)	−4.99 (3.08)	−0.63 (6.50)	−5.01 (3.83)

[a]n, number of sampled individuals with observed genotype.

Table I). For Run 2, the posterior probability for at least one additional locus in addition to PS2 and APOE was estimated to be 82.8%. The largest of the additional QTLs contributes 5.5% of the total variance in age-at-onset, or 64.3% of the residual genetic variance, after accounting for PS2, and accounts for more of the genetic variance than does APOE. In Run 2 there is also evidence for an additional, smaller, QTL contributing 1.4% to the total variance, although the existence and contribution of such additional QTLs is less secure since it is more sensitive to analysis conditions. These results provide evidence that at least one additional unlocalized QTL in addition to APOE plays a role in onset of AD in the VG families.

While much of the variance appears to be due to genetic factors, environment factors also appear to play a role. The estimated residual variance was fairly stable across the different runs, with the square root of the variance ranging from 6.5 to 7.5, and variance contributions accounting for 7.5 and 11.8% of the total variance in Runs 1 and 2, respectively. Family effects were less stable to the analysis model, and explained 25.8 and 8.6% of the variance for Runs 1 and 2, respectively.

DISCUSSION

We show here that there is evidence for genetic modifiers of age-at-onset of autosomal dominant AD in the VG families. Although the major determinant of disease is a single PS2 mutation, one of these loci appears to be APOE with evidence for at least one additional unlocalized QTL. The effects estimated for APOE are similar to our previous estimates in late onset families [Daw et al., 2000], and the estimated contribution to the variance of the additional QTLs is similar to or somewhat larger than that of APOE. Furthermore, our estimates of family-specific effects and residual variance indicate that environmental factors may play an appreciable role in age-at-onset of early-onset AD.

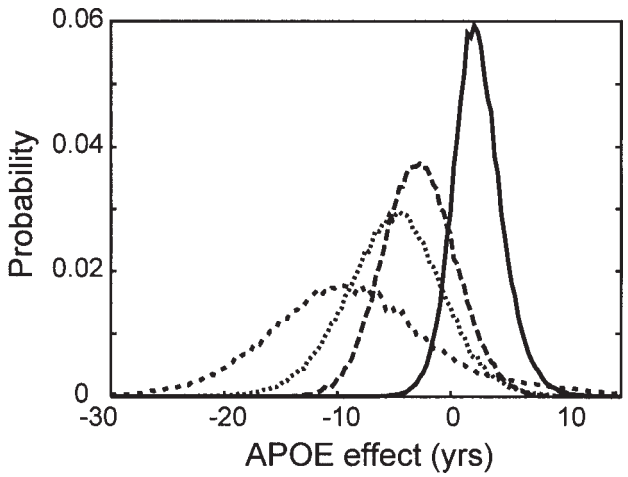

Fig. 2. Posterior distributions for APOE genotype effects relative to the baseline mean from Run 2. Results for ε4/ε4 (short dashed line),?ε2/ε3 (dotted line), ε3/ε4 (long dashed line) and ε3/ε3 (solid line) and are shown. Other genotypes were too rare to use for estimating effects.

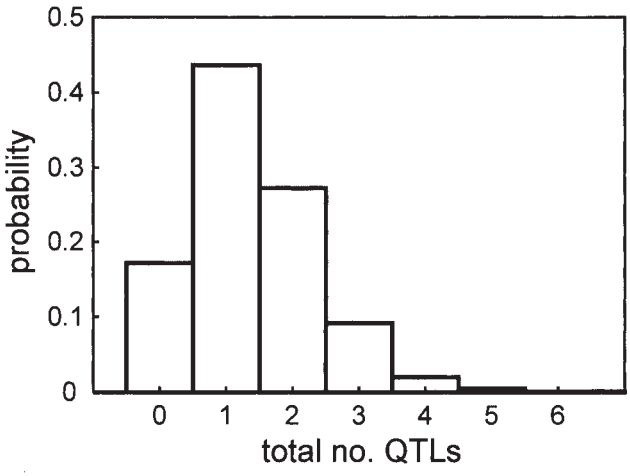

Fig. 3. Posterior distribution for the number of unmapped QTLs affecting age-at-onset for Run 2.

Our results suggest that the APOE ε4 effect in the VG families is similar to its effect in LOAD families [Daw et al., 2000]. These results are consistent with those reported recently for another sample with a single PS1 mutation [Pastor et al., 2003], which used standard survival analysis methods based on unrelated individuals, to present evidence that genotypes containing an ε4 allele had earlier age-at-onset than genotypes without an ε4 allele. In particular, as for LOAD, we see a dose effect for the ε4 allele relative to the ε3 allele, with a decline in onset of 5–6 years per ε4 allele. However, the results here are somewhat more sensitive than the previous analysis to details of the analysis in two ways. First, the sample size here is smaller than that of the LOAD sample studied earlier. Because of this, the uncertainty in the parameter estimates is higher, and there is insufficient data to make conclusions about the relative dose effects of the ε4 and ε2 alleles. Second, the current analysis required adjustment for the PS2 effect. This major gene effect is large compared to effects of additional modifier QTLs. This makes choice of the prior distribution of gene effects more difficult than in analysis of LOAD with its smaller and more uniform QTL effects. This, in turn makes it more difficult to be certain of the precise relationship among APOE genotypes since choice of the prior distribution has some effect on the estimates.

An intriguing result of this oligogenic segregation and linkage analysis is the suggestion that a locus in addition to APOE affects age-at-onset. This locus may be possible to localize in the current families once a genome screen is completed, and may be one of the loci suggested by an earlier analysis of LOAD [Daw et al., 2000]. Differences between the results in the two analyses may be explained by differences regarding whether or not a family effect was included as a covariate, or the effect of ascertaining through multiplex affected pedigrees in the presence versus absence of a PS2 mutation. The results here also suggest that simultaneous estimation of covariate effects in the context of use of the full pedigree data may improve estimation of all effects: we found it possible to estimate the effects of APOE genotypes here in an analysis of the full dataset, while in a previous analysis that did not use

the data fully, there was no evidence of a significant APOE contribution [Levy-Lahad et al., 1995a]. These better estimates of model parameters should improve our ability to detect and localize additional trait loci.

The estimation of a relatively high environmental component to the variance raises issues. While there clearly is a major AD gene segregating in these VG pedigrees, there was also evidence for non-genetic influences on age-at-onset. Some of this environmental variance may be due in part to measurement error of age-at-onset, but the commonly-accepted estimate of 1–2 years for possible measurement error would not account for all of the residual variance estimated here. Thus real environmental differences may also contribute. For example, Pastor et al. [2003], studying a large PS1 mutation kindred, found that urban living and high education lower the onset age when compared to low education and rural living, respectively. These results are also consistent with an environmental contribution to disease onset. The estimate of ~20% of the variance in age-at-onset explained by non-genetic factors, in the form of family effects and residual variance, suggests that even in these families segregating a major gene form of AD, it may eventually be possible to intervene to delay the age-at-onset.

The results here, coupled with recent results for PS1 [Pastor et al., 2003], show that APOE affects the age-at-onset of monogenic forms of AD caused by PS2 and PS1 mutations. These findings have important implications for understanding the mechanisms causing AD. Recent work demonstrated that presenilins are part of a multiprotein complex that acts as a γ-secretase, a complex that performs one of the proteolytic cleavages needed to produce Aβ from APP [Rogaev et al., 1995; Goutte et al., 2002; Edbauer et al., 2003; Takasugi et al., 2003]. PS1 and PS2 mutations result in production of a longer form of Aβ, which is more pathogenic, possibly because it more rapidly aggregates into the fibrils found in amyloid plaques. Thus PS1/PS2 mutations act to cause AD through Aβ, which is clearly a pathogenic molecule. How ApoE influences this process is unknown. One hypothesis is that ApoE binds Aβ directly and participates in the removal and eventual degradation of Aβ. In this model, the ε2 and ε3 ApoE isoforms are more effective than the ε4 isoform in removing Aβ, which is consistent with the increased amyloid plaque density seen in ε4-positive AD subjects [Rebeck et al., 1993; Schmechel et al., 1993]. If ApoE-mediated Aβ clearance rates are isoform-dependent, and this process is important as AD progresses, APOE genotypes should influence AD rate-of-progression. However, studies on APOE genotype effects on rate-of-AD progression have given inconsistent results [Frisoni et al., 1995; Growdon et al., 1996; Lehtovirta et al., 1996; Stern et al., 1997; Craft et al., 1998]. Thus if ApoE is involved in Aβ clearance, the critical effect may be in the prodromal phase of the disease, either prior to the appearance of detectable clinical symptoms in cognitively normal subjects [Flory et al., 2000; Caselli et al., 2001] or during the mild cognitive impairment phase of the disease [Dik et al., 2000].

In LOAD, where monogenic mutations are not known, APOE genotypes also influence age-of-onset. In this more common form of AD, even though Aβ-containing plaques are part of the pathology, it is not clear that disease initiation is caused by Aβ. The first AD-related pathology observed in brains from normal subjects is neurofibrillary tangles in the transenthorinal/enthorinal cortex region, not Aβ deposits [Braak and Braak, 1997]. This early accumulation of aggregated tau could compromise neuronal tracts leading to the hippocampus resulting in a set of neurons more sensitive to Aβ-induced injury. Since ApoE appears to function as a neuronal injury response protein in brain [Poirier et al., 1991], the APOE genotype may influence how well these neurons can respond to an insult [Nathan et al., 1994], such as exposure to excess Aβ. Thus APOE-influenced neuronal susceptibility, as part of the prodromal phase, may precede Aβ effects. A similar ApoE mechanism could also be how APOE genotypes influence PS1/PS2 mutation. A report that mildly cognitively impaired APOE ε4 positive subjects decline more rapidly than ε4-negative subjects [Dik et al., 2000] is consistent with both the Aβ clearance and the early neuronal injury model. The rate-of-progression through this presymptomatic phase could be influenced by APOE genotypes and appear as an age-of-onset effect. Though the mechanism of ApoE action in AD is not clear, the influence of APOE genotypes on both PS1/PS2 mutation cases and the more common form of late-onset AD strongly supports the hypothesis that AD is caused by a pathway that has at least some common components in all forms of the disease. If the same unmapped genes are involved in all forms of AD, identification of such genes may be easier.

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