Complete Genomic Screen in Late-Onset Familial Alzheimer Disease

Evidence for a New Locus on Chromosome 12

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Context.—Four genetic loci have been identified as contributing to Alzheimer disease (AD), including the amyloid precursor protein gene, the presenilin 1 gene, the presenilin 2 gene, and the apolipoprotein E gene, but do not account for all the genetic risk for AD.

Objective.—To identify additional genetic risk factors for late-onset AD.

Design.—A complete genomic screen was performed (N=280 markers). Critical values for chromosomal regional follow-up were a *P* value of .05 or less for affected relative pair analysis or sibpair analysis, a parametric lod score of 1.0 or greater, or both. Regional follow-up included analysis of additional markers and a second data set.

Setting.—Clinic populations in the continental United States.

Patients.—From a series of multiplex families affected with late-onset (≥60 years) AD ascertained during the last 14 years (National Insititute of Neurological Disorders and Stroke–Alzheimer's Disease and Related Disorders Association diagnostic criteria) and for which DNA has been obtained, a subset of 16 families (135 total family members, 52 of whom were patients with AD) was used for the genomic screen. A second subset of 38 families (216 total family members, 89 of whom were patients with AD) was used for the follow-up analysis.

Main Outcome Measures.—Linkage analysis results generated using both genetic model-dependent (lod score) and model-independent methods.

Results.—Fifteen chromosomal regions warranted initial follow-up. Follow-up analyses revealed 4 regions of continued interest on chromosomes 4, 6, 12, and 20, with the strongest results observed for chromosome 12. Peak 2-point affecteds-only lod scores (n=54) were 1.3, 1.6, 2.7, and 2.2 and affected relative pairs *P* values (n=54) were .04, .03, .14, and .04 for *D12S373*, *D12S1057*, *D12S1042*, and *D12S390*, respectively. Sibpair analysis (n=54) resulted in maximum lod scores (MLSs) of 1.5, 2.6, 3.2, and 2.3 for these markers, with a peak multipoint MLS of 3.5. A priori stratification by *APOE* genotype identified 27 families that had at least 1 member with AD whose genotype did not contain an *APOE*4* allele. Analysis of these 27 families resulted in MLSs of 1.0, 2.4, 3.7, and 3.3 and a peak multipoint MLS of 3.9.

Conclusions.—A complete genomic screen in families affected with late-onset AD identified 4 regions of interest after follow-up. Chromosome 12 gave the strongest and most consistent results with a peak multipoint MLS of 3.5, suggesting that this region contains a new susceptibility gene for AD. Additional analyses are necessary to identify the chromosome 12 susceptibility gene for AD and to follow up the regions of interest on chromosomes 4, 6, and 20.

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ALZHEIMER DISEASE (AD) is a complex genetic disorder and represents the most common form of dementia in the elderly. More than 4 million persons in the United States are affected with AD and that number continues to increase as the population ages. The cost in ancillary and nursing home care for patients with AD is more than $40 billion annually,[1] while the additional psychological and physical burden to patients and family members is immeasurable.

For editorial comment see p 1282.

Fortunately, considerable progress has been made in unraveling the complex etiology of AD, primarily using the powerful tools of genetic linkage analysis.[2] To date, 4 loci have been identified as contributing to AD, including the amyloid precursor protein gene (*APP*),[3] the presenilin 1 gene (*PS1*),[4] the presenilin 2 gene (*PS2*),[5,6] and the apolipoprotein E (*APOE*) gene.[7-9] The *APP*, *PS1*, and *PS2* genes cause early-onset (<60 years) autosomal dominant AD. *APOE* also is associated with early-onset sporadic AD,[10,11] and is the only gene to be confirmed as associated with the more common familial and sporadic late-onset (≥60 years) AD. The effect of *APOE* on susceptibility to AD has been confirmed in multiple racial groups and ethnic populations worldwide.[12] The *APOE*4* allele increases risk for and decreases the age of AD onset,[13] whereas the *APOE*2* allele decreases risk.[14] However, these 4 loci (*APP*, *PS1*, *PS2*, and *APOE*) do not account for all the genetic risk for AD,[15,16] indicating that there are additional unidentified AD loci.

The purpose of the present study was to identify additional major genetic effects in late-onset AD. We completed a genomic screen using a multistage, multianalytical approach. Our study is multistage because no inferences are made

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Table 1.—Data Set Configuration: Number of Affected Relative Pairs*

Affected Relative Pairs†	No. (No. Sampled With DNA)	
	Genomic Screen Data Set (n=16 Families)	Follow-up Data Set (n=38 Families)
Patients per family, mean	5.3 (3.3)	3.5 (2.3)
Sibpairs	100 (46)	134 (68)
Avuncular pairs	40 (7)	31 (10)
Cousin pairs	15 (9)	7 (4)
Parent-child pairs	32 (2)	30 (2)

*All possible affected relative pair combinations scored.
†Over all families in set.

until a second independent data set is tested, and is multianalytical because our approach combines the use of genetic model-dependent (ie, assumption of dominant or recessive inheritance) and model-independent (ie, no assumptions regarding the genetic model) methods of linkage analysis.

METHODS

Family Data

During the past 14 years, we have ascertained and sampled multiplex white families affected with late-onset AD (>1 patient with AD per family) (present sample, N=220 families) with a total of 450 affected individuals (affecteds) sampled for genetic studies. All sampled individuals diagnosed as having probable AD were examined by a neurologist or associated personnel of the Joseph and Kathleen Bryan Alzheimer's Disease Research Center (ADRC) at Duke University, Durham, NC, the Massachusetts General Hospital (MGH) ADRC, Boston, the University of California, Los Angeles Neuropsychiatric Institute, or the Indiana Alzheimer's Disease Center's National Cell Repository, Indianapolis. The clinical diagnosis of AD was made using the National Institute of Neurological Disorders and Stroke–Alzheimer's Disease and Related Disorders Association diagnostic criteria.[17]

A subset of 54 of these families (n=16 for the genomic screen; n=38 for the follow-up) was chosen for inclusion in a genome-wide screen and follow-up analysis to look for additional major genetic effects in late-onset AD. The 54 families were chosen because of their large size, the substantial number of family members with a clinical[17] and/or pathological[18] diagnosis of AD (ie, extended family history of AD, not just affected sibpairs [AD patients who are siblings]), and the number of AD patients with DNA samples available. DNA samples of the 54 families were negative for mutations in the *APP* gene and the common *PS1* mutations, and showed no evidence of linkage to either of the *PS1* and *PS2* loci. Age at onset of AD was defined as previously described.[7] The mean (SD) age at onset for the patients with AD in the 54 families was 72.7 (6.5) years.

The families were divided into 2 subsets, 1 for the initial genomic screen and 1 specifically for the follow-up analysis. The first set contained 16 families (genomic screen data set) and was selected to maximize efficiency for the genomic screen by allowing us to perform genotype testing on as few individuals as possible while still preserving the majority of the power to detect linkage in the families analyzed. Thus, these 16 families were selected for the genomic screen because they were the largest families available at the time of initiation of the genomic screen. Examination of the families after selection for family size revealed that 6 of the original 16 genomic screen families had at least 1 patient with AD who did not have an *APOE*4* allele, suggesting that *APOE*4* may not play a major role in the risk for AD in these families. The 16 families included 135 family members with DNA samples and 52 AD patients with DNA samples. The 16 families had an average of 5.3 (range, 3-9) patients with AD per family and an average of 3.3 (range, 2-5) AD patients with DNA samples per family. The 38 families in the follow-up data set were chosen for the follow-up of interesting regions that were identified in the genomic screen. The 38 families in the follow-up data set included 216 family members with DNA samples and 89 AD patients with DNA samples. The combined data set included 351 family members with DNA samples. These families were selected at the time of follow-up from the then available families based on the same criteria used to select the first 16 families analyzed in the genomic screen. The number of patients and affected relative pairs (ARPs), including cousin pairs and avuncular pairs (aunt/nephew or niece, uncle/nephew or niece), in addition to sibpairs within the families is shown in Table 1. The families contained an average of 3.5 (range, 2-10) patients with AD per family and an average 2.3 (range, 2-6) AD patients with DNA samples per family. In addition to the 234 known affected sibpairs (114 with DNA), there were 71 affected avuncular pairs (17 with DNA), 22 affected cousin pairs (13 with DNA), and 62 parent-child pairs (4 with DNA) in the combined screening and follow-up data set. These data (Table 1) indicate that the families were often multigenerational in structure and not limited to affected sibpairs. All pairwise combinations of relative pairs were used in the ARP counts shown in Table 1.

DNA Analysis

Genomic DNA was extracted from whole blood using methods described previously.[7,19] Marker genotyping was performed by means of fluorescence imaging (Molecular Dynamics SI Fluorimager, Duke University)[20] or silver staining (MGH).[21] Paternity was confirmed by examining a series of linked polymorphic markers (haplotypes) over several chromosomal regions. Microsatellite marker loci for the genomic screen were chosen for analysis based on a combination of high heterozygosity, approximate 10- to 15-centimorgan (cM) spacing, and ease of use (N=280 markers). X chromosome markers were not examined as there is no evidence for X chromosome involvement in AD.

Statistical Analysis

The families were analyzed for linkage with a multianalytical approach that used genetic model-dependent (lod score)[22] and model-independent (ARP [SimIBD[23]] and sibpair [SIBPAL, ASPEX][24-27]) linkage methods.[23-27] Analysis of ARPs was performed in addition to the sibpair studies because many of the families contained ARPs in addition to affected sibpairs (Table 1). Given the suboptimal structure of most AD pedigrees (ie, many deceased family members without DNA information because of the late age of AD onset), our goal was to capture as much linkage information in the families as possible.

Genetic model-dependent methods make assumptions about the mode of inheritance (dominant or recessive) penetrance (ie, a fully penetrant trait vs a nonpenetrant or age-dependent penetrant trait). The use of genetic model-dependent methods is a powerful approach to gene mapping when the necessary assumptions are met. However, the power (the ability to detect linkage) is decreased markedly if the incorrect assumptions are made. Because in genetically complex diseases such as late-onset AD a disease gene model cannot be assumed with certainty, model-dependent methods must be interpreted with caution. Thus, genetic model-independent methods such as sibpair and ARP analyses, which examine allele sharing among ARPs, offer an attractive and complementary approach to linkage analysis.

The VITESSE program package was used in the lod score analysis.[22] An autosomal dominant model was chosen because of the extensive evidence for vertical transmission in the families in our study (Table 1). For the lod score approach in the genomic screen, both age-dependent and low-penetrance (affecteds-only) models were used for analysis with the marker loci. The age curve used in the age-dependent analysis for assigning probabilities of carrying the *AD* gene to at-risk family members (children of pa-

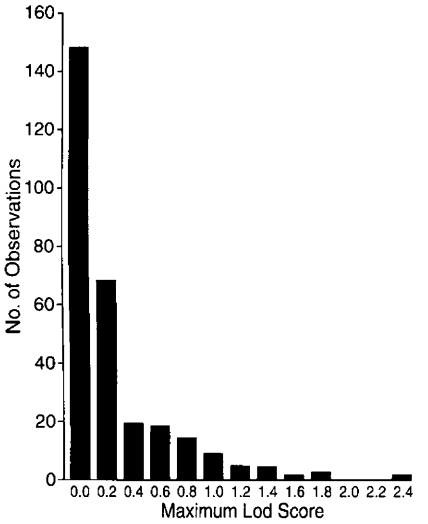

Figure 1.—Distribution of maximum lod scores for each marker in the genomic screen. Thirty markers (10.7%) had lod scores of 1.0 or greater.

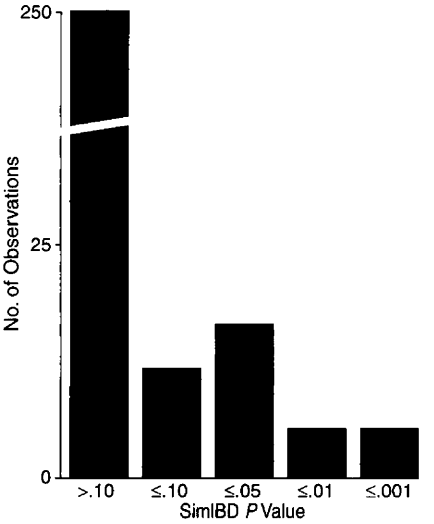

Figure 2.—Distribution of SimIBD *P* value for each marker in the genomic screen. Twenty-nine markers (10.4%) had *P* values of .05 or less. Because each *P* value was determined empirically given the pedigree and marker data, no generalized statistic is possible.

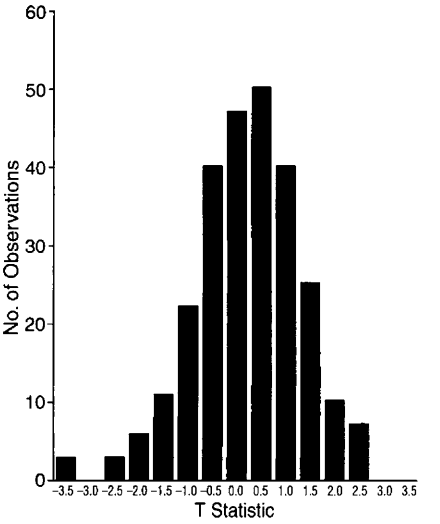

Figure 3.—Distribution of T statistic for sibpair (ASPEX) sharing for each marker in the genomic screen. Nineteen markers (6.8%) had T statistics of 1.645 or greater. The T statistic is normally distributed so that values of 1.645 or greater have associated 1-sided *P* values of .05 or less.

tients with AD) was generated assuming a normal distribution of age at onset of AD with a sample mean (SD) of 72.7 (6.5) years. An affecteds-only lod score analysis is a conservative approach when using a genetic model-dependent linkage method in a complex genetic trait. For the affecteds-only analysis, genotypic data were included on all sampled individuals but AD phenotypic data were limited to affected individuals.

The disease allele frequency assumed for the AD locus was 0.001. Marker allele frequencies were estimated using a series of 50 to 100 unrelated white control subjects. The SimIBD program[23] was used for the ARP analyses, the SIBPAL program in the SAGE program package[27] was used in the sibpair analyses in the genomic screen, and the ASPEX program package, which performs exclusion mapping and multipoint maximum lod score (MLS) analysis,[24-26] was used for the sibpair analysis in the follow-up studies. The order of the markers used in the multipoint analysis was determined from published sources (Collaborative Human Linkage Center, available at http://www.chlc.org) and by additional analysis in our laboratory on a subset of 20 of the largest families from the Centre d'Etude Polymorphisme Humaine[28] (M.A.P.-V. and J.L.H., unpublished data, 1997).

The critical values used to consider a region as interesting for follow-up analysis were a nominal *P* value of .05 or less for ARP and sibpair analyses and a 2-point or multipoint lod score of 1.0 or greater for either the age-dependent or the affecteds-only lod score analysis. A multipoint lod score is a lod score that is based on analyzing multiple mapped markers simultaneously for linkage. Using these criteria, a region was marked as "interesting" if a marker exceeded the critical values on 2 of 3 tests or if any one critical value was met for 2 or more adjacent markers in a 30-cM region. Interesting regions then were grouped into 3 different levels to efficiently prioritize laboratory follow-up. Level 1 regions reached critical values on all 3 linkage tests (lod score [either model], ARP, sibpair). Level 2 regions reached critical values on 2 tests. Level 3 regions reached critical values for 1 test for at least 2 more markers in a 30-cM region or had a multipoint lod score (either MLS or VITESSE) of 1.0 or greater.

Follow-up analyses of the interesting regions included genotyping at least 2 additional flanking markers and filling in all missing data, thereby capturing more than 95% of the potential genotypic information at each locus. Additional analyses were performed in both the genomic screening (n=16) and follow-up (n=38) data sets. These analyses included parametric 2-point lod score analysis using an affecteds-only model and assuming a 5% error rate in diagnosis of patients with AD[7] (misdiagnosis parameter), ARP (SimIBD), and sibpair analysis (MLS). A region remained interesting if there was evidence for linkage in the follow-up data set and if the overall evidence for linkage was stronger or equivalent to the initial finding.

In addition, the entire data set (n=54) was stratified into 3 family tiers based on the *APOE* genotypes of diagnosed affected individuals. Tier 1 families were those with at least 1 patient without an *APOE*4* allele. Tier 2 families were those in which genotyped patients all possessed at least 1 *APOE*4* allele. Tier 3 families were those in which all AD affecteds were *APOE 4/4* homozygotes. The stratification paradigm was developed before the availability of linkage results and was not applied to the data until after the completion of the follow-up studies inclusive of the elimination of laboratory errors by duplicate typing and haplotype analysis.

RESULTS

We genotyped 280 marker loci that covered the entire autosomal genome including the *APOE* gene and surrounding region on chromosome 19. Figure 1 shows the distribution of the 2-point parametric lod scores generated from the genomic screen using either model (affecteds-only or age-dependent). Only 30 markers (10.7%) generated lod scores of 1.0 or greater. Figure 2 shows the SimIBD *P* values for the genomic screen; 29 markers (10.4%) had *P* values of .05 or less. Figure 3 shows the distribution of the T statistic for the sibpair sharing results. Only 19 markers (6.8%) had *P* values of .05 or less (a value that corresponds to T≥1.645). Extreme departures from nominal significance levels were not observed; however, this is not unexpected especially if only 1 or a few AD loci are detected. Although some markers reached critical values in common for each linkage test (thus defining regions for follow-up), most markers reached critical value on only a single test. Fifteen regions on 12 chromosomes (1-8, 10, 14, 15, and 20) met our cri-

Table 2.—Follow-up Analysis (n=38 Families)

Region	Interesting Markers	Peak 2-Point Lod Score*	ARP (SimIBD)† *P* Value	Sibpair (SIBPAL)† *P* Value
Chromosome 4	*D4S1629*	>1.0	.03	.06
	D4S2368	<1.0	.13	.02
Chromosome 6	*D6S1004*	>1.5	<.001	.05
	D6S1019	>1.0	<.001	.02
	D6S391	<1.0	.01	.14
Chromosome 12	*D12S373*	>1.5	.04	.03
	D12S1057	>1.0	.03	.02
	D12S1042	>2.0	.14	<.001
	D12S390	>2.1	.04	.02
Chromosome 20‡	*D20S94*	<1.0	.50	.03

*Affected individuals only, autosomal dominant model.
†Program package used for analysis.
‡Multipoint maximum lod score (*D20S173*, *DS20171*, and *D20S94*) of 1.0 or greater.

Table 3.—Summary Results for Chromosome 12*

Marker	Follow-up Analysis (n=54 Families) 2-Point Maximum Lod Score†‡	APR (SimIBD) *P* Value†	Maximum Lod Score§ (ASPEX)	Families With at Least 1 Genotyped AD Patient Without an *APOE*4* Allele (n=27) 2-Point Maximum Lod Score‡	ARP (SimIBD) *P* Value	Maximum Lod Score§ (ASPEX)
D12S373	1.3 (0.3)	.04 (.71)	1.5	0.9	.13	1.0
D12S1057	1.6 (1.2)	.03 (.12)	2.6	2.9	.04	2.4
D12S1042	2.7 (1.2)	.14 (.51)	3.2	2.2	.06	3.7
D12S390	2.2 (1.2)	.04 (.10)	2.3	2.9	.16	3.3

*ARP indicates affected relative pair; AD, Alzheimer disease; and SimIBD and ASPEX, program packages used for analyses.
†Results for families in the genomic screen data set (n=16) are given in parentheses.
‡Affected individuals only, autosomal dominant model, with 5% misdiagnosis.
§Peak multipoint maximum lod score is 3.5 for genomic screen and follow-up analyses (n=54 families) and 3.9 for families with at least 1 genotyped AD patient without an *APOE*4* allele.

teria for initial follow-up analysis and were prioritized into 3 groups: level 1 regions (chromosomes 2, 10, and 12), level 2 regions (chromosomes 3, 5, 6, and 15), and level 3 regions (chromosomes 1, 4, 7, 8, 14, and 20). Chromosome 19 also met criteria for initial follow-up. Further examination of the chromosome 19 findings indicated that these results were attributable to the *APOE* locus on chromosome 19 and thus the region of chromosome 19 was not included in the extended follow-up.

Follow-up analysis in the data set of the 38 families resulted in 4 of the 15 regions remaining of interest (chromosomes 4, 6, 12, and 20) (Table 2). Of these regions, the region on chromosome 12 presented the strongest evidence for linkage in the follow-up analysis and the overall combined data set. These data were verified using duplicate genotyping at both MGH and Duke University, duplicate readings of the resulting marker data, and haplotype analysis to identify recombinants that could be indicative of laboratory error. Stratification of the 54 families based on *APOE* typing resulted in 27 tier 1 families, 27 tier 2 families, and no tier 3 families. The mean age at onset was 72.7 in both tiers with an associated SD of 7.3 and 5.7 in tiers 1 and 2, respectively.

The results for the 2 chromosome 12 markers genotyped as part of the original genomic screen (*D12S1042* and *D12S373*) and the additional centromeric markers (*D12S1057* and *D12S390*) genotyped as part of the follow-up analyses are shown in Table 3. These markers span approximately 30 cM near the centromeric region of chromosome 12. The maximum parametric lod score (affecteds-only model, 0.05 misdiagnosis parameter), SimIBD (ARP), and MLS (sibpair) results for these 4 markers over the entire data set and the results for the 27 tier 1 AD families are shown in Table 3. The MLS curves for the combined genomic screening and follow-up data sets (Figure 4) and for the tier 1 families (Figure 5) show peak multipoint MLSs are 3.5 and 3.9, respectively.

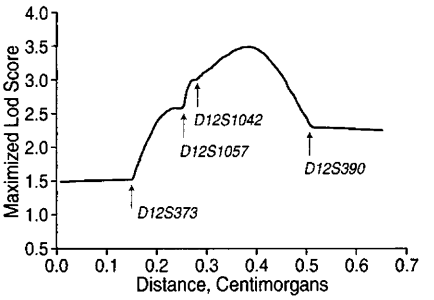

Figure 4.—Maximum lod score of the chromosome 12 markers in the combined genomic screen (n=16 families) and follow-up data set (n=38 families).

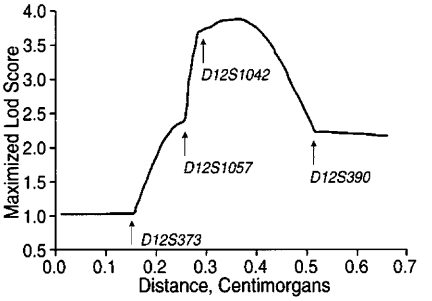

Figure 5.—Maximum lod score plot of the chromosome 12 markers in the tier 1 families (n=27) from the combined genomic screen (n=16 families) and follow-up data sets (n=38 families). Tier 1 families are the subset of families with at least 1 genotyped individual per family without an *APOE*4* allele.

COMMENT

The ability to identify additional genetic factors for AD and then examine their interaction with both *APOE* and potential environmental factors is critical to fully dissecting the etiology of this complex disease. Our studies have shown that, at most, *APOE* accounts for 50% of the total genetic effect in AD,[16] with a substantial amount of genetic variation still unexplained. Linkage analysis has been a powerful approach for unraveling the first 50% of this effect and remains the method of choice for unraveling the remaining genetic effect.

The various methods we used (lod score, ARP, and sibpair) do not examine the data in a fully independent fashion. Instead, these methods extract overlapping subsets of the complete information from the available data set, and the different methods can each provide additional information. For example, the MLS sibpair approach examines sharing only among affected sibpairs whereas the SimIBD approach examines all ARPs, including aunts, uncles, and cousins. Smalley et al[29] have shown that when the genetic model is unknown, a mixture of relative pairs can provide the most powerful approach to detecting linkage. Thus, each method has advantages and disadvantages, and a balanced approach may best decipher the unknown etiologic factors of the disease in question.

Because AD is a complex disorder, we chose a multianalytical approach to this problem that uses both model-dependent and a model-independent linkage techniques. Our first pass through the genome was not intended to identify with certainty new susceptibility genes for AD but was structured to highlight regions of interest for follow-up analysis. Thus, our critical values chosen for the follow-up criteria (lod score ≥1.0, nominal SimIBD

and sibpair P values $\leq .05$) are not used to establish linkage but to ensure a low probability of type II error (concluding that there is no linkage when there is in fact linkage). These criteria resulted in identifying 15 regions of interest for follow-up. Follow-up analysis in the second data set resulted in 4 regions of primary interest. One region, near the centromere on chromosome 12, generated by far the strongest results. The lod score, ARP, and sibpair analysis of both the genomic screen and the follow-up data set strongly support a major genetic effect in this region. The MLS obtained from sibpair analysis may be interpreted similarly to a parametric lod score.[25,26] Therefore, the peak multipoint MLS obtained from the chromosome 12 data (MLS of 3.5) exceeds the classic criterion lod score (≥ 3.0) for declaring linkage. The effect of this locus appears to be concentrated in tier 1 families, ie, those families with several patients without an *APOE*4* allele. Although these results are not based on formal statistical testing, they suggest that the effect of the chromosome 12 locus is greatest in families in which the *APOE*4* allele plays a minimal role in AD.

The purpose of our study was to identify additional major susceptibility loci for AD. For this purpose, we chose as our genomic screening set the largest multiplex families affected with late-onset AD.

These families also tended to have several members with AD who did not have the *APOE 4/4* genotype. Therefore, these families had little influence of or risk for AD from the *APOE* locus. In addition, the family structure selected for the analysis extended beyond just sibpair data. These factors must be considered when identifying additional follow-up data sets.

This region on chromosome 12 contains many known genes, any of which now becomes a candidate locus for AD. One interesting and highly plausible candidate is the low-density lipoprotein receptor–related protein (*LRP1*) locus (*LRP1* is the major receptor for APOE in neurons) for which evidence for genetic association has been previously reported.[30] However, *LRP1* maps about 10 cM distal to our peak region (data not shown). We have examined *LRP1* in detail and found no evidence of significant linkage or association in our familial or sporadic AD data sets.[31]

In summary, we have completed a genomic screen in late-onset AD to identify additional AD susceptibility loci. The data were analyzed with a multianalytical approach using several methods of analysis. Based on our a priori criteria, we identified 15 regions of the genome that were targeted for our initial follow-up analysis. Follow-up analysis of these original 15 regions resulted in 4 regions on chromosomes 4, 6, 12, and 20 that remained interesting, with the strongest results lying near the centromere of chromosome 12. Additional analyses of this region are in progress and include genotyping of new markers in the region, analysis of a comparable data set, association studies, and identification of potential candidate genes for analysis. Further follow-up of the regions on chromosome 4, 6, and 20 is also ongoing. Finding these additional AD loci will foster new studies of the etiology of AD, including the examination of interactions among genes as well as the interactions between genetic and potential environmental causes. These studies should help to unravel the mystery of this common, genetic disease of older adults, and eventually should lead to effective therapies.

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