Supplementary Text

Supplementary Materials and Methods:

DNA isolation:

Genomic DNA was isolated from blood samples for JS and RS subjects using AutoGen technology (AutoGen, Inc, Holliston, MA) and from cerebellum tissue for AUT subjects using Wizard ® Genomic DNA Purification Kit (Promega Corp, Madison, WI).

SNP selection:

Eight of the additional 22 SNPs were in conserved regions of *GAPDH* and *GAPDHS*. To select these conserved region SNPs, we first identified all known SNPs in the NCBI database (gene +/- 10kb flanking sequence) and selected those that were in regions with >70% interspecies conservation (human and mouse) in 100 bp sliding windows. Of these SNPs, we identified those that had a known MAF of >1% in Caucasian populations. We then narrowed this number further according to LD information available from the International HapMap Project (www.hapmap.org). When two SNPs were known to be in LD (r2 >0.8) the SNP with greater % conservation was selected. We were able to successfully genotype 5 conserved region SNPs in *GAPDH* and 3 in *GAPDHS*.

Genotype data for thirteen of the additional 22 SNPs were obtained from our published LOAD GWAS that utilized the Illumina HumanHap300 platform(Carrasquillo, et al., 2009). There were 6 *GAPDH* and 7 *GAPDHS* SNPs identified from our LOAD GWAS that resided within these genes +/- their 20 kb flanking sequence. Because pGAPD did not have a defined gene location at NCBI at the time of the study, we were unable to select either conserved region or LOAD GWAS SNPs for this gene.

In addition a further *GAPDHS* SNP was selected that did not meet the above criteria but was chosen as it was highlighted in the initial *Li et al*., study (rs12984928) (Lee, et al., 2008,Li, et al., 2004,Lin, et al., 2006).

In summary, we assessed 11 SNPs in *GAPDH* and 11 in *GAPDHS* in addition to the 3 key SNPs.

Genotyping:

Genotype concordance between Illumina and Sequenom platforms was determined to be 99.9% for 25 SNPs (2138 subjects) that were genotyped on both platforms for quality control purposes. Our comparisons between the TaqMan and Sequenom technologies have previously yielded an inter-assay concordance rate of 99.5% (unpublished data).

The initial 3 SNPs (rs3741916, rs2029721, rs4806173) were genotyped using the Applied Biosystems 7900 Taqman platform. Of the 22 additional SNPs, 13 had genotype data available from the Illumina Hap300 LOAD GWAS (Carrasquillo, et al., 2009,Reiman, et al., 2007), 6 were genotyped as part of a Sequenom pool and 3 were genotyped by Applied Biosystems Taqman assays.

The three SNP's from the initial study(Li, et al., 2004) were genotyped in subjects of all ages to allow for replication analysis of the previously reported associations. The additional 22 SNP were assessed in subjects with ages at diagnosis/evaluation/death below the series mean (ages 60-78). This is because our LOAD GWAS that provided the genotypes for the 13 SNPs is restricted to younger subjects (ages 60-80).

Linkage disequilibrium (LD) analysis:

Linkage disequilibrium between the SNPs was determined and plotted using Haploview(Barrett, et al., 2005). Haplotype blocks were defined using the Solid Spine method.

Haplotype analysis:

All SNPs within each haplotype block were analyzed using the haplo.stats program (Schaid, et al., 2002). Only subjects with non-missing alleles for all SNPs were included in the analysis. The score.bin.adj function was used to calculate score statistics for each haplotype with an expected minimum count of 10 and an additive model adjusted for covariates *APOE*, age and gender. Haplo.stats generates the score statistics for each haplotype and global score statistic for the set of haplotypes within each block.

Supplementary Results:

All SNPs were analyzed for Hardy-Weinberg equilibrium and had a Hardy-Weinberg p-value >0.001 in the controls (supplementary *Table A*).

Analysis of additional SNPs:

As described in Supplementary Methods, we analyzed 11 SNPs in *GAPDH* and another 11 in *GAPDHS*, in addition to the 3 key SNPs. Eight of these 22 additional SNPs resided in evolutionarily conserved regions of these genes (5 in *GAPDH* and 3 in *GAPDHS*). Another 13 SNPs were chosen from our LOAD GWAS (6 in *GAPDH* and 7 in *GAPDHS*). One other *GAPDHS* SNP was included because it was emphasized in the *Li et al*. study(Li, et al., 2004). These SNPs were assessed only in younger subjects with age at diagnosis/evaluation/death below the series mean (ages 60-78), because our LOAD GWAS, which provided genotypes for 13 of the SNPs, was restricted to a younger age group (60-80).

Along with rs3741916, rs2029721, and rs4806173, these 22 SNPs were analyzed by logistic regression analysis using an additive model with gender, age at diagnosis/evaluation/death and presence of an *APOE4* allele as covariates. In the combined series (JS/RS/AUT), this analysis of young subjects (*Table A*, Supplemental Data) yielded p values of 0.258 – 0.965 for the 22 additional SNPs. Since these SNPs yielded no associations that were highly suggestive or as significant as rs3741916 (aka 1136666), we did not pursue them further.

Haplotype and multi-locus genotype (MLG) analysis:

Analysis (solid spine of LD) by HaploView(Barrett, et al., 2005) of the genotypes for all 25 SNPs (3 key SNPs initially genotyped and 22 subsequent SNPs) in our 60-78 year age group was used to identify LD blocks in *GAPDH* (Figures 2 a-b) and *GAPDHS* (Figure A1-2). At the *GAPDH* locus two haplotype blocks were defined: Block 1 which encompasses 7 SNPs 5' to 3' from rs917634 to

rs7971637 and block 2 which encompasses 4 SNPs 5' to 3' from rs1136666 (aka rs3741916) to rs1060619. Two haplotype blocks were also defined at the *GAPDHS* locus: Block 1 which encompasses 5 SNPs 5' to 3' from rs2106446 to rs1029387 and block 2 which encompasses 6 SNPs 5' to 3' from rs4806173 to rs2251124.

Using haplo.stats (Schaid, et al., 2002), haplotypes were identified and their frequencies inferred using the expectation maximization approach implemented in the haplo.em function. Haplotype analysis was performed for all series combined (Supplementary Data, *Table B*). No individual haplotypes were identified that achieved nominally significant association with LOAD in our combined series. Likewise none of the haplotype blocks showed nominally significant global association with LOAD.

Li et al (Li, et al., 2004)reported Multilocus Genotype (MLG) associations with the three SNPs we have genotyped in all of our series (rs3741916, rs2029721 and rs4806173). We analyzed these 3 SNPs in our series using this (MLG) approach but we were unable to identify any significant MLG associations in our series (Supplementary Data, *Table C*).

Supplementary References:

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Supplementary Results:

						Logistic Regression Additive Model	
SNP (Locus)	Chr	Base Position	AD N (MAF)	Con N(MAF)	HW P-value Con	OR (95%CI)	p-value
rs917634^	12	6,501,430	631 (0.48)	1047 (0.47)	0.4950	1.03 (0.88: 1.19)	0.743
rs2072373^*	12	6,502,149	633 (0.20)	1045 (0.20)	0.0332	0.96(0.80: 1.16)	0.707
rs10849482^	12	6,505,535	623 (0.23)	1032 (0.24)	0.0042	0.93 (0.78: 1.11)	0.418
rs740850*	12	6,508,377	662 (0.28)	1129 (0.25)	0.9373	1.10 (0.93: 1.30)	0.271
rs740851^	12	6,508,611	624 (0.43)	1039 (0.46)	0.0525	0.92 (0.79: 1.07)	0.258
rs7316224	12	6,508,927	679 (0.28)	1142 (0.26)	0.9383	1.10 (0.93: 1.29)	0.281
rs7971637	12	6,513,590	693 (0.19)	1159 (0.21)	0.1078	0.91 (0.76: 1.09)	0.312
rs1136666*	12	6,514,252	855 (0.27)	1988 (0.29)	0.0627	0.89 (0.77: 1.02)	0.093
rs1060621*	12	6,514,957	680 (0.20)	1126 (0.21)	0.0226	0.93 (0.78: 1.12)	0.444
rs1060620*	12	6,514,983	691 (0.21)	1152 (0.22)	0.2654	0.93 (0.78: 1.11)	0.409
rs1060619^*	12	6,515,042	630 (0.21)	1023 (0.21)	0.0622	0.97 (0.81: 1.17)	0.775
rs10849488^	12	6,524,311	628 (0.27)	1042 (0.28)	0.3169	0.97 (0.82: 1.15)	0.757
rs2029721*	12	61,435,611	695 (0.40)	1189 (0.38)	0.6213	1.14 (0.98: 1.31)	0.090
rs2106446^	19	40,703,924	630 (0.34)	1045 (0.33)	0.8890	0.98 (0.83: 1.15)	0.815
rs3815018^	19	40,706,458	630 (0.09)	1046 (0.09)	0.3613	0.91 (0.69: 1.19)	0.492
rs17705633	19	40,707,492	697 (0.17)	1160 (0.18)	0.1594	0.93 (0.77: 1.13)	0.486
rs2285513^	19	40,708,038	631 (0.46)	1045 (0.45)	0.6619	1.04 (0.89: 1.22)	0.592
rs1029387^	19	40,712,481	630 (0.17)	1044 (0.18)	0.1641	1.02 (0.84: 1.24)	0.853
rs4806173*	19	40,716,765	699 (0.39)	1203 (0.38)	0.3280	0.98 (0.85: 1.14)	0.830
rs12984928*	19	40,721,692	683 (0.37)	1143 (0.36)	0.0022	1.00 (0.87: 1.16)	0.965
rs2239942^	19	40,722,288	624 (0.21)	1042 (0.22)	0.8579	0.93 (0.77: 1.13)	0.479
rs11882238	19	40,723,238	671 (0.36)	1114 (0.36)	0.0273	0.98 (0.84: 1.14)	0.814
rs12977572	19	40,734,371	694 (0.04)	1159 (0.04)	0.7177	1.09 (0.75: 1.56)	0.659
rs2251124^	19	40,736,364	606 (0.39)	981 (0.38)	0.0081	1.00 (0.86: 1.18)	0.925
rs748213^	19	40,740,783	632 (0.51)	1045 (0.49)	0.3533	1.07 (0.92: 1.25)	0.378

Table A: Single SNP associations in the combined Mayo Clinic series ages 60 to 78. ^SNPs genotyped as part of a LOAD GWAS(5) *SNPs that have been reported in at least one of the previous studies (8-10). All SNPs were analyzed for Hardy-Weinberg equilibrium and had a Hardy-Weinberg p-value >0.001 in the controls.

Locus	Block	SNPs	Haplotype	Sequence	AD (freq)	Con (freq)	Adj p-value
GAPDH: Chr12	1	1-7	1	2112121	0.255	0.278	0.320
			2	111111	0.285	0.294	0.570
			3	2111212	0.010	0.007	0.585
			4	1121211	0.246	0.227	0.524
			5	2211212	0.198	0.184	0.233
				Global p-val (adj)		0.536	
GAPDH: Chr12	2	8-11	1	2122	0.002	0.005	0.151
			2	1111	0.685	0.713	0.154
			3	1222	0.012	0.012	0.804
			4	1122	0.012	0.008	0.539
			5	2221	0.006	0.004	0.439
			6	2111	0.089	0.076	0.373
			7	2222	0.193	0.180	0.290
				Global p-val (adj)		0.549	
GAPDHS: Chr19	1	1-5	1	11121	0.116	0.125	0.318
			2	11112	0.014	0.018	0.515
			3	11111	0.268	0.266	0.684
			4	21122	0.157	0.150	0.919
			5	21121	0.172	0.182	0.814
			6	11211	0.174	0.165	0.544
			7	12111	0.093	0.086	0.458
			8	11212	0.003	0.002	0.369
				Global p-val (adj)		0.892	
GAPDHS: Chr19	2	6-11	1	111111	0.255	0.257	0.479
			2	111221	0.042	0.044	0.626
			3	211112	0.017	0.018	0.838
			4	111211	0.097	0.096	0.867
			5	221112	0.362	0.366	0.731
			6	112211	0.221	0.213	0.659
				Global p-val (adj)		0.984	

Table B: Haplotype analysis using haplo.stats: All Mayo Clinic series, Ages 60-78. Only subjects with non missing genotypes for all SNPs within each block were used.

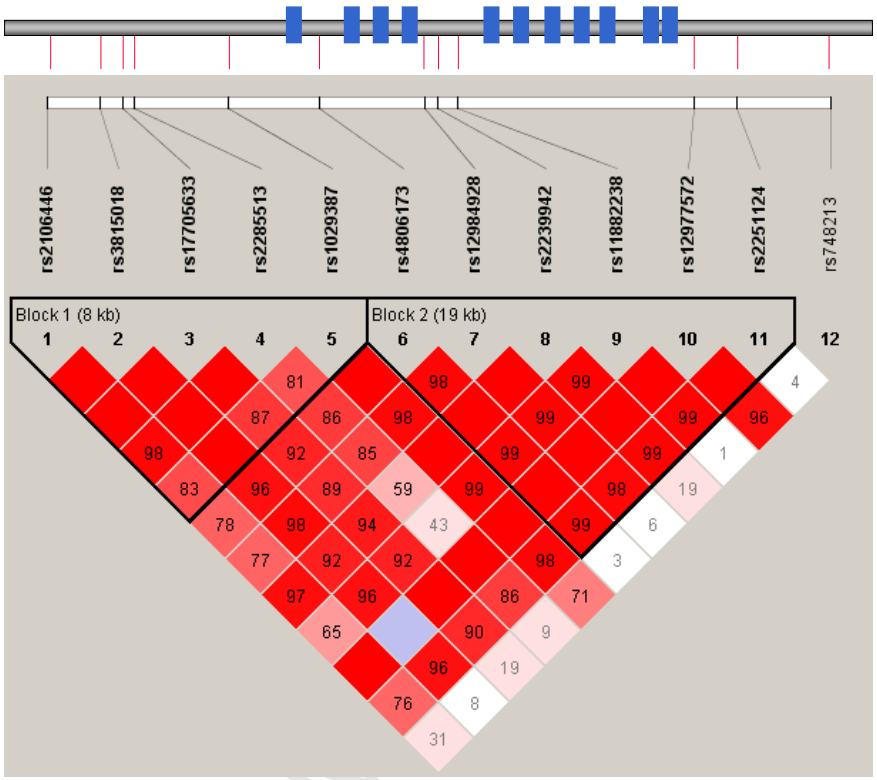
Sample	MLG	Case	Control	OR	OR 95% CI	P-value
JS/RS/AUT	12-11-11	93	133	0.92	0.64-1.33	0.818
	12-12-11	125	165	1	nc	
	12-22-11	41	59	0.92	0.58-1.45	
JS	12-11-11	33	75	1.07	0.60-1.92	0.385
	12-12-11	41	100	1	nc	
	12-22-11	13	33	0.96	0.41-2.11	
RS	12-11-11	29	32	1.09	0.54-2.22	0.751
	12-12-11	39	47	1	nc	
	12-22-11	19	15	1.53	0.64-3.68	
AUT	12-11-11	31	26	0.48	0.21-1.08	0.908
	12-12-11	45	18	1	nc	
	12-22-11	9	11	0.33	0.10-1.04	

Table C. Analysis of multilocus genotypes for the 3 key SNPs. (rs3741916-rs4806173-rs2029721)

Multilocus genotype analysis. In the combined Mayo Clinic series there are 4027 subjects with non-missing genotypes at all three key SNPs. Fixing the rs3741916 genotype as CG and the rs2029721 genotype as GG, there were 619 subjects with non-missing data for all three loci. 100 are 22, 290 are 12 and 226 are 11. P-values calculated using the Armitage trend test implemented in PLINK.

A1

A2



Figures A1-2: Linkage disequilibrium in the combined Mayo Clinic series at the *GAPDHS* locus. *SNP = Single nucleotide Polymorphism. A1. Exons are represented with blue boxes and SNPs are represented with red lines. A2. LD was estimated and haplotype blocks were defined using the "Solid Spine" method implemented in HAPLOVIEW. Darker shades of red indicate increasing strength of LD (D').*