Table 1
Demographics of brain donor subjects

	No. of subjects (F, M)	AAD		*APOE* genotype					
		Mean (SD)	Range	ε2*2	ε2*3	ε2*4	ε3*3	ε3*4	ε4*4
Cases	213 (128, 85)	83.5 (8.0)	57–102	0	8	1	105	82	17
Controls	370 (145, 225)	81.3 (7.2)	70–105	1	32	1	302	34	0

F, female; M, male; AAD, age at death of brain donor subjects; SD, standard deviation.

(DYNACOM, Japan; http://www.dynacom.co.jp/), as described in detail elsewhere [41].

Multiple logistic regression analysis was conducted to reveal the risk effects of significant SNPs identified in a SNP case-control study. For the SNPs, adjusted OR with 95% CI as to age, gender, and the carrier status of the *APOE* ε4 allele was computed. Subjects with an undetermined SNP genotype were excluded, with 554 subjects remaining. For this analysis, we used SPSS version 15.0 software (IBM).

We considered $p < 0.05$ to be statistically significant.

RESULTS

Association of SORL1 SNPs with LOAD

To determine whether or not *SORL1* exhibits a reproducible association with LOAD in Japanese, we genotyped 19 SNPs (Supplementary Table 1) in 583 neuropathologically characterized brain donor subjects (Table 1). Genetic associations were examined in a SNP case-control study (Table 2), followed by multiple logistic regression analysis of each significant SNP (Supplementary Table 2).

In the HapMap database, SNP rs4598682 was not available and remaining 18 SNPs were found to be polymorphic in Japanese (Supplementary Figure 1). Fifteen SNPs met HWE ($p > 0.05$) in both the LOAD and control subjects, however, one SNP, rs689021 (SNP 9), in the cases and three SNPs, rs11218343, rs17125523, and rs3737529, in the controls were deviated from HWE (Supplementary Table 1). Five SNPs, rs985421, rs12364988 [SNP 7], rs4598682, rs3781834, and rs3781836, showed significance after multiple test correction ($p < 2.63\text{E-}03$ [$= 0.05/19$]) (Table 2). We observed the most significant association for SNP rs985421: $p = 3.21\text{E-}04$ and OR (95% CI) = 0.58 (0.43–0.78).

Multiple logistic regression analysis strongly supported evidence that all five significant SNPs are associated with LOAD after adjustment as to age, gender and carrier status of the *APOE* ε4 allele (Supplementary Table 2).

LD mapping and haplotype-based case-control study

On *in silico* LD mapping using the HapMap genotype data (Release 24 on NCBI build 36, dbSNP build 126) for Japanese in Tokyo (JPT), it was found that *SORL1* could be roughly divided into two large LD blocks. Three significant SNPs, rs985421, rs12364988 (SNP 7), and rs4598682, encompassed by a 5' LD region were sandwiched between recombination hot spot (RHS)-1 and RHS-2, and the remaining two SNPs, rs3781836 and rs3781836, by a 3' LD region sandwiched between RHS-2 and RHS-3 (Fig. 1). Strong LD was detected between significant SNPs within both LD regions (Supplementary Figure 2). Then, haplotype block estimation involving Gabriel et al.'s and four gamete methods [40] and case-control haplotype analysis were carried out to elucidate the effects of multiple SNP combinations as to disease risk (Supplementary Figure 2). Four and three haplotype blocks were observed with Gabriel et al.'s (Supplementary Figure 2A) and the four gamete (Supplementary Figure 2C) methods, respectively. Various haplotypes exhibited significance on case-control haplotype analysis (Supplementary Figures. 2B, D). The most significant haplotype, a-c-C-G-g-G-C-G (m-m-M-M-m-M-M-M, rs985421-rs12364988-rs668387-rs689021-rs4598682-rs641120-rs12285364-rs2298813), was found in haplotype block 1 (Supplementary Figure 2D) revealed by means of the four gamete method: permutation $p = 2.00\text{E-}04$ and OR (95% CI) = 0.58 (0.43–0.78).

DISCUSSION

Here, we were able to genetically replicate the association of *SORL1* with LOAD using Japanese neuropathologically well-characterized brain donor subjects (Table 1). Through a SNP-based case-control association study (Table 2) and multiple logistic regression analysis (Supplementary Table 2), it was found that five of the 19 SNPs genotyped are associated with LOAD: one SNP, rs12364988 (SNP 7), was from the initial paper [5], and the remaining four,

Table 2
Genetic associations of *SORL1* with AD

dbSNP	SNP No.[a]	Allele		No. of subjects						*P*[b]	OR (95% CI)[c]
		M	m	Cases			Controls			(df = 1)	
				MM	Mm	mm	MM	Mm	mm		
rs4935774	1	T	c	156	49	7	269	93	5	6.99E-01	1.07 (0.76 - 1.50)
rs985421	-	G	a	144	61	7	194	146	26	***3.21E-04***	0.58 (0.43 - 0.78)
rs12364988	7	T	c	113	84	15	149	173	45	***1.65E-03***	0.66 (0.51 - 0.86)
rs668387	8	C	t	54	95	63	94	192	83	2.38E-01	1.16 (0.91 - 1.47)
rs689021	9	G	a	60	88	59	105	183	77	2.54E-01	1.16 (0.91 - 1.47)
rs4598682	-	A	g	144	60	7	197	145	26	***3.87E-04***	0.58 (0.43 - 0.79)
rs641120	10	G	a	60	93	58	105	183	76	2.59E-01	1.15 (0.91 - 1.46)
rs12285364	12	C	t	134	69	9	264	98	7	1.92E-02	1.44 (1.06 - 1.97)
rs2298813	13	G	a	132	71	9	263	99	6	9.29E-03	1.50 (1.10 - 2.04)
rs2276346	15	G	t	171	40	0	307	56	6	8.83E-01	1.03 (0.69 - 1.56)
rs11218343	-	A	g	100	93	19	143	194	32	1.34E-01	0.83 (0.64 - 1.07)
rs3781834	-	A	g	147	55	9	195	155	19	***4.77E-04***	0.59 (0.44 - 0.80)
rs3781836	-	G	a	144	56	9	195	152	19	***1.20E-03***	0.61 (0.45 - 0.83)
rs2282647	-	C	g	141	58	9	199	151	18	4.88E-03	0.66 (0.49 - 0.89)
rs17125523	-	T	c	131	69	12	188	163	18	4.95E-02	0.76 (0.57 - 1.01)
rs3824968	23	A	t	53	109	50	85	198	83	8.83E-01	0.98 (0.77 - 1.25)
rs3737529	-	G	a	131	69	12	189	162	18	5.60E-02	0.77 (0.58 - 1.02)
rs2282649	24	C	t	51	108	53	86	199	83	7.66E-01	1.04 (0.82 - 1.32)
rs1010159	25	C	t	53	108	51	85	196	86	8.38E-01	0.98 (0.77 - 1.24)

P passing Bonferroni correction for multiple testing ($p < 2.63E-03$ [= 0.05/19]) are shown in italicized boldface type highlighted in light-gray. M, major allele; m, minor allele; MM, major allele homozygote; Mm, heterozygote; mm, minor allele homozygote; df, degree of freedom. [a]Rogaeva's SNP ID [5]. [b]Cochran-Armitage trend test. [c]OR (95% CI) for the minor allele of each SNP.

rs985421, rs4598682, rs3781834, and rs3781836, from our GWAS [36]. We further obtained evidence that minor alleles of all these SNPs have a protective effect as to LOAD (Table 2 and Supplementary Table 2). Our data firmly support the positive association observed in the initial [5] and following replication studies involving Caucasians [18–23, 26, 27, 29, 30]. Recent large-scale transethnic meta-analysis involving over 30,000 subjects, including Caucasians and Asians, demonstrated that several *SORL1* SNPs are associated with LOAD [42]. Overall, it is likely that *SORL1* is associated with LOAD beyond ethnicity, although the disease risk effect of this gene (OR, 1.0–2.0) is evidently moderate compared to that of *APOE* (OR, 3.0–5.0) [43, 44].

In a *SORL1* genomic region spanning about 182 kb, two extended LD structures were roughly observed in the 5' and 3' regions (Fig. 1). It was found from the HapMap data that they are formed from three recombination hot spots, RHS1, RHS2, and RHS3 (Fig. 1). In our samples, association signals were located in both LD regions (Fig. 1), which is consistent with previous studies [5, 18, 20]. As suggested by Rogaeva et al. [5] and others [18, 20], allelic heterogeneity obviously exists in the *SORL1* region. Given the two rough LD structures and the presence of three recombination hot

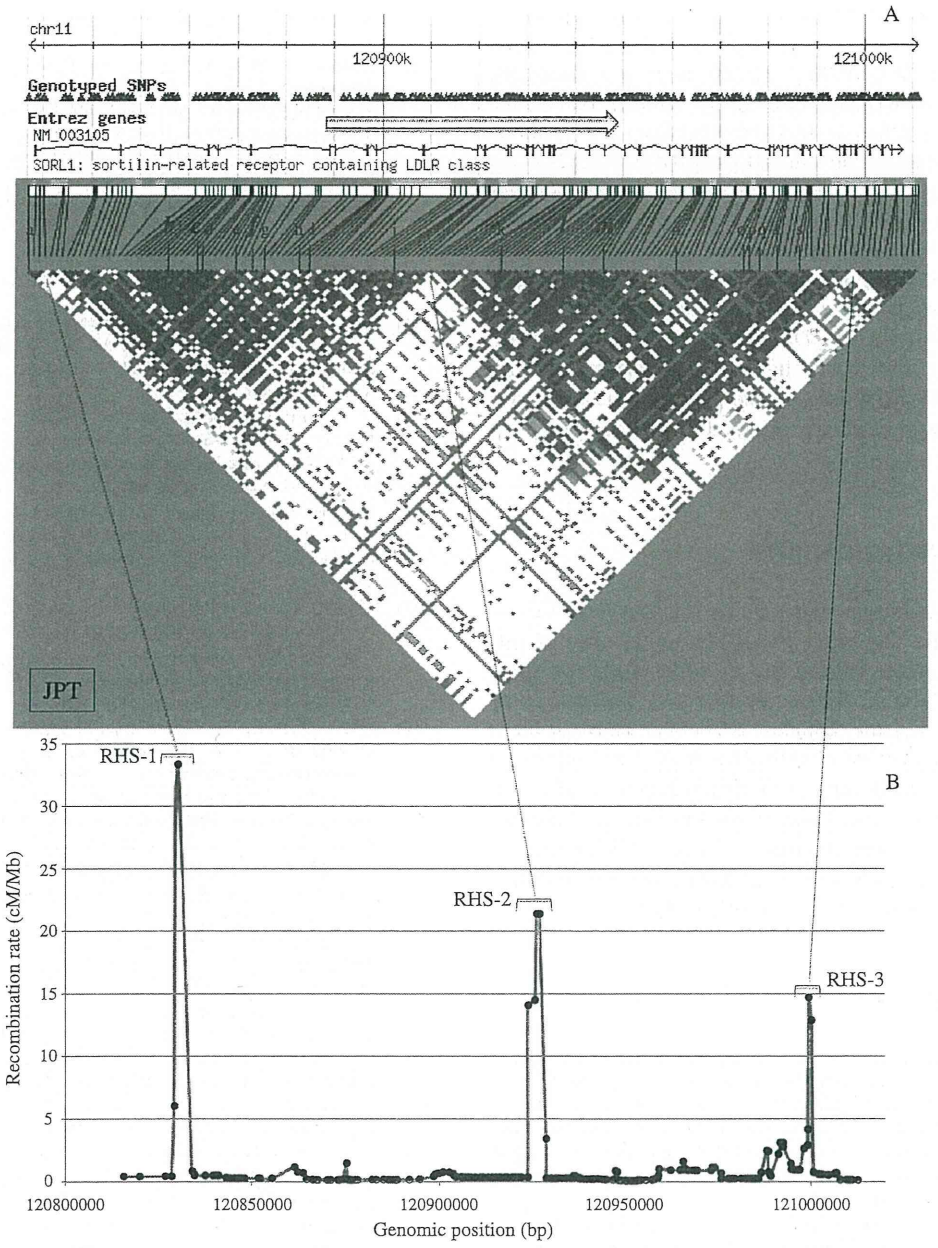

Fig. 1. LD structures and recombination hot spots in and around *SORL1*. A) For *in silico* LD mapping of *SORL1*, we used JPT genotype data with HWE $p > 0.001$ and minor allele frequency >0.01 from HapMap release 24 on dbSNP build 126 (NCBI build 36). The transcription orientation of *SORL1* is indicated by the horizontal non-filled arrow. Vertical short lines labeled with lower-case letters, a–s, indicate the genomic positions of the 19 SNPs examined in this study: a, rs4935774 (SNP 1); b, rs985421; c, rs12364988 (SNP 7); d, rs668387 (SNP 8); e, rs689021 (SNP 9); f, rs4598682; g, rs641120 (SNP 10); h, rs12285364 (SNP 12); i, rs2298813 (SNP 13); j, rs2276346 (SNP 15); k, rs11218343; l, rs3781834; m, rs3781386; n, rs2282647; o, rs17125523; p, rs3824968 (SNP 23); q, rs3737529; r, rs2282649 (SNP 24); and s, rs1010159 (SNP 25). Since genotype data for Japanese for SNP rs4598682 (genomic position: 120,881,161 bp) were not available in the HapMap database (Release 24), SNP rs7105365 (genomic position: 120,882,005 bp), located adjacent to SNP rs4598682, is labeled 'f' (dotted lines) instead. Significant SNPs are indicated by bold italicized letters (b, c, f, l, and m). B) Regional recombination rate data were obtained from Phase II HapMap estimated from phased haplotypes in HapMap Release 22 (NCBI build 36). The three recombination hot spots (RHS) are labeled RHS-1, RHS-2, and RHS-3, respectively.

spots within *SORL1* (Fig. 1), it is strongly suggested that there are two genomic regions showing association with LOAD in *SORL1*. Hence, there is a possibility that a cis-interaction causing an increased risk and/or protective effect as to LOAD between known/novel independent variants in and around *SORL1* exists.

Through exome sequencing in autosomal dominant early-onset AD index cases without a mutation in *APP*, *PSEN1*, or *PSEN2* in some families, Pottier et al. [45] identified novel non-synonymous variants of *SORL1*. In this study we were able to apparently replicate the association of *SORL1* with neuropathologically characterized LOAD. In the future, the relationship between *SORL1* variants and pathogenesis of the AD brain would be clearly revealed by neuropathological analyses.

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Supplementary Data

SORL1 is Genetically Associated with Neuropathologically Characterized Late-Onset Alzheimer's Disease

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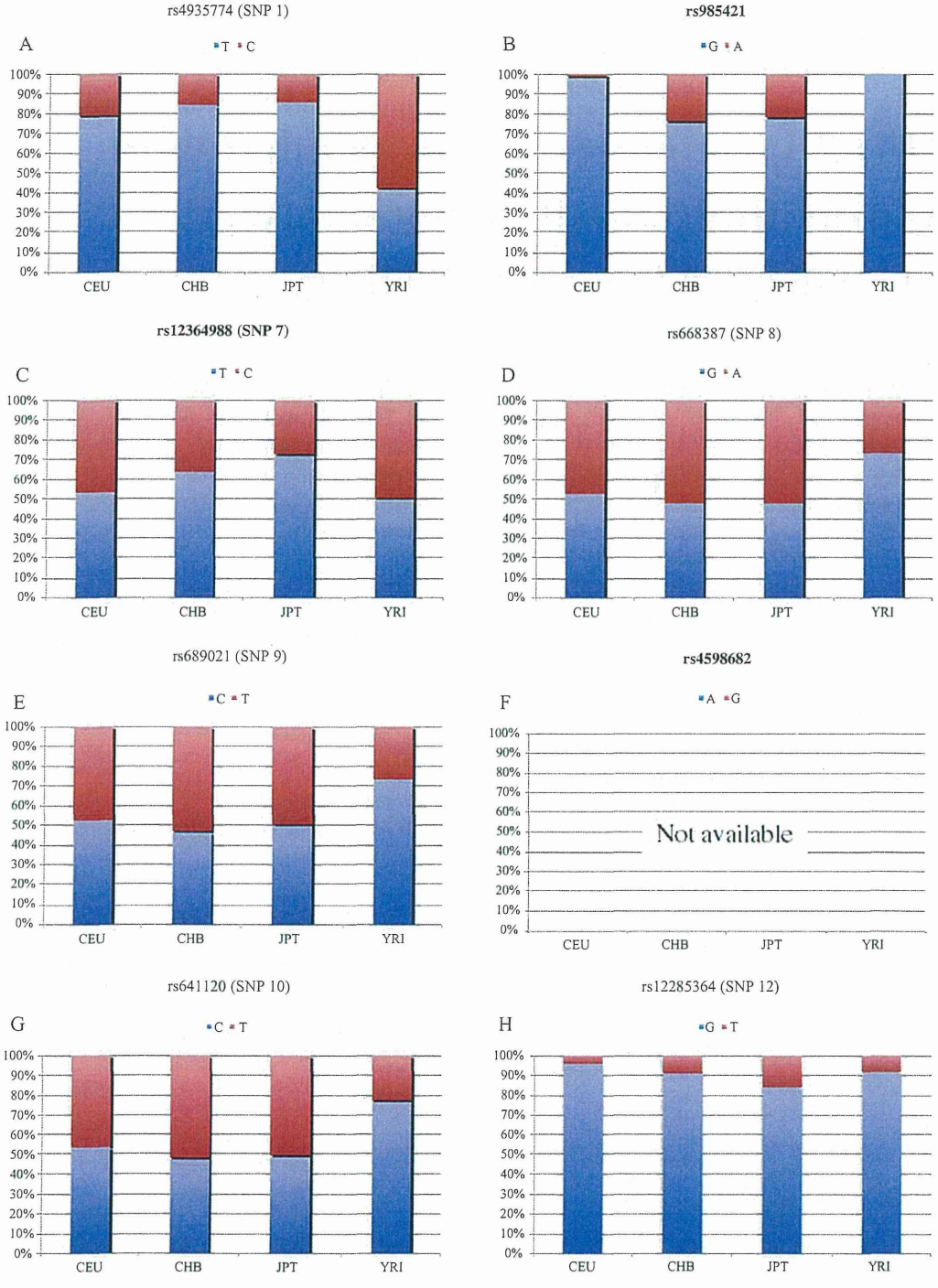
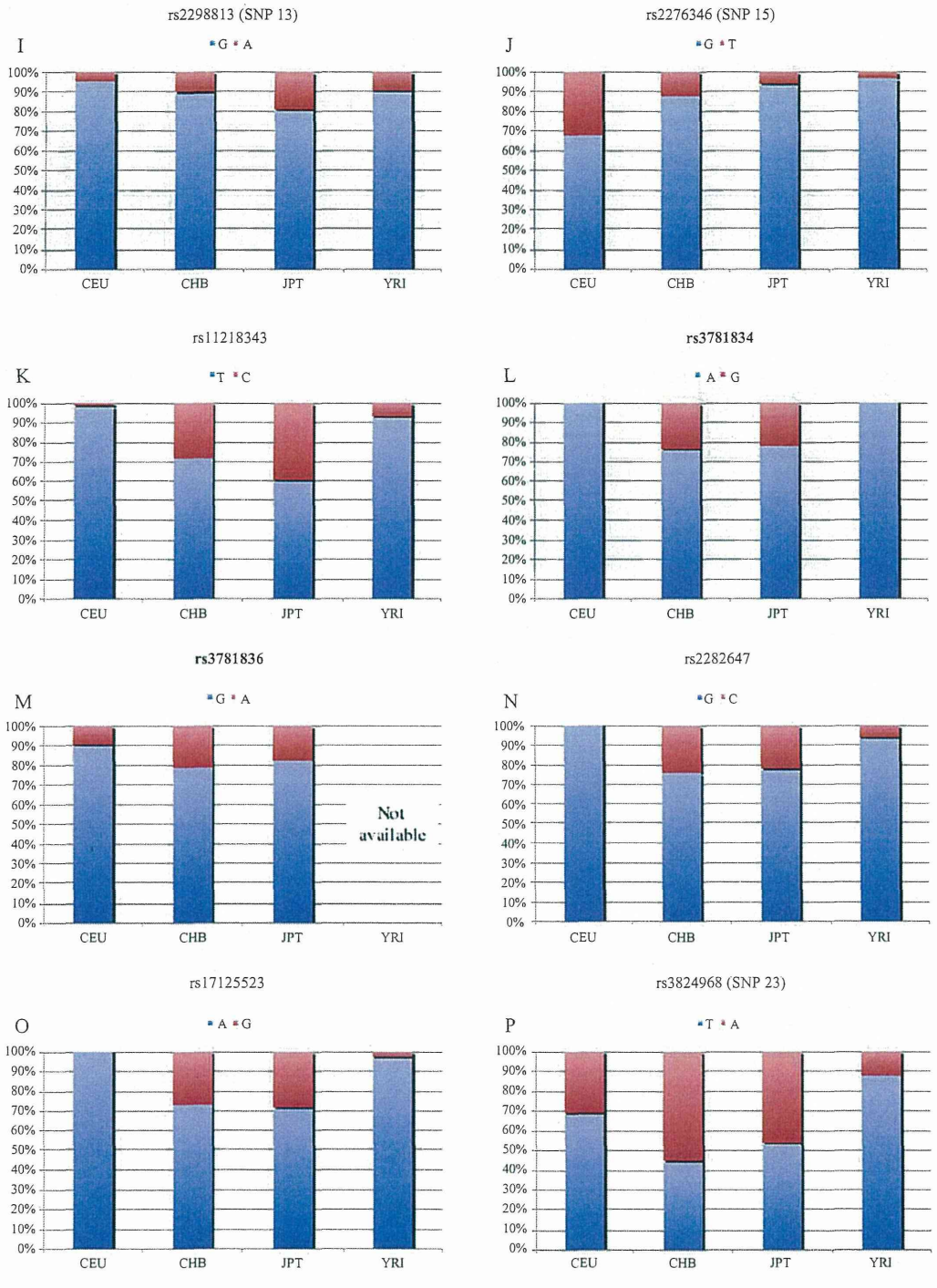
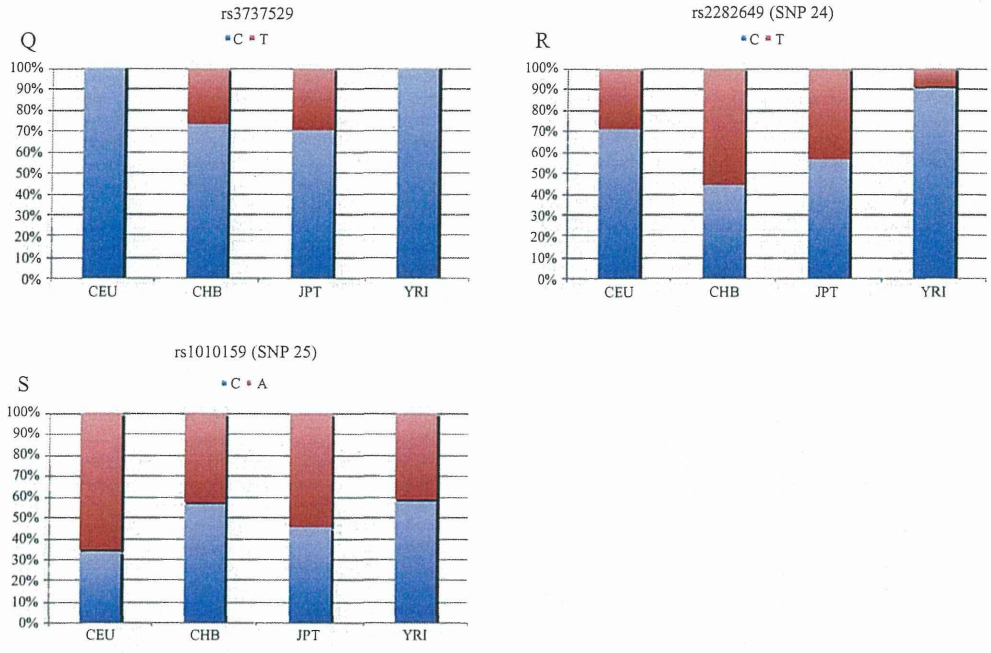


Supplementary Figure 1. Allelic frequency data from HapMap (Release #24) concerning the 19 SNPs examined in this study. The allelic frequencies for four populations, US Utah residents of northern and western European ancestry (CEU), Han Chinese in Beijing (CHB), Japanese in Tokyo (JPT), and Yoruba in Ibadan (YRI), Nigeria, are presented. Five significant SNPs, rs985421, rs12364988 (SNP 7), rs4598682, rs3781834, and rs3781836, found in a single SNP case-control study (Table 2 and Supplementary Table 2) are shown in boldface. Allelic frequency data for all four populations for SNP rs4598682 and those for YRI for SNP rs3781836 were not available in the HapMap database (Release #24).

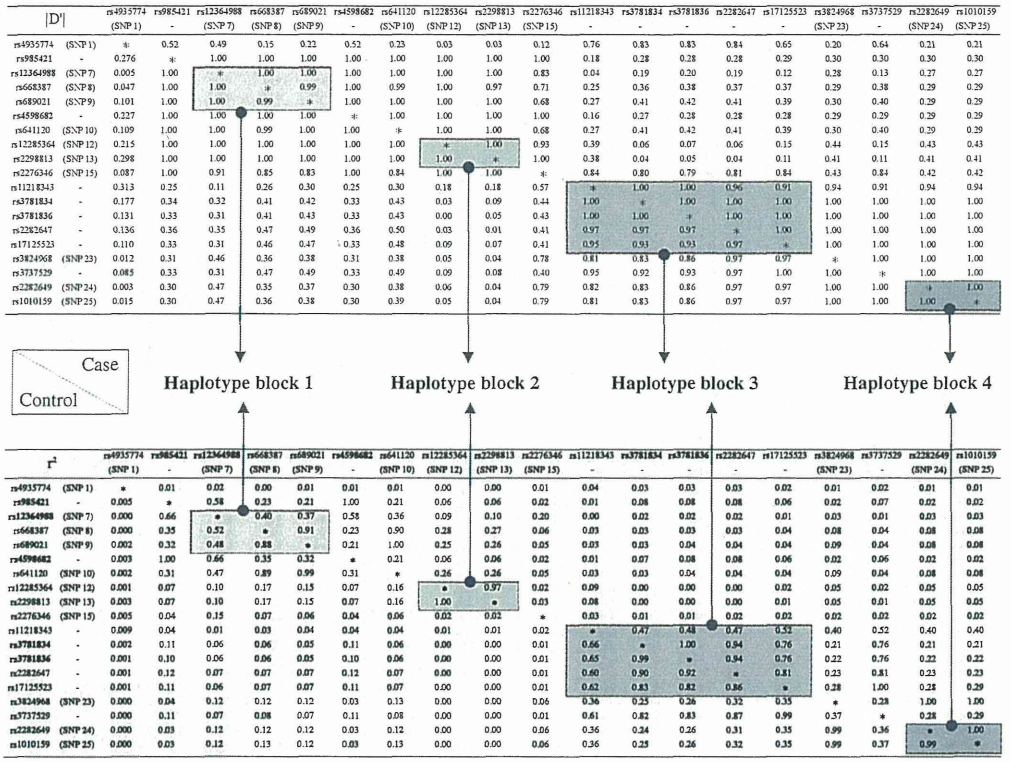


Supplementary Figure 1. (*Continued*)



Supplementary Figure 1. (*Continued*)

A

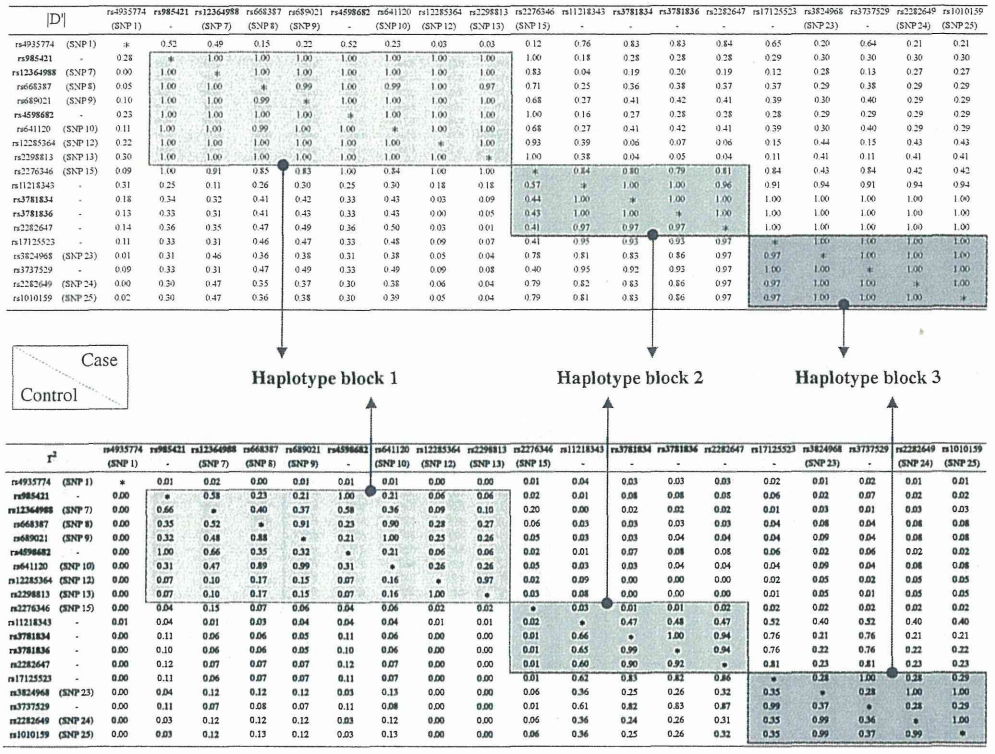


B

Haplotype block	Haplotype	Frequency		No. of estimated alleles		Permutation *P*	OR (95% CI)
		Cases	Controls	Cases	Controls	(10,000)	
1	M-m-m	0.499	0.459	213	340	2.05E-01	1.18 (0.93 - 1.49)
	m-M-M	0.268	0.358	114	265	**1.40E-03**	0.65 (0.50 - 0.85)
	M-M-M	0.211	0.156	90	115	**1.92E-02**	1.46 (1.07 - 1.98)
	M-m-M	0.022	0.028	9	20	6.92E-01	0.78 (0.35 - 1.72)
	Global *P*	-	-	-	-	**5.20E-03**	-
2	M-M	0.792	0.847	337	627	**1.63E-02**	0.68 (0.50 - 0.93)
	m-m	0.209	0.153	89	113	**1.63E-02**	1.47 (1.08 - 1.99)
	Global *P*	-	-	-	-	**1.63E-02**	-
3	M-M-M-M-M	0.689	0.655	294	484	2.18E-01	1.18 (0.91 - 1.52)
	m-m-m-m-m	0.177	0.254	75	188	**1.70E-03**	0.63 (0.47 - 0.85)
	m-M-M-M-M	0.107	0.077	46	57	8.34E-02	1.45 (0.96 - 2.18)
	m-M-M-M-m	0.027	0.014	11	11	1.85E-01	1.76 (0.75 - 4.09)
	Global *P*	-	-	-	-	**6.20E-03**	-
4	m-M	0.505	0.497	215	368	8.57E-01	1.03 (0.81 - 1.31)
	M-m	0.495	0.503	211	372	8.57E-01	0.97 (0.76 - 1.23)
	Global *P*	-	-	-	-	8.57E-01	-

Supplementary Figure 2. LD analysis. LD measures, |D'| and r^2, for the 19 *SORL1* SNPs examined were computed. Significant SNPs, i.e., rs985421, rs12364988 (SNP 7), rs4598682, rs3781834, and rs3781836, are depicted in boldface. The upper-right half shows AD cases and the lower-left half controls. Haplotype blocks were estimated using Gabriel et al.'s (A) and four gamete (C) methods [1], and are highlighted in grey-colored serial boxes. The results of the haplotype case-control study for each haplotype block are presented in B (Gabriel et al.'s method) and D (four gamete method). Permutation *p* (10,000 iterations of random sampling) of less than 0.05 are depicted in boldface. M, major allele; m, minor allele.

C



D

Haplotype block	Haplotype	Frequency		No. of estimated alleles		Permutation *P*	OR (95% CI)
		Cases	Controls	Cases	Controls	(10,000)	
1	M-M-m-m-M-m-M-M	0.500	0.459	213	340	1.99E-01	1.18 (0.93 - 1.49)
	m-m-M-M-m-M-M-M	0.176	0.269	75	199	**2.00E-04**	0.58 (0.43 - 0.78)
	M-M-M-M-M-M-m-m	0.212	0.154	90	114	**1.64E-02**	1.47 (1.08 - 2.00)
	M-m-M-M-M-M-M-M	0.094	0.090	40	67	8.35E-01	1.04 (0.69 - 1.57)
	M-M-m-M-M-M-M-M	0.019	0.027	8	20	4.25E-01	0.69 (0.30 - 1.58)
	Global *P*	-	-	-	-	**2.00E-03**	-
2	M-M-M-M-M	0.599	0.571	255	423	3.78E-01	1.12 (0.88 - 1.42)
	M-m-m-m-m	0.176	0.247	75	183	**5.40E-03**	0.65 (0.48 - 0.88)
	M-m-M-M-M	0.131	0.091	56	67	**2.74E-02**	1.52 (1.04 - 2.22)
	m-M-M-M-M	0.094	0.091	40	67	8.29E-01	1.04 (0.69 - 1.57)
	Global *P*	-	-	-	-	**1.17E-02**	-
3	M-M-M-m-M	0.505	0.495	215	366	7.63E-01	1.04 (0.82 - 1.32)
	m-m-m-M-m	0.218	0.271	93	200	5.17E-02	0.75 (0.57 - 1.00)
	M-m-M-M-m	0.277	0.235	118	174	1.23E-01	1.25 (0.95 - 1.64)
	Global *P*	-	-	-	-	8.92E-02	-

Supplementary Figure 2. (*Continued*)

Supplementary Table 1
SNP information

dbSNP	SNP No.[a]	Genomic position (bp)[b]	SNP interval (bp)	SNP position	Allele				GSR	HWE *P*	
					M	Frequency	m	Frequency		Cases	Controls
rs4935774	1	120,826,964	40,562	Upstream of 5' UTR	T	0.857 (0.86)	c	0.143 (0.14)	99.3%	0.2711	0.5136
rs985421	-	120,867,526	5,310	Intron 5	G	0.764 (0.78)	a	0.236 (0.22)	99.1%	0.8151	0.8952
rs12364988	7	120,872,836	295	Exon 6 (H269H)	T	0.674 (0.72)	c	0.326 (0.28)	99.3%	1.0000	0.7333
rs668387	8	120,873,131	3,199	Intron 6	C	0.502 (0.48)	t	0.498 (0.52)	99.7%	0.1327	0.4663
rs689021	9	120,876,330	4,831	Intron 6	G	0.525 (0.50)	a	0.475 (0.50)	98.1%	***0.0367***	0.9163
rs4598682	-	120,881,161	5,014	Intron 6	A	0.766 (N/A)	g	0.234 (N/A)	99.3%	0.8117	1.0000
rs641120	10	120,886,175	12,261	Intron 6	G	0.527 (0.49)	a	0.473 (0.51)	98.6%	0.0980	0.9160
rs12285364	12	120,898,436	458	Intron 9	C	0.829 (0.84)	t	0.171 (0.16)	99.7%	1.0000	0.6867
rs2298813	13	120,898,894	20,792	Exon 11 (T528A)	G	0.828 (0.80)	a	0.172 (0.20)	99.5%	1.0000	0.4186
rs2276346	15	120,919,686	21,111	Intron 13	G	0.907 (0.93)	t	0.093 (0.07)	99.5%	0.2271	0.1069
rs11218343	-	120,940,797	10,353	Intron 22	A	0.665 (0.60)	g	0.335 (0.40)	99.7%	0.7498	***0.0029***
rs3781834	-	120,951,150	2,398	Intron 24	A	0.771 (0.78)	g	0.229 (0.22)	99.5%	0.2250	0.1065
rs3781836	-	120,953,548	13,255	Intron 25	G	0.770 (0.82)	a	0.230 (0.18)	98.6%	0.2376	0.1367
rs2282647	-	120,966,803	12,646	Intron 30	C	0.772 (0.77)	g	0.228 (0.23)	98.8%	0.3520	0.1311
rs17125523	-	120,979,449	1,683	Intron 32	T	0.749 (0.71)	c	0.251 (0.29)	99.7%	0.4294	***0.0241***
rs3824968	23	120,981,132	1,894	Exon 34 (A1584A)	A	0.504 (0.47)	t	0.496 (0.53)	99.1%	0.7834	0.1431
rs3737529	-	120,983,026	1,142	Intron 36	G	0.750 (0.71)	a	0.250 (0.30)	99.7%	0.4294	***0.0246***
rs2282649	24	120,984,168	4,443	Intron 38	C	0.501 (0.57)	t	0.499 (0.43)	99.5%	0.8907	0.1441
rs1010159	25	120,988,611	-	Intron 39	C	0.501 (0.46)	t	0.499 (0.54)	99.3%	0.8907	0.2113

The allele frequency and genotyping success rate (GSR) of each SNP were computed by combining AD and control subjects: allele frequency values derived from HapMap JPT data (Release 24/Phase II on NCBI build 36) are shown in parentheses. HWE *p* of less than 0.05 are shown in boldface. M, major allele; m, minor allele; N/A, not available. [a]Rogaeva's SNP ID [2]. [b]According to NCBI build 36.

Supplementary Table 2
Multiple logistic regression analysis of significant *SORL1* SNPs

dbSNP	SNP No.[a]	Allele		No. of subjects						Genotype: MM_{ref} vs Mm vs mm			
		M	m	Cases			Controls			Global *P*	OR_{MM} (ref.)	OR_{Mm} (95% CI)	OR_{mm} (95% CI)
				MM	Mm	mm	MM	Mm	mm				
rs985421	-	G	a	136	58	6	188	140	26	**0.009**	1.00	0.55 (0.36 - 0.84)**	0.39 (0.14 - 1.10)
rs12364988	7	T	c	106	80	14	142	168	44	**0.019**	1.00	0.64 (0.42 - 0.97)*	0.40 (0.19 - 0.85)*
rs4598682	-	A	g	136	58	6	188	140	26	**0.009**	1.00	0.55 (0.36 - 0.84)**	0.39 (0.14 - 1.10)
rs3781834	-	A	g	139	52	9	186	149	19	**0.001**	1.00	0.43 (0.27 - 0.67)***	0.49 (0.18 - 1.29)
rs3781836	-	G	a	139	52	9	188	147	19	**0.001**	1.00	0.44 (0.28 - 0.69)***	0.49 (0.19 - 1.31)

Global *p* of less than 0.05 are shown in boldface. M, major allele; m, minor allele; MM, major allele homozygote; Mm, heterozygote; mm, minor allele homozygote; ref, reference; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$. [a]Rogaeva's SNP ID [2].

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