Linkage analysis of Alzheimer disease with psychosis

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Abstract—Although a portion of risk for late-onset AD (LOAD) is attributable to APOE, the search for other loci is ongoing. The authors hypothesize that psychotic symptoms with LOAD (LOAD+P) identify a potentially more etiologically homogeneous form of AD. Linkage analysis of families with LOAD+P identified one significant and several suggestive novel linkage signals, which bolsters the conjecture of greater etiologic homogeneity.

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In the search for the genetic basis of AD, one proven strategy has been to define subphenotypes with more homogeneous genetic origins. Grouping families by age of onset has uncovered mutations causing early-onset AD and revealed the impact of *APOE*-ϵ4 alleles on late-onset AD (LOAD) liability.[1] But *APOE* alleles are neither necessary nor sufficient to cause LOAD, so investigators have searched for other loci and additional subphenotypes to find those loci.[2] We previously hypothesized that the manifestation of psychotic symptoms with LOAD could define such a critical subphenotype. Here we explore this possibility using linkage analysis.

Psychotic symptoms, such as hallucinations and delusions, have a cumulative incidence of 40 to 60% among patients with LOAD, and identify a severe phenotype with greater cognitive deficits, faster cognitive and functional deterioration, liability to aggressive behavior, and premature institutionalization. Analysis of National Institute of Mental Health (NIMH) AD Genetics Initiative data revealed familial aggregation of psychotic symptoms with LOAD (LOAD+P).[3] For broad and narrow definitions of psychosis, the odds of LOAD+P among siblings of LOAD+P probands vs siblings of LOAD probands without psychosis were 2.4 and 3.2. In light of the evidence, we hypothesized that linkage analysis of families with LOAD+P might yield new signals not evident from other LOAD genome scans.

By using samples from the NIMH AD Genetics Initiative and genotyping short tandem repeats at average intervals of 16.3 centimorgan (cM), Kehoe et al.[4] performed a genome-wide linkage analysis of LOAD. Only individuals from families who had two or more siblings with probable or definite LOAD were genotyped. Their analyses identified some suggestive linkage signals, many revealed after accounting for the effect of *APOE* on liability by conditioning on the presence of ϵ4 alleles (LOAD+ϵ4). Drawing on the same data, we adopted a similar approach, anticipating that identification of novel linkage signals would be facilitated by first accounting for the genetic variance contributed by *APOE*-ϵ4. Thus, we report the first genome scan examining LOAD, using families with two or more LOAD+P siblings who were also *APOE*-ϵ4 carriers (LOAD+P+ϵ4).

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Methods. The original Kehoe et al.[4] study genotyped only individuals from families who had two or more siblings with probable or definite LOAD. Thus, our follow-up analyses were restricted to families 1) who had two or more members diagnosed with LOAD (definite or probable), 2) whose DNA had been genotyped for the battery of short tandem repeats, and 3) who had two or more members determined to have psychotic symptoms (LOAD+P). In addition, we further partitioned these families into a subset having two or more individuals with ϵ4 alleles (LOAD+P+ϵ4).

Subjects' psychotic symptoms were characterized dur-

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Table *Family structure, in terms of the number of affected siblings per family, for the LOAD+P+ϵ4 and LOAD+P sample*

No. siblings affected per family, n	LOAD+P+ϵ4 families, n	LOAD+P families, n
2	35	56
3	6	8
4	1	1
Total	42	65

LOAD+P+ϵ4 = psychotic symptoms with late-onset AD with ϵ4 allele.

ing initial and follow-up evaluation by semistructured interviews. In a subset of subjects, this assessment was augmented by ratings on the Brief Psychiatric Rating Scale.[5] Subjects were classified as having psychosis if they demonstrated either the presence of more than one psychotic symptom or the presence of psychotic symptoms during more than one assessment (i.e., narrow definition of our earlier report[3]).

APOE genotypes were characterized previously.[6] Sixty-five families with LOAD+P were identified. Partitioning the sample by ϵ4 genotype yielded 42 families who were classified as LOAD+P+ϵ4 (table). (Although 44 families with LOAD+P+ϵ4 were identified from the public data set, two families were eliminated based on new information provided by Deborah Blacker [personal communication, 2001], a member of the Alzheimer Disease Genetics Initiative.) Linkage analyses were performed on the LOAD phenotype. For short tandem repeat allele frequencies, we used those estimated by Kehoe et al.[4] using SPLINK. Likewise, we used the genetic map given by the NIMH Alzheimer Disease Genetics Initiative (additional information related to this article can be found on the *Neurology* Web site. Go to www.neurology.org and scroll down the Table of Contents of the July 9 issue to find the title link for this article). Linkage analyses were implemented using GeneHunterPlus[7] module ASM, which assumes a linear model for risk to obtain a refined version of the original GeneHunter model.[8]

Because of the late onset of AD, identity-by-descent for affected siblings must be inferred from allele frequencies. This source of uncertainty and an additional source because of the relative sparseness of markers are expected to impact the distribution of the test statistics substantially. Thus, to evaluate the results of the linkage analysis, we first performed simulations to determine the distribution of test statistics when no disease loci were present. To do so, we used the SIMULATE package.[9] We generated 10,000 data sets for all 23 chromosomes, and determined two thresholds for maximum LOD scores (MLS) following Lander and Kruglyak[10]: the MLS expected to be achieved, on average, once from 20 genome scans (genome-wide significant linkage) and once per genome scan (genome-wide suggestive linkage). These simulations yielded critical values for genome-wide significant and suggestive linkage of 3.18 and 1.70.

Results. For our principal analysis, namely families with LOAD+P+ϵ4 (figure), we found one significant and two suggestive linkages. The significant finding, MLS = 3.52, falls on chromosome 2p, near marker D2S1356 (64.3 cM according to the Marshfield map, http://research.marshfieldclinic.org/genetics). The suggestive linkages fall on chromosome 6, near marker D6S1021 (112.2 cM, MLS = 2.01), and 21, at marker D21S1440 (36.8 cM; MLS = 1.94). For the total LOAD+P sample, one suggestive linkage (MLS = 2.51) was found on chromosome 6 and corresponded to the same peak that occurred by analysis of the families with LOAD+P+ϵ4.

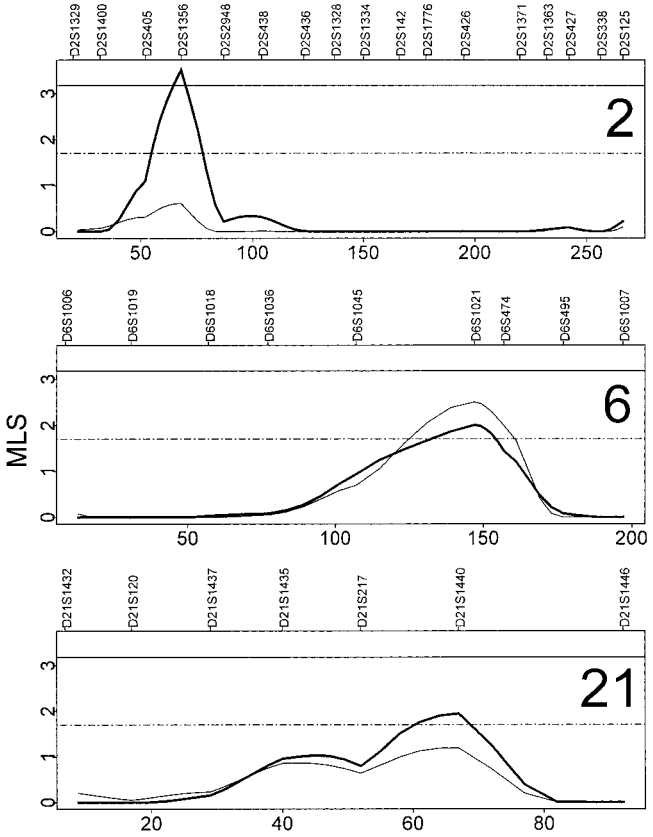

Figure. Multipoint maximum LOD scores (MLS) for families with psychotic symptoms with late-onset AD with ϵ4 allele (LOAD+P+ϵ4) (solid, thick line), and all LOAD+P families (solid, thin line). Map distances on x-axis (in centimorgans) are those given by the National Institute of Mental Health Alzheimer Disease Genetics Initiative. Solid and dashed reference lines on the y-axis indicate critical values for genome-wide significant (solid) and suggestive (dashed) linkage. (For MLS plots for all other chromosomes see the supplementary data on the Neurology *Web site. Go to www.neurology.org and scroll down the Table of Contents for the July 9 issue to find the title link for this article.)*

Discussion. The significant linkage signal on chromosome 2p, produced by analysis of families with LOAD+P+ϵ4, has no equivalent finding in AD literature. Interestingly, some support for the region can be garnered from schizophrenia literature. A suggestive linkage signal in this region has been reported in a set of extended Palauan families; follow-up multipoint studies have continued to support these results (references 11 to 22 can be found on the

Neurology Web site as part of the supplementary data. The unabridged Discussion section, which contains the reference citations, can also be found at www.neurology.org). Likewise, highly suggestive linkage has been found in the same region in a large sample of families of European ancestry.

The suggestive linkage on 6q for the LOAD+P+ϵ4 sample, and in the total LOAD+P sample, was more distal from the best linkage signal produced in other analyses of AD families. In the original Kehoe et al. analysis, they found an MLS = 1.4, near D6S1018, at 80 cM. A weak signal for linkage to chromosome 6 has also been reported near markers D6S1004 and D6S391, at roughly 90 cM. Interestingly, linkage to the more distal region of chromosome 6q identified in the current study has also been found in studies of schizophrenia.

We have previously proposed that multiple genes, no single one of which is a major locus for idiopathic psychoses, could increase the risk for psychosis onset in the context of neurodegenerative and neurodevelopmental conditions. The convergence of linkage findings on chromosomes 2p and 6q in schizophrenia and LOAD+P is consistent with this hypothesis. However, this interpretation should be made cautiously. Despite the common psychosis phenotype, there are substantial clinical and neurobiologic differences between the two disorders, and the chromosomal region in which linkage has been detected in these two conditions is broad. Moreover, alternative models are possible. For example, variants in genes may contribute major risk for idiopathic psychoses such as schizophrenia and for AD+P risk, or variants in genes that increase the liability to onset of LOAD could increase risk for psychosis.

Although other studies have found evidence for linkage on 21q,[4] their evidence comes from the ϵ4-negative families, whereas our evidence derives from LOAD+P+ϵ4 families. (We note the 21q result may not be robust because of discrepancies among maps of this region. Supplementary information related to this article can be found on the *Neurology* Web site). Chromosome 21 harbors two genes previously implicated in neurodegenerative disorders, namely superoxide dismutase 1 (*SOD1*), which physically maps between 30 and 40 Mb, and the amyloid beta (A4) precursor protein (*APP*), which maps between 23 and 24 Mb. In our analysis, the maximum LOD score occurs near the marker D21S1440, which maps to 35 to 36 Mb (see http://genome.ucsc.edu and http://bioinformatics.weizmann.ac.il/udb for mapping details). Although *SOD1* is slightly closer than *APP* to the peak linkage signal, we could not rule out (or in) either gene as the source of the linkage signal on this chromosome. Contribution of these genes to psychosis risk would be consistent with the model in which genes that increase the liability to onset of LOAD also increase the risk for psychoses. Although one study suggested the possibility of *APP* mutation contributing to schizophrenia, studies of the most important LOAD risk gene, *APOE*, have not found an association with schizophrenia or LOAD+P risk.

The regions of significant and suggestive linkage on chromosomes 2p and 6q also contain many genes expressed in the CNS, although none are known to be strongly associated with neurodegenerative illness. Of interest given the potential association of *SOD1* with neurodegenerative illness, the *SOD2* gene localizes to the region of suggestive linkage detected on chromosome 6q. Although *SOD2* is not clearly associated with neurodegenerative illness, *SOD2* nullizygous mice develop spongiform encephalopathy.

We have hypothesized that LOAD+P, which marks a more severe and progressive form of LOAD, could define a more homogeneous form for genetic study. Our previous results on the familial aggregation of LOAD+P support this proposal.[3] Although not definitive, the significant and suggestive linkages reported here bolster the case. Together they suggest that the characterization of psychosis in LOAD could open a new avenue for productive exploration of the genetic basis of LOAD.

Appendix

Data and biomaterials were collected in three projects that participated in the National Institute of Mental Health (NIMH) Alzheimer Disease Genetics Initiative. From 1991–1998, the principal investigators and coinvestigators were: Massachusetts General Hospital, Boston, MA, U01 MH46281, Marilyn S. Albert, PhD, and Deborah Blacker, MD, ScD; Johns Hopkins University, Baltimore, MD, U01 MH46290, Susan S. Bassett, PhD, Gary A. Chase, PhD, and Marshal F. Folstein, MD; University of Alabama, Birmingham, AL, U01 MH46373, Rodney C.P. Go, PhD, and Lindy E. Harrell, MD. Genotypes were generated from biomaterials in the NIMH Alzheimer Disease Genetics Initiative in the laboratories of: Alison Goate, PhD, Department of Psychiatry, Washington University School of Medicine, St. Louis; John Hardy, PhD, Mayo Clinic, Jacksonville; Mike Owen, MD, PhD, Department of Psychological Medicine, University of Wales College of Medicine, Cardiff.

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