Genome-wide linkage analyses of non-Hispanic White families identifies novel loci for familial late-onset Alzheimer's disease

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Abstract

INTRODUCTION—Few high penetrance variants that explain risk in Late-onset Alzheimer's disease (LOAD) families have been found.

METHODS—We performed genomewide linkage and identity-by-descent (IBD) analyses on 41 non-Hispanic Caucasian families exhibiting likely dominant inheritance of LOAD, and having no mutations at known familial AD loci and a low burden of *APOE* ε4 alleles.

RESULTS—Two-point parametric linkage analysis identified 14 significantly linked regions, including three novel linkage regions for LOAD (5q32, 11q12.2-11q14.1 and 14q13.3), one of

which replicates a genomewide association LOAD locus, the *MS4A6A-MS4A4E* gene cluster at 11q12.2. Five of the 14 regions (3q25.31, 4q34.1, 8q22.3, 11q12.2-14.1 and 19q13.41) are supported by strong multipoint results (LOD* 1.5). Non-parametric multipoint analyses produced an additional significant locus at 14q32.2 (LOD*=4.18). The 1-LOD confidence interval for this region contains one gene, *C14orf177*, and the miRNA *Mir_320*, while IBD analyses implicates an additional gene *BCL11B*, a regulator of brain-derived neurotrophic signaling, a pathway associated with pathogenesis of several neurodegenerative diseases.

DISCUSSION—Examination of these regions following whole genome sequencing may identify highly penetrant variants for familial LOAD.

Keywords

Non-Hispanic White; Late Onset Alzheimer's Disease; linkage; high penetrance; identity-by-descent; familial; genetics

1. BACKGROUND

While more than two dozen loci that contribute to late-onset Alzheimer disease (LOAD) have been identified [1], few genes with highly penetrant rare variants (e.g. *APP*, *PSEN1* and *PSEN2* in early-onset familial AD [2]) that explain risk in families heavily burdened with LOAD have been found. It is likely that rare variants contribute to complex disease, however [3], and recent reports implicating rare variants in *PLD3, APP* and *TREM2* [4–7] support their involvement in both sporadic and familial LOAD. Identification of additional rare mutations driving genetic risk in familial LOAD will help in defining new pathways for therapeutic and preventive treatments.

Linkage analyses in large multiplex pedigrees is a robust approach for identifying disease loci in the presence of allelic heterogeneity, and thus can be valuable for targeting regions for sequencing studies [8]. To identify genomic regions likely to contain rare (MAF 0.01) and low-frequency (0.01 MAF 0.05) LOAD risk and possibly protective genetic variants, a large number of well-characterized families were screened for inclusion in a linkage scan. The selected extended families are uniquely suited for discovery of genomic regions containing high penetrant Alzheimer's disease variants. We performed extensive parametric two-point and non-parametric multipoint linkage analysis on 385 individuals in 41 non-Hispanic Caucasian (NHC) families. Loci identified through this study can help prioritize regions of the genome for analyses of whole exome or whole genome sequence data from NHC LOAD families or case-control cohorts.

2. METHODS

2.1. Study samples

The 42 NHC families selected for linkage analyses are from five collections assembled by investigators at The University of Pennsylvania (8 Families), The University of Miami (12 Families), Case Western University (1 Family), the National Institute on Aging Late-Onset Alzheimer's Disease (NIALOAD) family study (17 Families), and the National Cell Repository for Alzheimer's Disease (NCRAD) (4 Families). Detailed descriptions of the

ascertainment and evaluation of subjects in these cohorts have been provided elsewhere [9–11]. To maximize the probability of detecting segregating novel rare variants, we developed several selection criteria including: (1) having four or more affected individuals with genomic DNA samples (2) exhibiting likely dominant inheritance of LOAD; (3) free of known mutations at established AD/FTD Mendelian loci (*APP, PSEN1, PSEN2, MAPT,* or *GRN*); and (4) reduced representation of the *APOE* ε4 allele. Criterion number 4 included prioritized selection of families with APOE ε2/ε2, ε2/ε3 and ε3/ε3 affected individuals (requiring at least one affected family member without any APOE ε4 allele and any affecteds with a single APOE ε4 must have age-at-onset (AAO) < 72). 385 individuals in the 41 NHC families ultimately analyzed (3-11 cases per pedigree) had genotyping data available and were included in the present analyses. 75.6% of families (31 of 41) have at least one autopsy confirmed LOAD case (Table 1).

2.2. Genotyping and quality control procedures

Genome-wide single-nucleotide polymorphism (SNP) genotyping was performed on several different platforms across the study cohorts, including the Illumina HumanHap 550, Illumina 1M, HumanOmniExpress, HumanOmniExpress Exome, and HumanOmni2.5 arrays. A call rate threshold of 98% was applied and the data were then merged to form a final linkage dataset for analysis. SNPs were only included in the analysis if they were present in at least 60% of samples. 319,409 SNPs were selected for analysis and aligned to the Rutgers Map v.3 [12]. Among this group of SNPs, 26,959 were excluded because the minor allele frequency (MAF) was less than 0.05 and/or the genotype distribution differed significantly ($P < 10^{-6}$ in controls) from Hardy-Weinberg equilibrium. An additional 919 SNPs not present in the HapMap CEU dataset were removed, reducing the number of SNPs available for analysis to 291,531 SNPs. More than three-fourths of these SNPs (77%; n = 225,250 SNPs) were present in 90 or more percent of samples. Checks for relatedness, Mendelian inconsistencies and gender based on X chromosome heterozygosity were performed using PLINK [13]. One sample was dropped due to Mendelian inconsistencies and one duplicate sample was removed. Principal components analysis using Eigenstrat [14] identified a family clustering with African American HapMap samples (eFigure 1). NIALOAD confirmed the family's African American ancestry through recontact with the ascertainment site. This family was removed from the analyses resulting in 41 families included in the linkage analyses reported here.

2.3. Statistical analyses

Autosomal and X-chromosome linkage analyses were performed using Merlin [15] and included parametric two-point affecteds-only and age-dependent penetrance models, and a non-parametric multipoint analysis. Parametric multipoint analysis was performed on significant overlapping regions between the families in this report and a companion analysis in Hispanics (Barral et al. in this issue). The package MINX (Merlin in X) was used for analysis of X-chromosome SNPs. Heterogeneity LOD (HLOD) models were applied to the two-point analyses to allow for detection of linkage in the presence of locus heterogeneity [16]. Whittemore and Halpern NPL-pair and NPL-all statistics [17], and Kong and Cox linear model logarithm of odds (LOD*) scores [18], were calculated for the non-parametric multipoint analysis.

Power analyses using SIMLINK[19] on the 41 families in the linkage analysis, with a dominant model and disease allele frequency of 0.001, showed we have >80% power to generate a LOD > 3 for a fully informative (alpha = 1) age-penetrance model with marker locus allele frequencies equal to 0.2 (MAXLOD = 5.62) and 0.4 (MAXLOD = 6.86). Using these same paramaters, the affecteds-only model has >80% power to generate a LOD > 2 with a marker locus frequency of 0.4 (MAXLOD = 3.61) and 0.71% power to produce a LOD > 2 with a marker locus frequency of 0.2 (MAXLOD = 3.00). Using a heterogeneity model (alpha = 0.5) reduced power to generate a LOD > 2 to 41% and 18% for the age-penentrance and affecteds-only models respectively (eTable 1).

Parameters for the parametric two-point models assumed dominant inheritance, a disease allele frequency of 0.001 and penetrances of 0.01, 0.90, and 0.90 (representing NN, NA, AA genotypes respectively). Age-dependent penetrances used in the analysis are listed in eTable 2. Two-point parametric analysis utilized all SNPs for each of the analyses. The non-parametric multipoint scan included a linkage disequilibrium (LD)-pruned set of 119,555, SNPs common to all genotype platforms. LD pruning was done using the independent pairwise LD pruning option in Plink (default settings). Mean distance between markers for the set of non-parametric multipoint markers is 4.55 cM. As some pedigrees were too large for MERLIN to perform nonparametric linkage analysis, uninformative family members (based on an individual's position in the pedigree and/or absence of genotyping) were trimmed before performing analyses using the program PowerTrim [20]. Allele frequencies for all SNPs were based on CEU HapMap data [21].

A significance threshold of HLOD 3.5 was set for the parametric two-point linkage scans taking into account testing of two separate parametric models. This is above the Lander and Kruglyak recommendations for significance (LOD 3.3; P value = 4.9×10^{-5}) in LOD score analyses of dense marker genome-wide linkage scans [22], and approximates a level suggested by Camp and Farnham for testing of two independent two-point models [23]. Multi-point significant and suggestive linkage thresholds were defined by LOD 3.60, P = 2.2×10^{-5} and LOD 2.20, P = 7.4×10^{-4}, respectively [22]. Linkage regions were considered independent if the locations of their peak HLOD or LOD* scores were separated by >20 cM. Linkage peaks were considered concordant with previous linkage peaks or linkage peaks reported in the companion Hispanic linkage analysis (Barral et al. 2014 in this issue), if they were 10 cM apart.

Follow-up analyses of significant multipoint results included haplotype segregation analysis and examination of overlapping identity-by-descent (IBD) sharing segments for families with a maximized within family LOD 0.59, corresponding to a nominal P value of 0.05, and 100% IBD sharing among all affecteds in the family. IBD segments were determined by estimating haplotypes in MERLIN followed by identification of IBD sharing regions using Olorin [24].

3. RESULTS

3.1. Dataset Characteristics

The selected families have an average of 8 affected individuals (range: 4-14), with an average of 5 genotyped affected individuals per family (range: 3-11) (Table 1). Mean AAO in affecteds was 75 ± 9.04, compared to a mean age of unaffecteds at last evaluation of 66 ± 12.29. *APOE* ε2, ε3, and ε4 frequency in affecteds (genotyped and ungenotyped) was 3%, 70%, and 27% respectively. This compares to an ε4 frequency of 38% in Caucasian LOAD individuals and 14% for controls [25], confirming the selection of LOAD families with reduced representation of the *APOE* ε4 allele. *APOE* genotype frequencies for affecteds were: ε2/ε2 = 0.5%, ε2/ε3 = 4.1%, ε2/ε4 = 0.9%, ε3/ε3 = 43.2%, ε3/ ε4 = 48.6%, and ε4/ ε4 = 2.7%.

3.2 Linkage Results

Two-point parametric linkage analysis identified 24 SNPs across 14 autosomal regions surpassing a significance level of HLOD   3.5 (*P* value = 3×10^{-5}) (Table 2, Figures 1 and 2). Nine of the 14 regions generated HLOD scores that exceeded 4.0, including 3q25.31 (HLOD = 4.15), 3q27.3 (HLOD = 4.58), 4p34.3 (HLOD = 4.46), 5q32 (HLOD = 4.10), 7p21.2 (HLOD = 4.19), 9p22.1 (HLOD = 4.21), 11q13.4 (HLOD = 4.74), 16q12.1 (HLOD = 4.05) and 19q13.41 (HLOD = 4.76). As expected based on the family selection criteria for exclusion of clustering of affected subjects who were predominantly *APOE* ε4, we did not observe linkage to the *APOE* locus. Age-dependent penetrance HLOD scores were generally lower than our affecteds-only HLOD scores. A majority of SNPs generated HLOD scores with alpha values equal to 1.0, suggesting modest contributions to individual loci by each family. No significant results were observed for the overall analyses on the X-chromosome, however two families had nominally significant LOD scores of 1.39 and 1.14 at Xq28 (rs5963398), the location of the highest HLOD score on the X-chromosome (HLOD = 2.07).

Non-parametric multipoint analyses identified an additional significant region at 14q32.2 (LOD* = 4.18) (Figure 2). Two-point results for this region were also supportive of linkage (HLOD = 2.82 at rs9323997; affecteds-only model). One other region produced suggestive multi-point results, 4q34.1-4q34.3 (LOD = 2.40), and was supported by a significant two-point linkage (HLOD = 4.46; affecteds-only model). Please see eTable 3 for the 1-LOD region and genes within this region. No significant multipoint results were observed on the X-chromosome.

3.3. Localization of region at 14q32.2

The resulting 1-LOD region [16] at 14q32.2 is a 0.78 Mbp segment between map positions 98.81 Mbp and 99.59 Mbp, and contains one gene (*C14orf177*) and one microRNA (miRNA), *Mir_320* according to the UCSC Genes Track [26]. The pseudogene ribosomal protein L3 psudeogene 4 (*RPL3P4*) also locates to this segment according to the Gencode database (version 19) [27]. Linked pedigree IBD sharing analyses among the four nominally significantly linked pedigrees (LOD*   0.59) isolated a 0.40 Mbp segment containing one gene, *BCL11B*, located just outside the 1-LOD region, and *Mir_320* (Figure 2). Haplotype segregation of these 4 pedigrees illustrated using the software program Progeny (Progeny

Software LLC, Delray Beach, FL www.progenygenetics.com) is shown in eFigures 2-5. Merlin software was used for haplotype construction and inference.

4. DISCUSSION

We report 14 significant two-point linkage regions (HLOD ≥ 3.5) and one significant multipoint region (LOD* ≥ 3.6) identified by analysis of 41 multiplex LOAD families that were selected on the basis of the absence of variants at known risk loci, apparent dominant inheritance of disease, and little evidence for association of LOAD with the *APOE* ε4 allele. Five of the 14 two-point regions are supported by multipoint results within a 1-LOD unit confidence interval with a minimum LOD* ≥ 1.5 (3q25.31, 4q34.1, 8q22.3, 11q12.2-14.1 and 19q13.41). The finding of numerous significantly linked loci instead of a few shared loci suggests that there is substantial locus heterogeneity within familial LOAD.

Three of the 14 two-point loci are novel linkage regions for LOAD (5q32, 11q12.2-11q14.1 and 14q13.3). The 11 other loci overlap previously reported LOAD regions including five significant loci (3q25.31, 7p21.2, 9p22.1, 11q12.2-11q14.1 and 19q13.41) also showing significant linkage (two-point HLOD ≥ 3.3, multipoint LOD* ≥ 3.6) or association (p ≤ 5 × 10^{-8}) in at least one previous report (Table 2). These include two loci reported in the largest LOAD GWAS to date, namely the 11q12.2-11q14.1 locus which overlaps the *MS4A6A-MS4A4E* association region and the 19q13.41 locus which contains *CD33* [1]. One additional GWAS locus (CR1 at 1q32.2) is near a significantly linked marker at 1q32.3. A check of our linked SNPs in these regions for association with LOAD in the IGAP GWAS summary statistics found no significant associations after correction for multiple testing (*P* ≥ 5 × 10^{-8}) (eTable 4) [1]. The 7p21.2 and 9p22.1 loci replicate significant linkage from reports that contain families used in the present analyses (Table 2). The number of pedigree members and the phenotypes and genotypes from the previous reports have been continuously expanded and updated since their previous linkage reports however, and these updates are most likely increasing power to localize linkage in this current report.

The locus at 14q32.2 is arguably our most robust result given its significant multi-point LOD* score supported by suggestive two-point scores. This locus is a considerable distance (~33 cM) from the *PSEN1* locus at 14q24. Suggestive linkage (Two-point LOD = 2.60) has been reported at 14q32 in an age-at-onset linkage analysis in Hispanic LOAD families [28]. The 1-LOD limit identified a region 0.78 Mbp in length containing *C14orf177* and *Mir_320,* both of which have some support for involvement in dementia-related disease and processes. *C14orf177* for instance, has been associated with risk for amyotrophic lateral sclerosis [29] and lipoprotein cholesterol levels [30], while members of the mir-320 microRNA family are significantly altered in sporadic AD brains [31] and associated with both neurite outgrowth [32] and neurodegeneration [33]. Evidence for genomic features with regulatory potential such as several ESTs and lincRNAs also exists in the region (based on UCSC genome browser data)[26], including one lincRNA in particular, TCONS_12_00008237, which is highly expressed in brain [34,35].

The region narrowed by IBD analysis in the subset of pedigrees most likely to be linked to 14q32.2 also includes *BCL11B*, which is a transcription factor and regulator of BDNF

signaling [36], a pathway associated with pathogenesis of several neurodegenerative diseases, including LOAD [37]. *BCL11B* is predominantly expressed in striatal neurons and may play an important role in adult neurogenesis [38], a process that when dysregulated may lead to AD [39]. *BCL11B* is thought to primarily reduce BDNF signaling [36], consistent with observations that BDNF serum and expression levels are decreased in AD [37,40,41], and high BDNF levels protect against AD [42,43]. A recent neuroimaging study implicated a role for BDNF in cognitive decline in LOAD patients [44], although AD genetic association studies of BDNF yielded conflicting results [40,45]. Finally, it has been suggested that BDNF-based drugs might be effective therapies for AD and other neurodegenerative diseases [46], with targeting of *BCL11B* interactions with BDNF even being suggested as a feasible therapeutic approach to elevate BDNF signaling in neurodegenerative drug development [36].

The accompanying study in Caribbean Hispanics by Barral et al [47] found strong evidence for linkage and association near one of our significant loci, 11q12.2-11q14.1. As noted above, the chromosome 11q12.2-11q14.1 locus is also a significant LOAD GWAS locus from Lambert et al. 2013 [1]. Parametric affecteds-only analysis of this region produced a multipoint peak LOD* of 1.18. An alpha of 0.17 suggests that only a small number of pedigrees are potentially segregating a variant in this region. A combined association and linkage analysis of all markers in this region using CAPL [48] produced no suggestive or significantly associated SNPs (data not shown). One possible explanation for this finding is lack of power for combined linkage and association analysis using these data. Several other significant loci from the Barral et al. study had suggestive linkage in our analyses, including 3q13.31 (HLOD = 3.31), 3q22.3 (HLOD = 3.18), 6q25.3 (HLOD = 3.02), 7p14.3 (HLOD = 3.05), and 14q12 (HLOD = 3.31) (eTable 5). The 3q22.3 region was previously reported as a potential locus for LOAD in a linkage study of a family containing four relatives with LOAD but without tau pathology (LOD = 4.1) [49], and in a genome-wide linkage study of Dutch families (LOD = 4.3; HLOD = 4.4) [50].

In summary, we report 15 significant regions for linkage, including novel evidence for linkage at 5q32, 11q12.2-11q14.1 and 14q13.3. Several of our regions overlap significant loci from previous LOAD analyses, including GWAS regions at *MS4A6A-MS4A4E* and *CD33*. Our strong multipoint result at 14q32.2 is particularly interesting, as it localizes to a region with a limited amount number of genomic candidates, most with plausible links to dementia-related processes and disease.

The 41 families included in these analyses are undergoing whole-genome sequencing (WGS) as part of the National Institute of Health's Alzheimer's Disease Sequencing Project (ADSP) [51]. ADSP WGS variants located in these linkage regions will be primary candidates for examination as contributing to risk or protection for LOAD. Analyses planned by the ADSP to identify these variants include: 1) combined linkage and association analyses, and 2) filtering for rare, damaging variants in shared familial segments. An ADSP replication phase will follow to confirm and validate candidate loci from the discovery phase.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Systematic review: Pubmed and Google Scholar were used to search for articles related to genetic linkage and genetic association analyses of Alzheimer's disease (AD). Additionally, we searched for literature relating our significant loci to AD and neurodegeneration through a search of each significant chromosomal band (and gene features from the significant multipoint result) and the terms "Alzheimer's" and "Neurodegeneration". Relevant research relating our significant loci to Alzheimer's or Neurodegeneration is cited.

Interpretation: These findings pinpoint several novel genomic regions linked to increased risk of familial AD, including a region on 14q32.2 containing a gene that regulates brain-derived neurotrophic signaling (BDNF) and the 11q12.2 region previously linked to AD through large genome-wide association analyses of LOAD.

Future directions: Identification of these loci as linked to familial AD provides an exciting opportunity to identify causal variants for LOAD through prioritization of these regions for analyses in forthcoming whole genome sequencing.
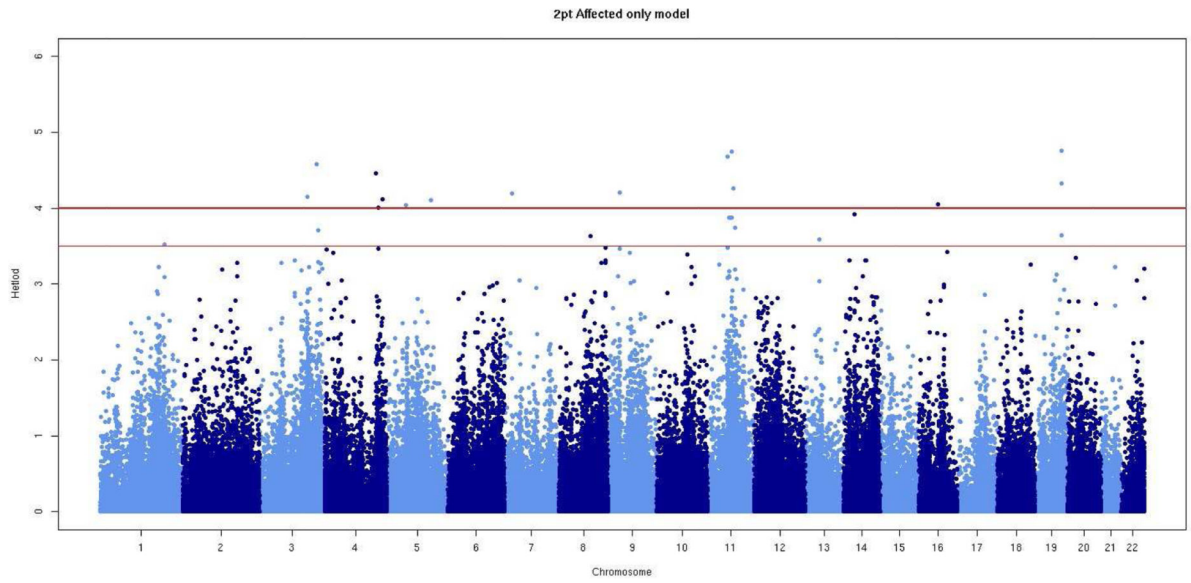
Figure 1.
Manhattan plot of parametric 2-point affecteds-only results (Red lines represent HLOD = 3.5 for significant linkage and 4.0 for highly significant linkage).
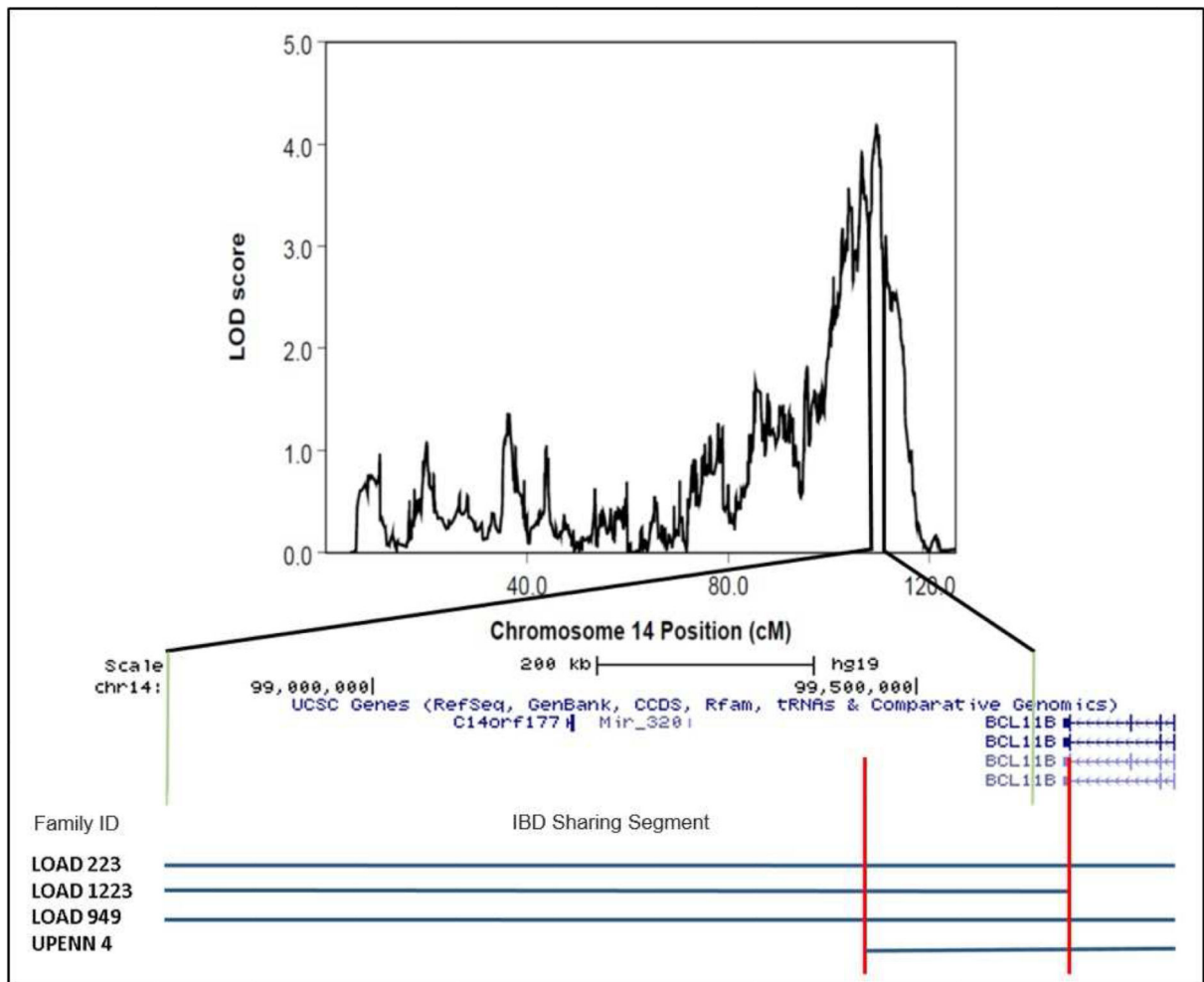
Figure 2.
Shared IBD segments among the four families with LOD* > 0.588 and full IBD sharing for all affected, genotyped family members) in the chromosome 14 linkage region. Red lines represent the minimum shared IBD segment region. Light green lines represent the 1-LOD confidence interval region.

Table 1

Demographic and clinical characteristics of families

Characteristics	
Number of families, n	41
LOAD cases sampled (%)/Unaffected sampled (%)	202 (52.4)/183 (47.5)
1 autopsy confirmed LOAD case in family (%)	31 (75.6)
Affecteds Sampled Per Family (n families)	
3 Affecteds	7
4 Affecteds	156
5 Affecteds	8
6-9 Affecteds	8
10+ Affecteds	3
Proportion of women, n (%)	246 (64%)
Age at onset of affecteds, years, mean (SD)	75 (SD 9.04)
Age at last examination of unaffecteds, years, mean (SD)	66 (SD 12.23)
APOE Allele Frequency in affecteds, n (%)	
ε2	11 (3%)
ε3	284 (70%)
ε4	109 (27%)

Table 2

Two-point parametric linkage analysis results with HLODs ≥ 3.5.

Chr	BP (Hg19)	Cytogenic Location	RS ID	Ref Allele	Alt Allele	Marker MAF	Affecteds Only Model			Age Penetrance Model			GWAS Locus * (Closest Gene)	Previous Evidence for Region †
							LOD	HLOD	Alpha	LOD	HLOD	Alpha		
1	212028969	1q32.3	rs28451400	C	T	0.07	3.52	3.53	1	2.03	2.03	1	1q32.2 (CR1)	
3a	156034741	3q25.31	rs498033	G	T	0.50	3.70	4.15	0.87	3.99	3.99	1		[52]
3b	186464055	3q27.3	rs6807774	A	G	0.46	4.54	4.58	0.95	2.85	2.85	1		
3b	187667808	3q27.3	rs2590453	A	G	0.36	3.71	3.71	1	2.52	2.52	1		
4	174854581	4q34.1	rs7670348	C	T	0.18	4.46	4.46	1	1.66	1.66	1		
4	179530781	4q34.3	rs1434035	C	T	0.24	4.01	4.01	1	1.61	1.61	1		
4	185382061	4q35.1	rs793798	G	A	0.34	4.11	4.11	1	2.52	2.52	1		
5a	41264243	5p13.1	rs391781	A	G	0.42	3.97	3.97	0.93	1.24	1.34	0.82		
5b	145636671	5q32	rs11953090	G	T	0.28	2.43	4.10	0.75	0.00	0.57	0.40		
7	16025340	7p21.2	rs13234986	A	G	0.32	4.19	4.19	1	2.24	2.24	1		[53]
8	101862078	8q22.3	rs4734484	C	T	0.29	3.63	3.63	1	0.93	1.01	0.81		
9	19051208	9p22.1	rs7022613	C	T	0.47	4.21	4.21	1	1.42	1.42	1		[54]
11	60047410	11q12.2	rs1426248	G	A	0.28	4.68	4.68	1	2.40	2.40	1		
	63197930	11q12.3	rs7934347	G	A	0.22	3.85	3.88	0.94	1.44	1.51	0.88		
	73317931	11q13.4	rs737586	A	G	0.17	4.74	4.74	1	2.15	2.15	1	11q12.2 (MS4A6A-MS4A4E)	
	75012535	11q13.4	rs506233	T	C	0.45	3.59	3.88	0.87	1.02	1.35	0.75		
	78879841	11q14.1	rs4359232	T	C	0.42	4.26	4.26	1	1.74	1.74	1		
	83831259	11q14.1	rs7108582	A	C	0.09	3.74	3.74	1	1.70	1.70	1		
13	46047499	13q14.13	rs2985987	G	A	0.14	3.56	3.56	0.93	2.54	2.54	1		
14	36681923	14q13.3	rs1766132	G	A	0.17	3.92	3.92	1	2.05	2.05	1		
16	52112372	16q12.1	rs3743795	T	G	0.32	3.23	4.05	0.84	2.23	2.23	1		

Chr	BP (Hg19)	Cytogenic Location	RS ID	Ref Allele	Alt Allele	Marker MAF	Affecteds Only Model			Age Penetrance Model			GWAS Locus * (Closest Gene)	Previous Evidence for Region †
							LOD	HLOD	Alpha	LOD	HLOD	Alpha		
19	52668414	19q13.41	rs919271	A	G	0.18	4.76	4.76	1	3.23	3.23	1		
	52670905	19q13.41	rs7246914	C	T	0.24	4.33	4.33	1	2.90	2.90	1	CD33 (19q13.41)	
	52699440	19q13.41	rs6509626	C	T	0.10	3.36	4.64	0.87	1.87	1.87	1		

BP = base pairs in GCHr17/Hg19

*
GWAS Locus based on Lambert et al. 2013[1]

†
Reference for previous evidence of linkage in region based on a significant two-point (LOD 3.3) or multipoint (LOD 3.6) score