

A Genome Screen of Successful Aging Without Cognitive Decline Identifies LRP1B by Haplotype Analysis

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Successful aging without cognitive decline (SA) is apparent in those who escape age-related illnesses, such as cardiovascular disease and dementia. To determine whether there are protective genotypes that increase the probability of successful cognitive aging, a genome-wide screen was conducted on subjects who were 85 years of older, had MMSE scores >26, and had no major illnesses. SNP 500K microarrays were used. The data from the microarrays was analyzed versus that from Alzheimer's patients. Three SNPs in the gene for the low density lipoprotein receptor-related protein 1B (LRP1B) had significant *P* values, after Bonferroni correction. Additional SNPs were analyzed in this very large gene. Haplotypes in intron 18 were significant for successful aging versus Alzheimer's patients; those haplotypes were not significant when Alzheimer's patients versus CEPH controls were analyzed. These results suggest that haplotypes in the gene LRP1B are significant/protective for successful aging without cognitive decline. © 2009 Wiley-Liss, Inc.

Key words: successful cognitive aging; Alzheimer's disease; SNP microarrays

INTRODUCTION

A gradual decline in physiological functions as well as susceptibility to age-related diseases, such as cardiovascular disease and dementia, occurs during normal aging. However, there are seniors who escape these age-related diseases. Moreover, there are seniors who are free of cognitive decline into and through very late old age. Very long life has a strong genetic component as centenarians cluster in families and their siblings are likely to live past age 85 [Perls et al., 2002]. Of particular interest are seniors who are over the age of 85 with preserved cognition. A genome-wide scan of the seniors with successful aging without cognitive decline versus late-onset Alzheimer's disease was undertaken, using the Affymetrix GeneChip[®] Human Mapping 500 K Array set. A significant set of SNPs in the gene, the low density lipoprotein receptor-related protein 1B (LRP1B), was identified, after Bonferroni correction.

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Additional SNPs in the LRP1B gene were analyzed and several haplotypes were found to be significant for successful aging without cognitive decline.

METHODS

Successful Aging Without Cognitive Decline

The successful aging without cognitive decline subjects (SA) were recruited from retirement villages and through community events. To be eligible, the subjects were 85 years or older and had an MMSE >26. (The MMSE or Mini Mental State Examination is a tool that assesses mental status [Folstein et al., 1975]. The MMSE consists of 11 questions which tests five areas of cognitive function. While the maximum score is 30, the score of ≤25 indicates cognitive impairment.) In addition, the subjects did not have any major illnesses, such as cardiovascular problems, diabetes, obesity, or major cancer diseases, nor was there any dementia in their families.

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Some of the subjects had skin cancers that were treated; they were considered eligible for the study. Most of the subjects had normal cholesterol levels. While some subjects used walkers to ambulate, they were all very interested in the research, asked intelligent questions, had an active exercise program, and looked quite physically fit. Most had at least college degrees and had higher level positions when they were working.

NAS Subjects

The VA Normative Aging Study (NAS) is a multidisciplinary longitudinal investigation of the aging process. It was started in the Department of Veteran Affairs in 1963 at the VA outpatient clinic in Boston. The subjects were primarily veterans from World War II and the Korean War. The veterans went through a screening process consisting of three phases, all relating to the health of the participant. From the initial phase of 6,000 men, 2,280 were selected for the study, based on good health and geographic stability. (Women were not available for the study.) The ages of the veterans ranged from 22 to 82 years at the time of entry (average age: 42 ± 9 years). Some 74% were born between 1915 and 1934. Most of the veterans were Caucasian (<2% African American), had a high school diploma (86%), and were from a higher socioeconomic status than the general Boston population. As of 1997, 26% had died, 8% stopped participation, and 6% were lost to follow-up assessment [Brady et al., 2001].

The veterans in the study had physical exams every 3–5 years which consisted of multiple biomedical parameters, including blood pressure, glucose, and cholesterol levels. In addition to other psychosocial information, many of the veterans had neuropsychological testing, starting in 1993, including the Mini Mental Status exam (MMSE) [Folstein et al., 1975], and the CERAD battery (word list learning, delayed recall, and figure copying). The stored DNA samples were from approximately 1,200 veterans and included data from their physical exams (blood pressure, glucose, homocysteine, and cholesterol) as well as results from cognitive testing. Of a closely followed subgroup of 1,088 veterans, 94 had an MMSE < 23; 294 had MI; 57 had strokes; 437 had cancer; 201 had diabetes; 5 had Parkinson's disease. Of the remainder with MMSE > 24, 367 were < 65 years of age; 362 were 65–70; 352 were 70–79; 89 were > 79 years of age. We have analyzed the group over age 80 with known MMSE's > 25 and normal cognitive assessments. Moreover, the medical information on cholesterol, homocysteine, glucose levels, blood pressure readings, were normal and there was no evidence of diabetes, cancer, or cardiovascular problems.

Alzheimer's Patients

The samples consist of 227 Alzheimer's patients. The clinical diagnosis of probable Alzheimer's disease was made according to NINCDS-ADRDA criteria [McKhann et al., 1984], after a review of the medical records to verify a documented progressive decline in cognition and appropriate blood work to rule out other medical conditions, including thyroid and vitamin B12 deficiencies. In addition to these criteria, we also included a CT scan and/or MRI of the brain which showed cortical atrophy but no evidence of strokes or tumors. The patients were Caucasian, of European descent.

All participants or the authorized representatives of the patients gave consent for the study, in accordance with the Institutional Review Board guidelines.

The successful aging samples and those from the NAS study were analyzed together for population stratification with Structure 2.1; no significant differences were found for the data [Falush et al., 2003]. Thus both groups were classified as the successful aging without cognitive decline (SA). There was no population stratification in the Alzheimer's subjects.

Genotyping

Genomic DNA was extracted using the Qiagen QIAamp DNA blood midi kit (Qiagen, Inc., Valencia, CA) and suspended in low EDTA TE buffer. Aliquots were quantitated using the NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, DE). Samples (14 Alzheimer's patients from three extended families, 29 SA subjects, and 16 spouses and unaffected siblings from the three extended families) were diluted to 50 ng/μl and sent to Precision Biomarker Resources, Inc., (Evanston, IL) for genotyping, according to the manufacturers specifications (Affymetrix, Santa Clara, CA), using the GeneChip® 500K Mapping Array Set, consisting of two arrays (Nsp I, ~262,000 SNPs and Sty I, ~238,000 SNPs). Genotype calls were obtained from the Bayesian Robust Linear Model with Mahalanobis distance classifier genotype calling algorithm (BRLMM) on the Affymetrix platform. Among the 500,568 SNPs on the microarrays, 469,218 had call rates $\geq 95\%$ and HWE (Hardy–Weinberg equilibrium) $P > 0.001$, and were further analyzed. Gender calls were in accordance with the X chromosome genotype data and the known gender. Genotypic association was performed using the trial version of HelixTree software (Golden Helix, Bozeman, MT). Bonferroni corrections for multiple testing were made using the total number of samples on each array.

For stage 2 of the project, additional SNPs in the gene, LRP1B, were selected from the NCBI SNP database (www.ncbi.nlm.nih.gov/SNP). Three of the most significant SNPs from the microarray data and those seven in other LD blocks spanning the gene were selected from the database and were genotyped in the successful aging samples (32 samples) and in the Alzheimer's patients (227 subjects), using fluorescent-detected single base extension with the SNaPshot Multiplex kit (Applied Biosystems, Foster City, CA) as described [Huang and Poduslo, 2006]. Power analysis for the 227 Alzheimer's patients and the 32 SA subjects, using G*Power, was 0.84 [Purcell et al., 2003]. For stage 3, 10 additional SNPs in the 4 LD blocks surrounding the most significant SNPs from the database were selected. The trial version of HelixTree was used to determine the haplotypes and SAS was used for the genotypic, allelic, and haplotype association analysis.

RESULTS

The results from the microarrays indicated that there were three SNPs in a single gene that were significant (P), after Bonferroni correction (bP), when the Successful Aging samples were analyzed versus the Alzheimer's patients (Table I).

TABLE I. Successful Aging Versus Alzheimer's Disease

Probe set ID	db SNP	Chromosome	Position	<i>P</i>	<i>aP</i>	<i>bP</i>
A-1824685	rs12474609	2q22.1	141,446,369	5.93E-09	4.44E-08	2.45E-05
A-4234192	rs10201482	2q22.1	141,446,369	4.95E-06	9.09E-04	2.73E-03
A-1965117	rs980286	2q22.1	141,447,731	1.80E-07	1.22E-06	6.70E-04

P, raw *P*-value; *aP*, adjusted *P*-value; *bP*, Bonferroni-adjusted *P*-value.

The SNPs are located in the gene, low density lipoprotein-related protein 1B (LRP1B). The gene belongs to the low density lipoprotein receptor gene family. The gene is large, 1,906.41 kb, and has 93 introns.

For stage 2 of the analysis, these three SNPs plus 6 SNPs in other LD blocks spanning the large gene were selected for further analysis, again using the Successful Aging samples (32 samples) versus the Alzheimer's patients (227 samples). The additional SNPs included rs12467730, rs11901880, rs12466938, rs6748626 rs28483746, rs12474609 rs10201482, rs980286, rs13007735.

After analysis in stage 2, the SNPs from the microarray were still the most significant, indicating that this area of the gene was of interest. By chi-square analysis, the *P* values for rs12474609, rs10201482, and rs980286 were *P*=0.0042, 0.0009, and 0.009, respectively. Haplotype analysis of the three SNPs: T:C:T, gave a *P*=0.0034.

Encouraged by these results, 10 additional SNPs in four adjacent LD blocks around the SNPs from the microarray were selected for further analysis, to verify this region of interest. The additional SNPs included rs6732847, rs12053560, rs13016717, rs1346641 rs10928081, rs716000, rs716001, rs15845065, rs11888460, rs11904038.

The allelic comparisons and haplotypes are presented in Table II. The SNPs were in four linkage disequilibrium (LD) blocks, according to HAPMAP. LD was defined as having a pairwise $D' = 0.92$, instead of the usual 0.95. Those SNPs with a minor allele frequency (MAF) <10% were excluded. In addition to the Successful Aging samples, data was also obtained for the CEPH (Foundation Jean Dausset-Centre d'Etude du Polymorphisme Humain; www.cephb.fr/en/cephdb/) subjects from the NCBI website (www.ncbi.nlm.nih.gov), and both were analyzed versus the AD samples and versus each other (Table II).

The haplotypes for the Alzheimer's samples versus the Successful Aging without cognitive decline samples from the four LD blocks all have significant *P* values, ranging from 0.0012 to 0.0136, with low odds ratios ranging from 0.36 to 0.48. Thus the haplotype from Block 1 has *P*=0.0012; from Block 2, *P*=0.0052; from Block 3, *P*=0.0037; and Block 4, *P*=0.0136. The frequencies for the AD, CEPH, and SA for the haplotype in Block 1 were 78%, 80%, and 85%; for Block 2, they were 82%, 85%, and 89%; for Block 3, 78%, 79%, and 93%; for Block 4, 78%, 81%, and 86%, respectively. When the Alzheimer's samples versus the CEPH samples were analyzed, none of the haplotype blocks were significant. This area of the gene, LRP1B, has genetic variants significant/protective for successful aging without cognitive decline. Interestingly, 80% of the successful aging subjects were APOE3 and only 10% were APOE 2, suggesting

that in this group of subjects, APOE 2 may be less important for protection against AD. Of the 10% who were APOE 4, only one was APOE 4/4. The Alzheimer's patients were 43% APOE 4, 54% APOE 3, and 3% APOE 2.

DISCUSSION

Most studies of human aging have focused on centenarians and have examined survival or particular candidate genes for polymorphisms associated with longevity or avoidance of dementia. A polymorphism in the promoter region of the gene for APOC3 on chromosome 11q23 and one in the gene CETP (cholesterol ester transfer protein) on chromosome 16q21 have been identified in Ashkenazi Jews over the age of 99 years [Atzmon et al., 2006; Barzilai et al., 2006]. In the CETP study, subjects with MMSE's >25 were more likely to have the VV genotype [Barzilai et al., 2006]. Another study identified a locus on chromosome 4 at D4S1564 in centenarians as associated with longevity [Puca et al., 2001]. A recent study focused on successful cognitive aging and identified loci on chromosomes 1, 8, X, and Y as relevant [Zubenko et al., 2007]. Our study is unique in that we recruited seniors and veterans from the NAS study >85 years of age, with MMSE's >25, with no major illnesses, cholesterol levels and blood pressures under control, and generally no dementia in the family. Our genome-wide screen used the seniors with successful aging versus our late-onset Alzheimer's patients. Thus we identified haplotypes in the gene LRP1B as associated with successful aging without cognitive decline.

LRP1B belongs to the low density lipoprotein receptor gene family; it is located on chromosome 2q21.2. The gene is large, 1,906.41 kb, contains 93 introns, and 5 alternatively spliced mRNA's (AceView: <http://www.ncbi.nlm.nih.gov/IEB/Research/Acembly/av.cgi?c=geneid&org=9606&l=53353>). Of the three spliced mRNA's which encode good proteins, the "a" form is 16,535 bp, and the protein product is 4,636 aa. The protein has 4 extracellular ligand-binding domains which have different numbers of cysteine-rich ligand-binding repeats; it also has clusters of epidermal growth factor precursor repeats, and (F/Y)WDX spacer repeats. The 89 exons of LRP1B are nearly identical to those in LRP (low density lipoprotein receptor-related protein). The extra exons are exon 68 which encodes an additional ligand-binding repeat in domain IV, and exon 90, which encodes a 33 amino acid insertion in the cytoplasmic tail [Liu et al., 2001]. The SNPs of significance in this study are located primarily in intron 18 which is 30,219 bp.

LRP is widely expressed, has multiple functions, and interacts with multiple ligands. While LRP is expressed in liver, brain, and

TABLE II. Successful Aging Versus Alzheimer's Disease Versus CEPH Controls

SNP	AD	SA	CEPH	P SA/AD	Odds ratio	95% CI	P SA/CEPH	OR	95% CI	P AD/CEPH	OR	95% CI
Block 1												
rs6732847	253 (62%)	51 (80%)	135 (75%)	0.0069	0.42	0.22–0.80	0.57	1.3	0.65–2.22	0.0027	0.55	0.37–0.82
	153 (38%)	13 (20%)	45 (25%)									
rs12053560												
	46 (11%)	11 (17%)	27 (15%)	0.1822	0.62	0.30–1.26	0.17	1.18	0.55–2.53	0.21	0.72	0.43–1.21
	360 (89%)	53 (83%)	153 (85%)									
rs6748626												
	336 (83%)	60 (94%)	n/a	0.0248	0.32	0.11–0.91						
	70 (17%)	4 (6%)	n/a									
Haplotype												
				0.0012	0.36	0.19–0.68						
CTG												
Block 2												
rs13016717	347 (85%)	55 (86%)	157 (87%)	0.9209	0.96	0.45–2.05	0.07	0.9	0.39–2.05	0.32	0.86	0.51–1.45
	59 (15%)	9 (14%)	23 (13%)									
rs13446641												
	316 (78%)	52 (81%)	144 (80%)	0.5376	0.81	0.41–1.58	0.05	1.08	0.52–2.24	0.35	0.88	0.57–1.35
	90 (22%)	12 (19%)	36 (20%)									
rs10928081												
	348 (86%)	62 (97%)	158 (88%)	0.0129	0.19	0.05–0.81	0.04	4.32	0.99–18.91	0.5	0.84	0.49–1.41
	58 (14%)	2 (3%)	22 (12%)									
rs12474609												
	323 (80%)	68 (94%)	152 (86%)	0.0031	0.23	0.08–0.66	0.07	2.68	0.9–8.04	0.065	0.63	0.38–1.03
	81 (20%)	4 (6%)	24 (14%)									
Haplotype												
				0.0052	0.43	0.23–0.79	0.07	0.66	0.42–1.04	0.066	0.8	0.62–1.02
ATCA												
Block 3												
rs716000	361 (89%)	62 (97%)	160 (89%)	0.0485	0.26	0.06–1.09	0.06	3.88	0.88–17.07	1	1	0.57–1.75
	45 (11%)	2 (3%)	20 (11%)									
rs716001												
	307 (76%)	60 (94%)	120 (67%)	0.0016	0.22	0.08–0.61	0.0001	7.5	2.6–21.62	0.014	1.62	1.10–2.38
	95 (24%)	4 (6%)	60 (33%)									
rs10201482												
	298 (73%)	66 (92%)	143 (79%)	0.0008	0.25	0.11–0.60	0.01	3.42	1.28–9.09	0.12	0.71	0.47–1.09
	108 (27%)	5 (8%)	37 (21%)									
rs980286												
	303 (75%)	63 (88%)	141 (79%)	0.0175	0.42	0.20–0.88	0.13	1.84	0.84–4.03	0.23	0.77	0.50–1.18
	103 (25%)	9 (12%)	37 (21%)				<0.001	3.43	2.10–5.59	0.92	0.99	0.80–1.22
Haplotype												
				0.0037	0.36	0.18–0.74						
GCGG												

(Continued)

TABLE II. (Continued)

SNP	AD	SA	CEPH	P SA/AD	Odds ratio	95% CI	P SA/CEPH	OR	95% CI	P AD/CEPH	OR	95% CI
Block 4												
rs16845065												
G	342 (84%)	55 (86%)	157 (87%)	0.7269	0.87	0.41–1.86	0.07	0.9	0.39–2.05	0.35	0.78	0.47–1.31
C	64 (16%)	9 (14%)	23 (13%)									
rs11888460												
T	362 (89%)	62 (97%)	162 (90%)	0.0536	0.27	0.06–1.12	0.08	3.44	0.78–15.28	0.76	0.91	0.51–1.63
G	44 (11%)	2 (3%)	18 (10%)									
rs11904038												
C	300 (74%)	58 (91%)	143 (79%)	0.0035	0.29	0.12–0.70	0.04	2.5	1.00–6.24	0.15	0.73	0.48–1.12
T	106 (26%)	6 (9%)	37 (21%)									
rs13007735												
G	266 (66%)	53 (74%)	123 (69%)	0.1791	0.68	0.39–1.20	0.48	1.25	0.68–2.3	0.4	0.85	0.58–1.24
A	140 (34%)	19 (26%)	55 (31%)									
Haplotype												
GTCG				0.0136	0.4756	0.26–0.87	0.07	1.44	0.97–2.15	0.07	0.82	0.65–1.02

AD, Alzheimer's samples; SA, successful aging without cognitive decline; CEPH, European samples; data obtained from the NCBI website; OR, odds ratio; n/a, not available.

lung, LRP1B is most abundant in brain, thyroid, and salivary glands [Liu et al., 2001].

Both LRP and LRP1B are expressed at the cell surface and both bind and internalize similar ligands. However, the internalization rate is 15-fold slower or 80% less efficient for LRP1B when compared with LRP [Cam et al., 2004]. It has been shown that the protein product of LRP1B interacts with the β -amyloid precursor protein (APP) on the cell surface and binds soluble APP [Bu et al., 2006]. There is a threefold accumulation of APP at the cell surface, due to the slower endocytosis rate of the protein product of LRP1B. This accumulation of APP results in decreased production of A β 40 (amyloid- β peptide) and A β 42 [Cam et al., 2004]. If the haplotypes described in our study are involved with the decreased production of A β 42 in successful aging, LRP1B may become a new therapeutic approach for the treatment of Alzheimer's disease.

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REFERENCES

- Atzmon G, Rincon M, Schechter CB, Shuldiner AR, Lipton RB, Bergman A, Barzilai N. 2006. Lipoprotein genotype and conserved pathway for exceptional longevity in humans. *PLOS Biol* 4(4):e113.
- Barzilai N, Atzmon G, Derby CA, Bauman JM, Lipton RB. 2006. A genotype of exceptional longevity is associated with preservation of cognitive function. *Neurology* 67:2170–2175.
- Brady CB, Spiro A, McGlinchey-Berroth R, Milberg W, Gaziano JM. 2001. Stroke risk predicts verbal fluency decline in healthy older men: Evidence from the normative aging study. *J Gerontology Series B* 56:340–346.
- Bu G, Cam J, Zerbiniatti C. 2006. LRP in amyloid- β production and metabolism. *Ann NY Acad Sci* 1086:35–53.
- Cam JA, Zerbiniatti CV, Knisely JM, Hecimovic S, Li Y, Bu G. 2004. The low density lipoprotein receptor-related protein at the cell surface and reduces amyloid- β peptide production. *J Biol Chem* 279:29639–29646.
- Falush D, Stephens M, Pritchard JK. 2003. Inference of population structure using multilocus genotype data: Linked loci and correlated allele frequencies. *Genetics* 164:1567–1587.
- Folstein MF, Folstein SE, McHugh PR. 1975. Mini-mental state. A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* 12:189–198.
- Huang R, Poduslo SE. 2006. CYP19 haplotypes increase risk for Alzheimer's disease. *J Med Genet* 43:e42.
- Liu C-X, Li Y, Obermoeller-McCormick LM, Schwartz AL, Bu G. 2001. The putative tumor suppressor LRP1B, a novel member of the low density lipoprotein (LDL) receptor family, exhibits both overlapping and distinct

- properties with the LDL receptor-related protein. *J Biol Chem* 276:28889–28896.
- McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlen E. 1984. Clinical diagnoses of Alzheimer's disease: Report of the NINCDS-ADRDA work group under the auspices of the Department of Health and Human Services Task Force on Alzheimer's disease. *Neurology* 34: 939–944.
- Perls T, Wilmoth J, Levenson R, Drinkwater M, Cohen M, Bogan H, Joyce E, Brewster S, Kunkel L, Puca A. 2002. Life-long sustained mortality advantage of siblings of centenarians. *Proc Natl Acad Sci USA* 99:8442–8447.
- Puca AA, Daly MJ, Brewster SJ, Matisse TC, Barrett J, Shea-Drinkwater M, Kang S, Joyce E, Nicoli J, Benson E, Kunkel LM, Perls T. 2001. A genome-wide scan for linkage to human exceptional longevity identifies a locus on chromosome 4. *Proc Natl Acad Sci USA* 98:10505–10508.
- Purcell S, Cherny SS, Sham PC. 2003. Genetic power calculator: Design of linkage and association genetic mapping studies of complex traits. *Bioinformatics* 19:149–150.
- Zubenko GS, Hughes HB, Zubenko WN, Maher BS. 2007. Genome survey for loci that influence successful aging: Results at 10-cM resolution. *Am J Geriatr Psychiat* 15:184–193.