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Follow-Up Mapping Supports the Evidence for Linkage in the Candidate Region at 9q22 in the NIMH Alzheimer's Disease Genetics Initiative Cohort

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Other than the *APOE* peak at 19q13, the 9q22 region was identified in our original genomic scan as the candidate region with the highest multipoint lod score (MLS) in the subset of late onset Alzheimer's Disease (AD) families (MLS = 2.9 at 101 cM) from the NIMH Genetics Initiative sample. We have now genotyped an additional 12 short tandem repeats (STR) in this region. Multipoint analysis shows the region remains significant with an increase in the peak MLS from 2.9 to 3.8 at 95 cM near marker D9S1815, and the 1 LOD interval narrows from 21.5 to 11 cM. HLOD scores also provide evidence for significant linkage (4.5 with an $\alpha = 31\%$) with a further narrowing of the region to 6.6 cM (92.2–98.8 cM). Single nucleotide polymorphisms (SNPs) in the Ubiquilin1 gene (*UBQLN1*), located at 83.3 cM, have been reported to be significantly associated to AD, accounting for a substantial portion of the original linkage signal [Bertram et al., 2005]. Our analyses of the higher resolution genotype data generated here provide further support for the existence of a least one additional locus on chromosome 9q22. In an effort to pinpoint this putative AD susceptibility gene, we have begun to analyze SNPs in other candidate genes in and around this narrowed region to test for additional associations to AD.

KEY WORDS: chromosome; neurodegenerative; STR; genomic scan; SNPs

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INTRODUCTION

Alzheimer's Disease (AD) is a devastating neurodegenerative disorder of late life. Early onset (<65) AD occurs in less than 5% of cases, is usually familial, and exhibits an autosomal dominant mode of inheritance. Most cases of AD are late-onset (LOAD) with a complex inheritance involving multiple genes and/or environmental factors [Cummings and Cole, 2002; Bertram and Tanzi, 2004]. Apolipoprotein E (*APOE*), located on 19q, is the only gene that has consistently been demonstrated to be a major risk factor for the late-onset form [Blacker and Tanzi, 1998; Katzman et al., 1998]. *APOE* does not account for all the genetic variability; therefore, it is hypothesized that there are additional genes contributing to LOAD that remain to be identified [Kamboh, 2004]. Several genome-wide linkage scans [Pericak-Vance et al., 1997, 2000; Kehoe et al., 1999; Myers et al., 2002; Blacker et al., 2003; Lee et al., 2004; Sillen et al., 2006] and association studies [Zubenko et al., 1998; Hiltunen et al., 2001; Farrer et al., 2003] have identified multiple candidate regions on several chromosomes that may harbor these other LOAD genes.

The most significant linkage peak we found in our 9 cM full genome scan on the NIMH AD Genetics Initiative sample was at 19q13, consistent with the location of the *APOE* gene [Blacker et al., 2003]. The next most suggestive peak, identified at 9q22 near marker D9S283, had the highest multipoint lod score (MLS) in the subset of LOAD families (MLS = 2.9 at 101 cM). Evidence for linkage in the 9q21-31 region has also been confirmed in data sets containing NIMH and other LOAD families [Kehoe et al., 1999; Myers et al., 2002], and several linkage analyses of the data utilizing various covariates [Curtis et al., 2001; Olson et al., 2002; Holmans et al., 2005], although a recent genomic scan in a small, homogenous AD population did not find evidence for linkage to this region [Sillen et al., 2005]. We now report results for the follow-up mapping of this 9q22 candidate peak in the complete NIMH Genetics Initiative sample with additional short tandem repeat (STR) markers.

Our colleagues at MGH recently genotyped single nucleotide polymorphisms (SNPs) in Ubiquilin 1 (*UBQLN1*), a gene located at 83.3 cM, according to the Rutgers combined linkage map [Kong et al., 2004], and reported a strong association to AD in the NIMH set and in an independent set of sibships from the Consortium on Alzheimer's Genetics (CAG) [Bertram et al., 2005]. Although the association of these and other *UBQLN1* SNPs to AD risk and age of onset has been confirmed in case-control and family based samples [Slifer et al., 2005; Kamboh et al., 2006], a recent large study consisting of five AD case-control series did not find any evidence of association to AD for any of these SNPs in *UBQLN1* [Smemo et al., 2006]. The MGH group also showed an almost 50% reduction of the original

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linkage signal when families transmitting the risk allele (C) of one of the SNPs (UBQ-8i) to two affected individuals were removed. We have performed a formal test of the follow-up linkage data conditioned on *UBQLN1* genotype. Our results support the previous observation that *UBQLN1* does not account for all of the linkage signal in the 9q22 region. Therefore, we have begun family based association testing of SNPs in other AD candidate genes in this enhanced region.

MATERIALS AND METHODS

Study Population: NIMH AD Genetic Initiative Families

Subjects were collected as part of the NIMH-Genetics Initiative following a standardized protocol utilizing the NINCDS-ADRDA criteria for diagnosis of definite, probable, and possible AD [McKhann et al., 1984; Tierney et al., 1988]. A total of 468 families were ascertained. The primary structure of these families are affected sib-pairs and the ethnic make-up was primarily Caucasian (95%). In 437 of these families, the mean age of onset (MAO) of affected family members were above 50 (72.4, range = 50–97). (Those with MAO $\leq$50 were believed to be enriched for the *APP*, *PS1*, and *PS2* mutations, and were therefore dropped from the analyses.) The linkage and mapping results presented here are from a subset with a MAO $\geq$65 (LOAD families), identified in 320 families with the same primary structure and ethnic make-up as the total set of families. Blood was collected and sent to the NIMH repository at Rutgers University where genomic DNA was extracted from lymphocyte cell lines. The STRs used in the original scan were genotyped by the Center of Inherited Disease Research (CIDR), and were combined with additional STRs described below.

STR Genotyping

STRs flanking the 9q22 peak were chosen from the genomic database (www.gdb.org) and deCODE Genetics (www.nature.-com/ng/journal/v31/n3/extref/ng917-S13.xls). We used the sex average distances in the Rutgers combined linkage-physical map, Build 35 (compgen.rutgers.edu/mapomat) [Kong et al., 2004]. The genetic locations of the STRs in the recently updated Build 36.1 have not changed. A total of 21 additional STRs were identified for saturation of the 9q22-31 peak region between D9S922 and D9S934, inclusive (~80–128 cM). The mapping order for 3 of the 21 markers did not agree between the genomic database and deCODE maps and were therefore not genotyped. The remaining 18 additional STRs were genotyped for follow-up mapping, but only 12 were used in the final analysis (Table I). Five of the six markers removed from the analysis had an excessive number of genotyping and Mendelian errors. The other marker, D9S318, had an estimated heterozygosity of 0.44, which resulted in far greater variance in IBD estimates compared to the other markers (heterozygosity >0.6), and therefore led to a greater bias towards the null as shown by Schork and Greenwood [Schubert et al., 1995]. All genotypings were performed with the Beckman/Coulter CEQ 8000 capillary electrophoresis platform with WellRED dyes (Beckman/Coulter, Fullerton, CA). For PCR, dye labeled (Proligo, Boulder, CO) and M13-tailed primers (IDT, Coralville, IA) were used in a total volume of 20 μl with standard conditions [Boutin-Ganache et al., 2001]; Dye selection for each STR was based upon the sensitivity of each dye and the amplification efficiency and size range of each STR. After PCR, products were pooled into three groups for simultaneous analyses on the CEQ (www.beckman.com/resourcecenter/literature/BioLit/BioLi-tList.asp, misc. doc. #608113af). The ratio of each of the 18 flanking STRs in the pooled product was also determined by the dye selection criteria above (D9S152, D9S252, D9S1815, D9S1801, D9S51 = 1:1:2:1:2, respectively; D9S1877, D9S776, D9S1680, D9S257, D9S777, D9S1820 = 1:2:1:1.3:3:3, respectively; D9S318, D9S1803, D9S280, D9S1809, D9S176, D9S1690, D9S1784 = 1:3.5:1.3:3:7:1.2:2) so that ~1.0 μl of pooled product was added to the final reaction before running on the CEQ. Binning of alleles for automatic genotyping was performed with the CEQ software (ver. 6.0.75) to allow allele calling by the software. The allele sizes were read and confirmed by two separate readers.

TABLE I. Markers Used in the Study, and Their Positions on the Rutgers Combined Linkage-Physical Map

Position (cM)	Original	Follow-up
78.16	**D9S922**	
82.14		**D9S152**
83.32		**D9S1877**
85.15		D9S776
86.22		**D9S252**
87.77		**D9S1680**
89.48		**D9S257**
90.3		D9S777
91.94	**D9S283**	
93.95		**D9S1820**
94.45		D9S318
95.92		**D9S1815**
97.18		D9S1803
99.34		**D9S1809**
100.57		D9S280
101.7	**D9S910**	
102.5		D9S176
104		**D9S1690**
106.1	**D9S938**	
108.4		**D9S1784**
112.3		**D9S1801**
117.8	**D9S930**	
123		**D9S51**
126.4	**D9S934**	

Markers in bold were used in the final analyses.

Linkage Analyses

Model-free linkage analysis was performed using the program Genehunter Plus with extensions to calculate the Kong and Cox statistic [Kruglyak et al., 1996; Kong and Cox, 1997] and the program SIBPAL in the software package SAGE 4.5 [S.A.G.E., 2002] that incorporates affected, unaffected, and discordant pairs via the Haseman and Elston [1972] regression method. SIBPAL also allows covariates to be added to the analysis in a straightforward manner and was used to adjust for *APOE* genotype, gender, and education. The estimates of the proportion of alleles shared identical-by-descent (IBD) that we used for this technique were multipoint estimates calculated by the program GENIBD in SAGE 4.5 [S.A.G.E., 2002]. Maximum likelihood estimates of allele frequencies were calculated with the SAGE program FREQ taking into account the family relationships. Replicates were performed on selected samples and any Mendelian errors were detected with the SAGE program MARKERINFO, as well as detected implicitly by all analytical programs used here. Empirical genome-wide *P*-values for the linkage results were calculated by gene-dropping analysis [Sawcer et al., 1997; Kruglyak and Daly, 1998] using the program MERLIN with 1000 iterations [Abecasis et al., 2002]. Heterogeneity lod score statistics (HLOD) were obtained in GeneHunter Plus using an admixture parametric model [Terwilliger and Ott, 1994]. The parameters used here were identical to those described in the original scan, which was an age dependent, dominant inheritance model with a gene frequency of 0.02 [Blacker et al.,

2003]. A formal test of the amount of the linkage evidence accounted for by the *UBQLN1* locus was performed with the Genotype IBD Sharing Test (GIST) [Li et al., 2004a] and the program LAMP [Li et al., 2005].

RESULTS

Figure 1 shows the MLS and HLOD score statistic for the 9q22 peak region in the LOAD subset (320 families, MAO $\geq$65), from the original CIDR scan with six of the markers covering this region (Mean Interval Distance (MID) = 8 cM) (Fig. 1a) and from the current follow-up scan with 12 additional markers (MID = 2.7 cM) (Fig. 1b). The peak MLS increases substantially from 2.9 at 101 cM to 3.8 at 95 cM (near D9S1815). In addition, the interval that encompasses the maximum LOD score minus 1 LOD on either side of the maximum (1 LOD interval) narrows by almost 50%, from 21.5 to 11 cM**.** The empirical *P*-value through the 1 LOD region does not exceed 0.003 [Sawcer et al., 1997; Kruglyak and Daly, 1998; Abecasis et al., 2002]. The peak HLOD statistic score also increases from 2.3 with $\alpha = 26\%$ in the original scan (Fig. 1a) to 4.5 with $\alpha = 31\%$ in the final analysis (Fig. 1b). Importantly, the 1 LOD interval for the HLOD statistic in the final scan greatly narrowed from 27 to 6.6 cM (92.2–98.8 cM). We included *APOE*, gender, and education—all shown to be associated with the development of AD—as covariates in the Haseman–Elston (HE) model [Haseman and Elston, 1972]. SIBPAL analysis showed the regions under the peak that were significant before the covariate adjustment were still significant after covariate adjustment (e.g., for D9S1815, no covariates $P = 0.003$; with covariates $P = 0.007$). Therefore, even after controlling for these previously reported associations, there was still strong evidence for linkage in this region.

To test how much of the follow-up linkage signal was accounted for by *UBQLN1*, we used the programs GIST [Li et al., 2004a] and LAMP [Li et al., 2005]. For the GIST program the maximum statistic obtained was a weighted NPL score of 2.898 at 78 cM ($P = 0.08$), which is 5 cM proximal to the position of *UBQLN1*. At 83 cM, GIST did not indicate any evidence that the linkage signal was accounted for by the previously associated *UBQLN1* polymorphism, UBQ-8i ($P = 0.385$) [Bertram et al., 2005]. LAMP, which calculates evidence for various models of linkage and association involving a specific locus hypothesized to be associated with the observed trait, indicated significant evidence ($P = 0.006$) that at least one locus other than *UBQLN1* was the source of the evidence for linkage on 9q22.

DISCUSSION

Linkage Peak at 9q22

The peak MLS of 2.9 at 101 cM for the 9q22 region in the LOAD families of the original scan [Blacker et al., 2003] met the Lander and Kruglyak [1995] criteria for 'suggestive' linkage. Our follow-up mapping of the 9q22 region in the same LOAD families from the NIMH cohort used in the original scan and using an additional 12 STRs, results in a noticeable increase of the MLS to 3.8 at 95 cM. Additionally, the 1 LOD interval of support in the follow-up scan narrows substantially from 21.5 to 11 cM, which reduces the area to search for possible AD succeptibility genes by almost 50%. The empirical *P*-value through the 1 LOD region does not exceed 0.003 [Sawcer et al., 1997; Kruglyak and Daly, 1998; Abecasis et al., 2002]. Because of the likelihood of genetic heterogeneity in this complex disease, the HLOD score statistic [Terwilliger and Ott, 1994], which allows for linked and unlinked families in the sample, was performed on the original CIDR marker set and the follow-up set of markers. A substantial increase in the peak HLOD score for the LOAD families was observed in the follow-up set (HLOD = 4.5 with $\alpha = 31\%$) over the original scan (HLOD = 2.3 with $\alpha = 26\%$). The non-significant increase in α from 0.26 to 0.31 may reflect the additional information gained by a more dense set of markers. The additional narrowing of the 1 LOD interval from 11 to 6.6 cM reduces the search area by another 43%.

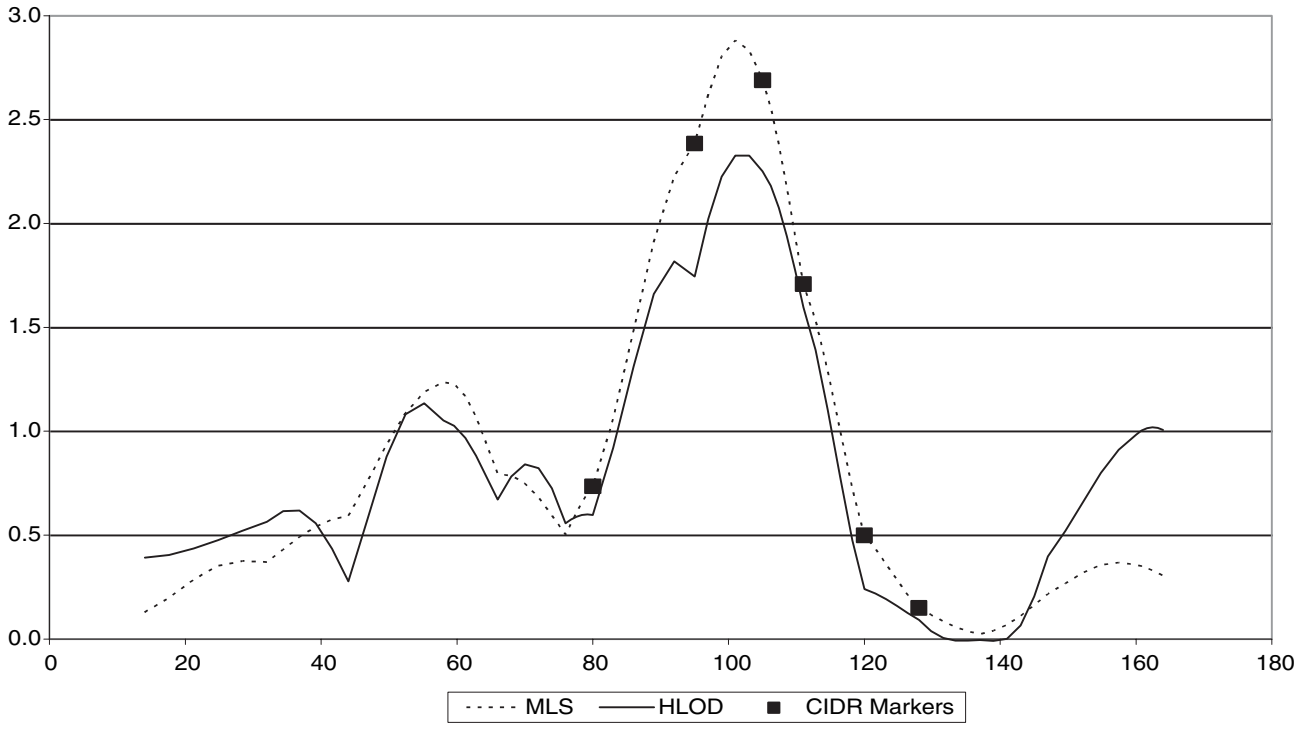
When the 12 additional markers are added to the original six markers, the resulting MID equals 2.7 cM. The addition of these 12 markers increased the information content (IC), which is dependent upon position and is calculated by GeneHunter Plus using all the families in the study. In the region we added the additional markers, the IC increased from 0.5 from the original scan to 0.7, as calculated by GeneHunter Plus. An IC estimate close to 0.7 is the theoretical maximum for sibpair families, which is the predominant structure in the NIMH families [Evans and Cardon, 2004], therefore the 12 STRs genotyped in the follow-up analysis were sufficient to saturate the region and genotyping of additional STRs was not required.

Inclusion of *APOE*, gender, and education-all shown to be associated with the development of AD—as covariates in the Haseman–Elston (HE) model [Haseman and Elston, 1972], led to similar results as those from Genehunter Plus, with the greatest evidence for linkage around 100 cM. Regions under the peak that were significant before the covariate adjustment were still significant after covariate adjustment.

This 9q22-31 region has also been confirmed as having MLS >1 in data sets containing NIMH with other LOAD families [Kehoe et al., 1999; Myers et al., 2002]. The Kehoe et al. [1999] study was the first to implicate this region, however it only included the smaller first release subset of NIMH families (n = 230, 543 definite and probable affected; 230 unaffected), while the LOAD subset derived from the final and complete set of NIMH families (n = 437, 930 definite and probable affected; 433 unaffected) was utilized here (A complete description of the family information can be found at http://zork.wustl.edu). Additionally, several groups have confirmed linkage to the 9q region in these families, utilizing different covariates and methods in the linkage analyses. When Curtis et al. [2001] applied a model-free linkage analyses method conditional on genotypes at established risk loci to the earlier dataset, the 9q linkage evidence was enhanced. Olson et al. [2002] noted inclusion of *APOE* genotype as a covariate in their analytical model reconfirmed the linkage signal at 9q in the same earlier NIMH dataset. Recently, Holmans et al. [2005] reported the 9q linkage signal significantly increased when mean rate of decline was included as a covariate in the final, complete NIMH set, and the significance remained after allowing for *APOE* effects. They also found some increase in the 9q linkage signal in a separate, but smaller cohort of samples from UK. Recently, a cohort of AD families from a relatively genetically homogenous population did not find any evidence for linkage to chromosome 9; however, the cohort was relatively small with 71 families [Sillen et al., 2006]. Based on the results of these previous scans utilizing various analytical models including families from the NIMH sibling cohort, and our current follow-up results from model-free, HLOD parametric, and Haseman–Elston analyses that controlled for *APOE*, gender, and education on the complete NIMH set of LOAD families, we hypothesize that there is at least one or more susceptibility genes linked to AD in this region.

UBQLN1

Ubiquilin 1 (*UBQLN1*) is a candidate gene located 10 cM proximal to our narrowed peak, around 83.3 cM. UBQLN1 is involved in protein degradation, interacts with presenilins 1 and 2, and colocalizes with neurofibrillary tangles in the brains of AD patients [Mah et al., 2000]. Recently, our



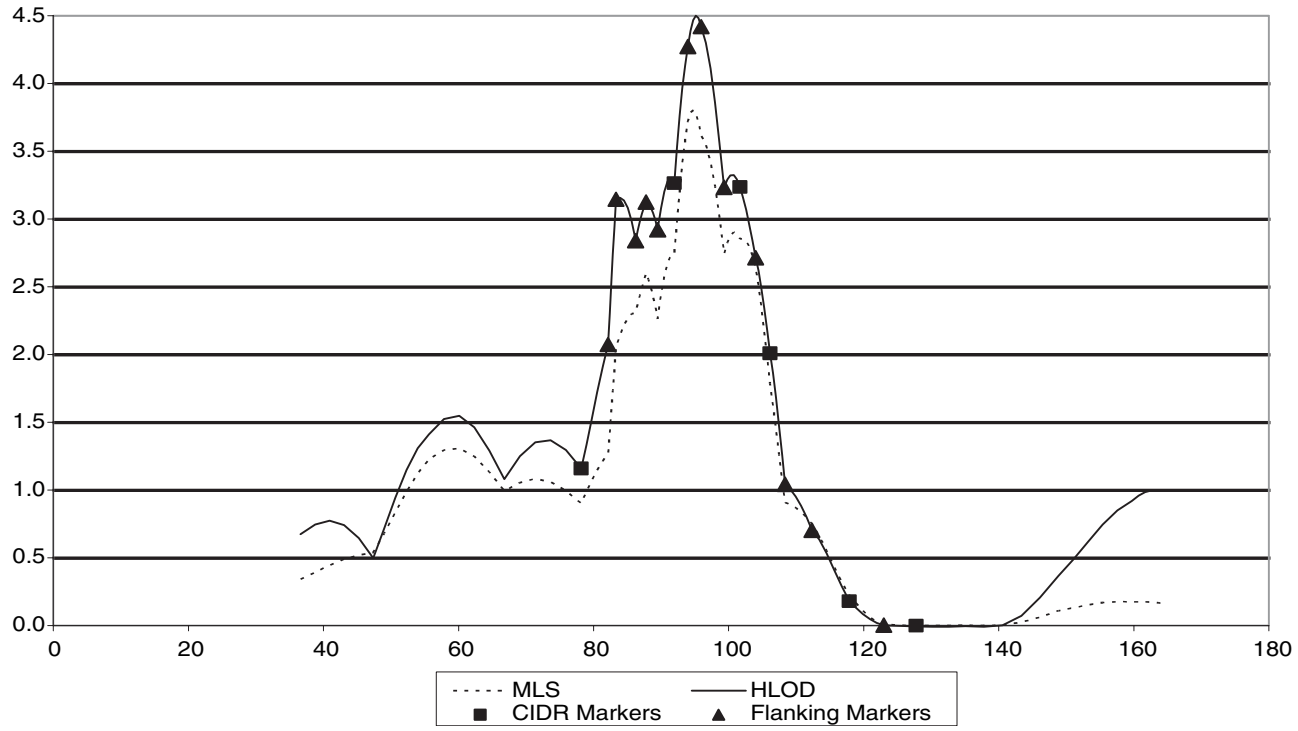


Fig. 1. MLS and HLOD scores for chromosome 9 in the late onset subset of NIMH families. **A**: The original genomic scan with the five CIDR markers located around the peak; (**B**) the current follow-up mapping of the 9q22 candidate region with 12 flanking markers.

colleagues at MGH genotyped several SNPs located in *UBQLN1* and found significant associations to AD in the NIMH sample and in an independent set of sibships from the Consortium on Alzheimer's Genetics (CAG) sample [Bertram et al., 2005]. Additional studies have confirmed association of these and additional *UBQLN1* SNPs in case-control samples and family based samples to AD risk and age of onset [Slifer et al., 2005; Kamboh et al., 2006]. However, a large study consisting of five AD case-control series did not find any evidence of association to AD for any of these SNPs [Smemo

et al., 2006]. The MGH group also found an almost 50% reduction with in the original linkage signal in this region when families transmitting the risk allele for one of the *UBQLN1* SNPs to two affected individuals were removed, and a shift q-ter of the residual linkage signal. The closest STR to *UBQLN1* that we genotyped is D9S1877, located at 83.3 cM, which has an MLS score of 2.1, and an HLOD of 2.98. The GIST program revealed that the previously associated UBQ-8i variant accounts for part of the linkage signal observed near 78 cM ($P = 0.08$; similar to the results of the original study), but there is no evidence for an UBQ-8i effect on the linkage signal near the newly added marker, D9S1877 at 83 cM ($P = 0.4$). Furthermore, LAMP indicated significant evidence ($P = 0.006$) that at least one other locus other than *UBQLN1* was the source of evidence for linkage to this 9q22 region. Taken together, these results support the earlier observation that the *UBQLN1* variants tested by Bertram et al. [2005] may not account for the entire linkage signal at 9q22, and that additional variants in this or other AD succeptibility genes remain to be identified in this region.

Other Candidate Genes

Candidate genes located within the 1 LOD region (92.2–98.8 cM) that we report here from our follow-up mapping and related to neuronal function or to biological pathways known to be involved in AD, such as Aβ production/processing, inflammation, or athersclerosis, are of first consideration to us. One such gene is serine palmitoyltransferase long chain 1 (*SPTLC1*), located at 96.5 cM, which codes for a key enzyme in sphingolipid biosynthesis including ceramide production [Bejaoui et al., 2001]. Ceramides are not only essential components of sphingomyelins which are structural moities of plasma membranes, but also play important roles in neuronal function by regulating rates of neuronal growth, differentiation, and death [Buccoliero and Futerman, 2003]. In addition, ceramides regulate both APP processing and Aβ generation by affecting the molecular stability of *BACE1*, the rate-limiting enzyme for the amyloidogenic pathway [Puglielli et al., 2003]. We genotyped three SNPs in introns 1 and 12 and the 3′UTR of *SPTLC1* in the NIMH set of families using family based association testing. Analysis of the SNPs singly or as a haplotype did not reveal any significant association to AD (data not shown).

There are inherent uncertainties in the parameters and methods used to narrow a peak in follow-up scans for complex traits, such that the estimate of a position of a gene can vary more than 20 cM [Lander and Schork, 1994; Altmuller et al., 2001; Papachristou and Lin, 2006]. Therefore, we have also identified three candidate genes located just outside our narrowed 1 LOD region (92.2–98.6 cM) that have strong biological relevance to AD neuropathological pathways: Neurotropic tyrosine kinase receptor 2 (*NTRK2*) located at 84.4 cM, transforming growth factor-beta receptor, type I, *TGFBRI*, located at 102 cM, and ATP-binding cassette, subfamily A, member 1, *ABCA1*, located at 106.9 cM. NTRK2 is the receptor for brain-derived neurotrophic factor (BDNF) [Soppet et al., 1991; Squinto et al., 1991]; both NTRK2 and BDNF regulate both short-term synaptic functions and long-term potentiation of brain synapses [Minichiello et al., 1999, 2002; Rico et al., 2002]. *TGFBR1* codes for a receptor that is activated by the binding of the multifunctional cytokine, TGFB1 to TGFBRII, [Heldin et al., 1997; Akiyama et al., 2000]. TGFB1 is a key regulator of the brain's responses to injury and inflammation [Mattson et al., 1997], and we recently reported a significant association of a promoter SNP in *TGFB1* to AD in the NIMH set of families [Dickson et al., 2005]. ABCA1 is a transporter molecule that mediates the efflux of cholesterol and other lipids out of the cell to lipid-depleted HDL apolipoproteins [Santamarina-Fojo et al., 2001; Oram and Heinecke, 2005]. APOE is involved in the binding and clearance of lipid-containing particles from plasma and the E4 allele of *APOE* is the strongest risk factor for AD and age of onset, after age [Blacker et al., 1997]. Using *ABCA1* knock-out mice, three separate groups recently reported the lack of *ABCA1* appears to decrease APOE levels and may increase amyloid deposition [Hirsch-Reinshagen et al., 2005; Koldamova et al., 2005; Wahrle et al., 2005].

We have begun investigating these candidate genes by genotyping SNPs in these genes and testing for association to AD. We genotyped two SNPs located in introns 5 and 22 of *NTRK2*, the R219K SNP in exon 7 of *ABCA1*, and a SNP located in the 3′ UTR of *TGFBR1* in the NIMH set of families. Using family based association implemented in the program FBAT [Lake et al., 2000; Horvath et al., 2001] under the dominant model, we only found a marginal association ($P = 0.06$) to AD for the intron 22 SNP (rs4412435) of *NTRK2*; Haplotype analysis of the *NTRK2* SNPs did not reveal any significant association to AD (data not shown). Similarly, a Finland study did not find any significant association of three SNPs in *NTRK2* to AD [Vepsalainen et al., 2005]; these SNPs are located 5′ to the intron 5 SNP that we investigated. Most of the case-control and family based association studies of 16 SNPs distributed throughout *ABCA1* have been negative for risk of development of AD in Caucasion and Hispanic subjects from European and North American populations [Wollmer et al., 2003; Katzov et al., 2004; Li et al., 2004b; Bertram et al., 2005; Kolsch et al., 2006; Shibata et al., 2006].

Future Direction

The lack of significant association to AD that we observed from the genotyping of 1–3 SNPs in these four candidate genes is not conclusive. Because of the linkage disequilibrium (LD) block structure in genes and chromosome regions, association of SNPs in a gene to a particular trait can vary; therefore, an association may still be present for SNPs located in other LD blocks in these genes [Gabriel et al., 2002]. According to HapMap [Int HapMap, 2003] (http://www.hapmap.org) looking in the CEPH reference population: the two variants we genotyped in *NTRK2* are haplotype tag (ht) SNPs located in blocks 2 and 19 of the 20 haploblocks of the gene, one of the three SNPs in *SPTLC1* we genotyped is a htSNP located in the first of three haploblocks in the gene while the other two are located in the third haploblock, the R219K SNP of *ABCA1* we genotyped is located in block 6 of approximately 16 haploblocks in the gene, and there is only one haploblock present in *TGFBR1*. The genotyping of additional tagged SNPs in *NTRK2*, *SPTLC1*, *ABCA1*, and *TGFBR1* and additional candidate genes from this region would be one way to more accurately determine if any significant association of these genes to AD exists. Using the Perlegen database of SNPs (http://www.perlegen.com) and HapMap, the LD block structure in this region can be analyzed for the identification of htSNPs to provide a SNP LD map of the region [Couzin, 2002; Terwilliger et al., 2002; Zhang and Jin, 2003; Huang et al., 2004; Schaid et al., 2004]. HapMap shows 160 haplotype blocks located in this 1 LOD region, ranging in size from 0.032 to 432 Kb. Combining data from both the Perlegen and HapMap project, the average SNP density in this 1 LOD region (92.2–98.8 cM) is ~1 SNP/2 Kb. If only htSNPs and SNPs not assigned to LD blocks are selected, we calculate the average density would decrease to 1 SNP/6.8 Kb. Therefore, we suggest high density SNP genotyping at an average density of ~1 SNP/5 Kb is required to capture the genetic variation within this 1 LOD region, which would then allow a family based association analytical approach to exploit the LD between disease risk alleles and marker alleles as a next

necessary step in localizing the LD segment to a smaller area or to candidate gene(s).

However, the identification of blocks and htSNPs is highly algorithim dependent and based on the SNPs chosen by HapMap [Hewett et al., 2002]. An alternative approach would be to perform dense SNP genotyping at regular intervals of ~1 SNP/5 Kb in the 1 LOD region (6.6 cM) [Gabriel et al., 2002; Hewett et al., 2002; Nsengimana et al., 2005] to assess any evidence for association/linkage to AD. With LD blocks as small as 0.032 Kb identified in this 1 LOD region, this too has the limitation of possibly missing an area that might contain SNPs associated with AD.

We have begun to follow these strategies for following-up the marginal association to AD that we found for the intron 22 SNP in *NTRK2*. As a first step, we are currently genotyping 12 additional SNPs, of which 11 are ht SNPs located in 6 additional LD blocks of *NTRK2*. The 14 total SNPs we have genotyped represents 40% of the block structure in *NTRK2*. Preliminary analysis indicates a significant association for haplotypes that include two intronic SNPs that are within elements that bind transcription and alternative splicing binding factors located in regions of *NTRK2* not reported by others. Detailed genotyping results for *NTRK2* will be presented in a later publication. We plan to continue with these strategies by genotyping additional SNPs at increased densities in *NTRK2* and the other candidate genes in this region for localizing the LD segment and the association to AD to a smaller area.

In conclusion, we have genotyped an additional 12 STRs in the 9q22-31 region (MID = 2.7 cM) in the NIMH AD Genetics Initiative cohort to follow-up our initial suggestive linkage findings for this area in this cohort [Blacker et al., 2003]. We now demonstrate significant linkage to this region in the LOAD families (MAO $\geq$65, MLS = 3.8, empirical $P = 0.003$) around 95 cM near D9S1815 and narrowing of the 1 LOD interval of support from 21.5 to 11 cM, reducing the area potentially harboring AD succeptibility genes by almost 50%. The peak remained significant even when covariates (*APOE*, gender, education) were included. HLOD scores, which allow for genetic heterogeneity, were noticeably increased from the original scan to a peak score of 4.5 with an $\alpha = 31\%$, and there was further narrowing of the 1 LOD support region to 6.6 cM. With supporting evidence for linkage to this region from other genomic scans, the increased evidence of linkage we find in the LOAD families with corresponding narrowing of the region, and the observation that the currently tested variants in *UBQLN1* do not account for all the linkage signal at 9q22, we conclude there is compelling evidence that additional variants in *UBQLN1* or other AD succeptibility gene(s) may be located within or adjacent to this 1 LOD region. We have found marginal association of one intronic SNP in another gene, *NTRK2*, near our 1 LOD region, to the NIMH cohort and preliminary analysis for genotyping 12 additional SNPs in *NTRK2* indicates significant association to AD for haplotypes containing SNPs from other regions of *NTRK2* that are located in different LD blocks. Therefore, we plan to perform dense SNP association testing in *NTRK2* and additional candidate genes from within and around this 1 LOD region, taking advantage of information from the HapMap project to perform haplotype analyses of htSNPs and SNPs not located in blocks, in order to localize the LD segment and association to AD to a smaller area for the identification of AD succeptibility genes in or adjacent to this 6.6 cM region on chromosome 9q22.

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