Fine Mapping of the Chromosome 12 Late-Onset Alzheimer Disease Locus: Potential Genetic and Phenotypic Heterogeneity

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Apolipoprotein E (APOE) is the only confirmed susceptibility gene for late-onset Alzheimer disease (AD). In a recent genomic screen of 54 families with late-onset AD, we detected significant evidence for a second late-onset AD locus located on chromosome 12 between D12S373 and D12S390. Linkage to this region was strongest in 27 large families with at least one affected individual without an APOE-4 allele, suggesting that APOE and the chromosome 12 locus might have independent effects. We have since genotyped several additional markers across the region, to refine the linkage results. In analyzing these additional data, we have addressed the issue of heterogeneity in the data set by weighting results by clinical and neuropathologic features, sibship size, and APOE genotype. When considering all possible affected sib pairs (ASPs) per nuclear family, we obtained a peak maximum LOD score between D12S1057 and D12S1042. The magnitude and location of the maximum LOD score changed when different weighting schemes were used to control for the number of ASPs contributed by each nuclear family. Using the affected-relative-pair method implemented in GENEHUNTER-PLUS, we obtained a maximum LOD score between D12S398 and D12S1632, 25 cM from the original maximum LOD score. These results indicate that family size influences the location estimate for the chromosome 12 AD gene. The results of conditional linkage analysis by use of GENEHUNTER-PLUS indicated that evidence for linkage to chromosome 12 was stronger in families with affected individuals lacking an APOE-4 allele; much of this evidence came from families with affected individuals with neuropathologic diagnosis of dementia with Lewy bodies (DLB). Taken together, these results indicate that the chromosome 12 locus acts independently of APOE to increase the risk of late-onset familial AD and that it may be associated with the DLB variant of AD.

Introduction

Alzheimer disease (AD [MIM 104300]) is a progressive neurodegenerative disorder with a complex etiology comprising genetic and environmental factors. More than 2 million individuals in the United States have AD; this figure is projected to quadruple in the next 50 years, as the population ages (Brookmeyer et al. 1998). Clinically, AD causes progressive memory loss and alters higher intellectual function (Guttman et al. 1999). Although provisional diagnosis of AD may be made on the basis of clinical symptoms, neuropathologic examination is required for confirmation of the diagnosis. At autopsy, AD is characterized by neurofibrillary tangles in the neurons of the cerebral cortex and hippocampus as well as by the deposition of amyloid in senile plaques and cerebral blood vessels (Wisniewski et al. 1993). Lewy bodies, the neuropathologic hallmark of Parkinson disease, are found in 15%–20% of autopsied individuals with a clinical diagnosis of probable AD (Hulette et al. 1995; McKeith et al. 1996). In a recent consensus paper (McKeith et al. 1996), it was recommended that the designation “dementia with Lewy bodies” (DLB) be used to describe this finding. Whether DLB is a variant of AD or is a separate yet phenotypically similar neurodegenerative disease is currently unknown.

Familial aggregation of AD has been noted for decades, suggesting a role for genes in the etiology of the disease. The results of studies focusing on large families with the rare, early-onset, autosomal dominant form of the disease led to the discovery that mutations in the amyloid precursor protein, presenilin 1, and presenilin 2 genes caused AD (Goate et al. 1991; Levy-Lahad et al. 1995; Rogaev 1995; Sherrington et al. 1995). Studies
of the more-common late-onset form of AD have determined that the apolipoprotein E gene (APOE) is a susceptibility factor for both late-onset familial AD and sporadic AD (Corder et al. 1993; Saunders et al. 1993; Strittmatter et al. 1993). Unlike the genes for early-onset AD, APOE is not a sufficient cause of the disease but, instead, modulates the risk of AD developing. The presence of the APOE-4 allele increases risk and decreases age at onset of the disease, whereas the APOE-2 allele has a protective function (Corder et al. 1993, 1994). Despite success in the detection of genes associated with age at onset of the disease, whereas the APOE-4 allele increases risk and decreases age at onset of the disease, whereas the APOE-2 allele has a protective function (Corder et al. 1993, 1994).

Recent research efforts have identified chromosome 12 as the most-promising location for a fifth AD gene. The functional candidate gene, LRP1 (LDL receptor–related protein), is located on chromosome 12q, and noncoding LRP1 polymorphisms have been associated with a slightly increased risk of AD in some studies (Kang et al. 1997; Lendon et al. 1997; Wavrant-De Vriese et al. 1997; Hollenbach et al. 1998; Kamboh et al. 1998) but not in all (Clatworthy et al. 1997; Fallin et al. 1997; Scott et al. 1998). In addition, the results of our recent complete genomic screen (Pericak-Vance et al. 1997) showed significant evidence for linkage to a pericentromeric region of chromosome 12; subsequent follow-up has detected evidence for linkage in a wider region of the chromosome (Scott et al. 1999).

Other investigators have recently replicated linkage to chromosome 12 (Rogaeva et al. 1998; Wu et al. 1998) in independent samples, strengthening the argument that an AD gene maps to this chromosome. Finally, the results of a study conducted with use of the National Institute of Mental Health (NIMH) AD Genetics Initiative data set showed a significant association with an insertion/deletion polymorphism in the α2-macroglobulin gene (A2M), which is another functional candidate gene for AD located on chromosome 12p (Blacker et al. 1998). However, this association has not been replicated either in several independent familial and case-control samples (Dow et al. 1999; Rogaeva et al. 1999; Rudrasingham et al. 1999) or in an independent examination of the publicly available NIMH AD Genetics Initiative data set (Rogaeva et al. 1999). Therefore, it appears likely that the AD gene located on chromosome 12 remains to be discovered. In the present study, we refine our original linkage (Pericak-Vance et al. 1997) by analyzing additional markers in the region, and we address the issue of genetic heterogeneity in the data set by weighting families by APOE genotype as well as by clinical and neuropathologic features.

Material and Methods

Description of Sample

During the past 15 years, more than 200 families with multiple individuals affected with AD ("multiplex families") have been ascertained for genetic studies of late-onset familial AD. All sampled individuals have had possible or probable AD diagnosed (by use of National Institute of Neurological Disorders and Stroke/Alzheimer's Disease and Related Disorders Association [NINDS/ADRDA] criteria [McKhann et al. 1984]) by a neurologist or by associated clinical personnel at the Joseph and Kathleen Bryan Alzheimer's Disease Research Center (ADRC) at Duke University Medical Center (DUMC), the Massachusetts General Hospital ADRC, the University of California at Los Angeles Neuropsychiatric Institute, or the Indiana Alzheimer's Disease Center National Cell Repository. Data collection and analysis for this study were performed according to protocols reviewed and approved by the institutional review boards of each participating institution.

A subset of 54 families was selected for a recently completed genomic screen and follow-up (Pericak-Vance et al. 1997) that implicated the region of chromosome 12 examined in more detail in the present study. A description of this data set may be found in the initial report (Pericak-Vance et al. 1997). Participants in this study are followed up longitudinally; clinical and neuropathologic data are continuously updated, and the most recent information was used in this analysis. Since the 1997 report appeared, affection status has changed for six individuals from six families: one individual with a previously unclear status is now considered to be affected, two unaffected individuals are now considered to have unclear status, and three individuals that were previously considered to be affected are now considered to have unclear status. In addition, two unaffected individuals from a seventh family were added to the data set. Therefore, although the data set used for this analysis contains the same families that were used in our previous study, it is not identical in terms of clinical or neuropathologic data.

Neuropathologic Studies

Consensus diagnostic criteria (McKhann et al. 1984) for AD indicate that postmortem neuropathologic examination is necessary to establish a definite diagnosis of AD. Therefore, autopsy confirmation of AD is obtained for genetic-study participants whenever possible. Assessment of AD neuropathology is performed according to standard Consortium to Establish a Registry for Alzheimer's Disease (CERAD) and National Institute on Aging and the Reagan Institute (NIA–Reagan) criteria.
Weighting schemes was compared with use of the Spairs evidence for linkage. The performance of these three LOD score (e.g., MLS, denoted as "MLS" by Hinds and Risch [1998]). ASPEX calculates a nonparametric maximum LOD score (for all affected pairs containing the first affected individual) in a nuclear family was examined. To control for the effect of including multiple (dependent) ASPs in a nuclear family was considered significant evidence for linkage). The performance of these three weighting schemes was compared with use of the $S_{\text{pairs}}$ statistic implemented in GENEHUNTER-PLUS (Kong and Cox 1997), where the maximum LOD score (denoted as "LOD*" by Kong and Cox) is calculated from estimates of allele sharing averaged across all possible affected pairs.

Because several extended families contained affected relative pairs other than sib pairs (Pericak-Vance et al. 1997), we selected GENEHUNTER-PLUS (Kruglyak et al. 1996; Kong and Cox 1997) to perform multipoint, nonparametric affected-relative-pair analysis. This extension of the GENEHUNTER program uses a hidden Markov model to examine allele sharing either at multiple markers averaged across all possible pairs of affected relatives ($S_{\text{pairs}}$) or simultaneously across all affected individuals in a pedigree ($S_{\text{all}}$). Because the $S_{\text{pairs}}$ statistic is more similar, compared with the $S_{\text{all}}$ statistic, to the approach implemented in ASPEX and because it is more robust in small samples, it was selected for use in this analysis. The nonparametric-linkage (NPL) test statistic is asymptotically normal, and statistical significance in large samples may be assessed by use of a standard normal distribution. GENEHUNTER-PLUS calculates a maximum-likelihood estimate of the NPL statistic under either a linear model or an exponential model; this estimate may then be converted to a "semiparametric" LOD score: LOD* (Kong and Cox 1997). For these analyses, LOD* was calculated by use of the exponential model, which is more robust in smaller data sets (Kong and Cox 1997). The maximum-likelihood estimate of LOD* was limited to positive values of the sharing parameter $\delta$, to ensure that positive LOD* scores reflected excess sharing of alleles among affected family members. Like the MLS statistic generated by ASPEX, LOD* may be interpreted as a traditional LOD score, and it is a less-conservative test of linkage in the presence of missing data, compared with the NPL statistic. Because of computational constraints on pedigree size, unaffected individuals were omitted from analysis in 13 pedigrees.

Potential interactions between APOE and chromosome 12 markers were evaluated by use of conditional linkage methods outlined elsewhere (Cox et al. 1999). Heterogeneity was assessed by weighting the by-family results on the basis of the proportion of affected individuals lacking an APOE-4 allele (APOE-4$^-$ weighting). Additive or epistatic interaction was tested by weighting the by-family results on the basis of the proportion of affected individuals possessing an APOE-4 allele (APOE-4$^+$ weighting). A similar approach was used to explore clinical heterogeneity. Families were stratified on the basis of the presence of neuropathologic features consistent with DLB. Families with at least one member classified as having DLB at autopsy ($n = 8$) were analyzed separately from the other 46 families in the screen set. To assess linkage conditional on both DLB and APOE, the by-family results in each subset were weighted on the basis of the proportion of affected individuals lacking an APOE-4 allele. Statistical significance of conditional
analyses was assessed by shuffling family-specific weights to create a null distribution, as originally described elsewhere (Cox et al. 1999).

**Results**

We genotyped 26 microsatellite markers spanning ~100 cM of chromosome 12. Map distances and maximum affecteds-only two-point LOD scores (with 5% misdiagnosis correction) assuming homogeneity (LOD) and heterogeneity (HLOD) are presented in table 1. Maximum LOD scores were generally positive across the entire region, and there was little difference in the maximum LOD score and HLOD for each marker. Maximum LOD scores were >1.0 for 9/14 markers located within our initial region of interest (Pericak-Vance et al. 1997), with the peak LOD = 1.48 (HLOD 1.71) at marker D12S1042.

Multipoint ASP analysis, by use of all possible ASPs, generated a peak MLS of 2.21 between markers D12S1057 and D12S1042, with a smaller peak near D12S368 (fig. 1). The fluctuation in LOD scores for chromosome 12 markers indicated possible genetic heterogeneity. Additional analyses were performed to control for differences in family size, the effect of APOE, and neuropathologic features, as a means of addressing heterogeneity.

In the first of these analyses, additional ASP analyses were performed with the use of various weighting schemes available in ASPEX and GENEHUNTER-PLUS (fig. 1). Considering only independent ASPs, the average of all ASPs, or only a single ASP per nuclear family progressively decreases the evidence for linkage at D12S1057. The most-extreme method of limiting the influence of families with many ASPs (considering only one ASP per nuclear family) also changes the location estimate—a peak of 0.77 is obtained at marker D12S1701. In the second analysis, GENEHUNTER-PLUS was used to examine allele sharing averaged across all affected relative pairs in each family. The overall LOD* plot (fig. 2) indicates that controlling for family size shifts evidence for linkage in a fashion similar to the use of only one affected relative pair in ASPEX; the peak LOD* is 1.40 between markers D12S398 and D12S1632.

To explore potential interactions between chromosome 12 linkage and APOE, we calculated LOD*, conditional on the proportion of affected individuals in each family possessing (APOE-4 wt) or lacking (APOE-4 ? wt) an APOE-4 allele (fig. 2). Consistent with a heterogeneity model, linkage results were strongest when APOE-4 wt weighting was used (peak LOD* = 2.43 at D12S1632). With APOE-4 ? wt weighting, LOD* scores remained low throughout the region (peak LOD* = 0.48 at D12S368). Simulation determined that the increase in LOD* seen with the use of APOE-4 wt weighting was statistically significant (P = .04), since only 40/1,000 replicates produced LOD* > 2.43.

In addition, we considered neuropathologic findings as a second potential indicator of genetic heterogeneity in this data set. Families were stratified into two groups, on the basis of the presence of at least one family member with autopsy findings consistent with the consensus criteria for DLB (McKeith et al. 1996). The peak LOD* = 2.18 that was obtained in the eight families with DLB occurs between D12S1042 and D12S1090, and the remaining 46 families generate a peak LOD* = 0.58 at D12S1632 (fig. 3). Simulation also determined that the increase in LOD* in the families with DLB was statistically significant (P = .035), with only 35/1,000 replicates generating a LOD* > 2.18.

The two groups of families (those with affected individuals lacking an APOE-4 allele and those with DLB) with more evidence for linkage to chromosome 12 were not independent, since six of the eight families with DLB also had affected individuals lacking an APOE-4 allele. Thus, we weighted the results in the eight families with DLB and those in the other 46 families on the basis of

**Table 1**

<table>
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<tr>
<th>Marker</th>
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<th>Maximum HLOD</th>
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* Used in multipoint analysis.
the proportion of affected individuals lacking an APOE-4 allele (fig. 4). In the families with DLB, the results were not significantly different from those obtained with stratification on DLB alone: a peak LOD* = 2.02 occurs between D12S1042 and D12S1090. However, in the remaining 46 families, the LOD* increases to 1.63 at D12S1632, indicating that evidence for linkage remains in this subset and is stronger when APOE-4 weighting is used.

**Discussion**

Following up the original linkage of AD to chromosome 12 (Pericak-Vance et al. 1997), by genotyping of additional markers and by performing multipoint ASP analysis, has generated a maximum LOD score that is lower than that originally obtained with a less-dense map of markers. This finding is consistent with our previous experience (Haines et al. 1993) in following up the initial linkage of AD to chromosome 19 (Pericak-Vance et al. 1991). In that effort, genotyping of additional markers and family members lowered the maximum LOD score and widened the region of interest. The difference in LOD scores was resolved by stratification of the families on the basis of age at onset (thereby reducing genetic heterogeneity), facilitating the identification of the APOE gene as a risk factor for late-onset AD.

Our effort to control for family size in ASP analysis clearly showed that including all ASPs in each nuclear family changed the magnitude of the peak-location score in this data set. The original peak MLS obtained by use of all possible ASPs was at D12S1057, and this MLS decreased with the use of each method of controlling for multiple ASPs per nuclear family. This observation indicated that the localization of the gene to 12p was influenced by the contribution of multiple independent ASPs from several large families, possibly inflating the evidence for localization of the chromosome 12 gene to that region. In fact, when using the most-stringent limitation of one ASP per nuclear family, we found that the peak MLS was at D12S1701, ~20 cM away and on the other arm of the chromosome. To further control for family size, we analyzed allele sharing averaged across all ASPs in each family, by use of the GENEHUNTER-PLUS program. Controlling for family size in this manner supported the MLS results when one ASP was used per family; the linkage peak occurred on chromosome 12q, close to D12S1632. The
results from both of these analyses suggest that analysis of extended pedigrees broken down into nuclear families and failure to consider the dependencies among ASPs may influence estimates of the disease-gene location.

Analysis of the chromosome 12 data, conditional on APOE genotype in affected family members, revealed that linkage to chromosome 12 markers was concentrated in the families with affected members lacking the APOE-4 allele (APOE-4\(^{\text{weighting}}\)), supporting a heterogeneity model. These data are consistent with those from our previous report (Pericak-Vance et al. 1997), in which we indicated that linkage to chromosome 12 was strongest in families with at least one affected individual with an APOE 2/2, 2/3, or 3/3 genotype.

We also considered separately the eight families with at least one individual that met consensus neuropathologic criteria for DLB (McKeith et al. 1996). These eight families alone provide evidence for linkage to a 20-cM interval bounded by D12S1057 and D12S1701. In contrast, the remaining 46 families show more-moderate evidence for linkage to the same 10-cM region on chromosome 12q, bounded by D12S1701 and D12S1632, detected in the overall data set.

These results indicate that estimates of the location of the chromosome 12 AD gene vary by family size, APOE genotype, and the presence of autopsy-confirmed DLB in the family. However, it is unclear whether these are independent effects in our data set or whether each is associated with the same genetic-risk factor. Evaluating linkage to chromosome 12 markers while considering APOE genotype and presence of DLB indicated that much of the linkage information in the data set comes from the six families with both DLB and individuals lacking an APOE-4 allele. However, the remaining 46 families without DLB still generate moderate evidence for linkage to 12q when APOE-4\(^{\text{weighting}}\) is used.

Family size and presence of DLB in the family are somewhat correlated in this data set, because the chances of obtaining autopsy confirmation of AD for at least one affected family member increases with both the number of affected individuals in the family and the length of time the family has been studied. In fact, the presence of DLB in the 24 families with autopsy-confirmed AD but with no DLB cannot be ruled out, since all affected individuals have not been autopsied. In addition, 22 families have no individuals with autopsy-confirmed AD enrolled in the study, and, therefore, they...
could not possibly be grouped with the families with DLB. Taking this information into account, the proportion of families with at least one individual with autopsy-confirmed AD and with autopsy-confirmed DLB (8/32 [25%]) is not much higher than previous estimates of the frequency of DLB in probable AD (10%–20%; Hulette et al. 1995; McKeith et al. 1996). The strong correlation between chromosome 12 linkage, APOE genotype, and presence of DLB makes it difficult to determine which factor is more indicative of the presence of the chromosome 12 locus: absence of an APOE effect or presence of DLB.

Because of this correlation and because evidence for linkage persists in the families without DLB when APOE-4* weighting is used, it would be unwise to stratify families on the basis of presence or absence of DLB and to focus fine-mapping efforts in the region of maximum linkage in each subset, without additional confirmation. Therefore, at present, the most-conservative approach to finding the chromosome 12 AD gene is to study the ~30-cM region from D12S1057–D12S1632. This region contains both the overall linkage peak on chromosome 12q as well as the centromeric region implicated by the eight families with DLB.

In effect, our efforts to fine map this region of linkage have been an exercise in data mining. We have considered several potential factors (family size, APOE, and presence of DLB) that may significantly alter observed linkage between markers on chromosome 12 and AD, and we have stratified our analyses accordingly, in an attempt to identify a combination of factors that defines a more-homogeneous subset of families in which there is linkage to chromosome 12. The application of neural networks and recursive partitioning to pattern recognition in genetic analysis is currently being explored (Falk et al. 1998; Lucek et al. 1998) and could provide a framework for such data mining in the future. Even so, the problem with data mining will remain: the solution generated is often one of many possible solutions, and, frequently, there is little evidence to recommend one solution over the other.

The results described in the present study highlight the difficulties of fine-mapping linked regions in a complex disease, where genetic and clinical heterogeneity likely impact estimates of the location and effect size. In the case of chromosome 12–linked AD, several studies have reported linkage to chromosome 12 but have highlighted different regions of maximum linkage (Pericak-Vance et al. 1997; Rogaeva et al. 1998; Wu et al. 1998; Kehoe et al. 1999) (fig. 5). Rogaeva and col-

![Figure 3](image-url)
leagues (Rogaeva et al. 1998) studied a data set of 53 families that included both ASPs and large, multigenerational families with late-onset AD. Their linkage studies focused on the region of chromosome 12 between D12S373 and D12S390 that was highlighted in our original report (Pericak-Vance et al. 1997). Evidence for linkage in their families was concentrated at either end of the interval, with the strongest linkage being located near D12S390. In contrast, others (e.g., Wu et al. 1998; Kehoe et al. 1999) have studied a collection of 230 families with ASPs from the NIMH AD Genetics Initiative data set. As part of a whole-genome scan, they detected evidence for linkage on chromosome 12p, near D12S391 (Wu et al. 1998; Kehoe et al. 1999). This region contains the A2M gene, which Blacker and colleagues had previously reported as being associated with AD in the NIMH data set (Blacker et al. 1998).

The different localizations of the chromosome 12 locus may be the result of differences in the ascertainment of families and the manner in which the data sets were stratified on the basis of APOE genotype. Although Rogaeva et al (Rogaeva et al. 1998) studied families of similar structure to those in our data set, they found the strongest evidence for linkage in “APOE-4 positive” families—contrary to our findings. However, they considered a family to be “APOE-4 positive” if >75% of the individuals possessed an APOE-4 allele; four of these families would have been classified as APOE-4 negative in our original report (Pericak-Vance et al. 1997). Thus, the results for APOE genotype may not conflict with ours. Wu, Kehoe, and colleagues (Wu et al. 1998; Kehoe et al. 1999) studied ASPs; the focus on nuclear families produces a data set with a family structure that is different from ours and may influence their estimate of the disease-gene location. The implication of the differences among the three studies is not entirely clear. Each could represent linkage to the same poorly localized gene. Alternatively, two AD loci could be located on chromosome 12: all three studies support linkage to chromosome 12p in at least a subset of families, and both the present study and a study done elsewhere (Rogaeva et al. 1998) detect evidence for a locus on chromosome 12q. A potential resolution of the conflict may rest in a consistent stratification of the families on the basis of APOE and further stratification of the data sets on the basis of presence of Lewy bodies in autopsied family members.

![Figure 4](image-url)
In addition to difficulties in fine mapping posed by heterogeneity, the results of recent studies indicate that genotyping of additional markers provides additional linkage information up to a map density of only 1–2 cM. The results of a recent study in sib pairs with multiple sclerosis (Feakes et al. 1999) showed that, once markers reached a density of 2 cM, fine mapping done by genotyping of additional markers became inefficient because of the increasing likelihood that map or genotyping errors would significantly lower the multipoint LOD scores. Although our laboratory minimizes genotyping errors by use of an extensive set of quality control measures (Rimmler et al. 1998), our analyses are still susceptible to errors in map order or distance. These findings are consistent with our previous experience with chromosome 19 linkage in AD (Haines et al. 1993).

Given these limitations of the use of linkage to refine a candidate region in a genetically heterogeneous data set, we believe that the approach that is most effective in finding the AD gene on chromosome 12 is a candidate-gene- and linkage-disequilibrium-based approach. Therefore, further fine mapping will use family-based tests of association and linkage (such as the sibling transmission/disequilibrium test) for regularly spaced microsatellite and single-nucleotide polymorphism markers in the region.

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**Electronic-Database Information**

The accession number and URLs for data in this article are as follows:

- **Marshfield Medical Research Foundation, Center for Medical Genetics**, [http://www.marshmed.org/genetics/](http://www.marshmed.org/genetics/) (for follow-up markers)

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