Brief Genetics Report

SLC12A3 (Solute Carrier Family 12 Member [Sodium/Chloride] 3) Polymorphisms Are Associated With End-Stage Renal Disease in Diabetic Nephropathy

Jae Hyeon Kim,[1,2] Hyoung Doo Shin,[3] Byung Lae Park,[3] Min Kyong Moon,[1,2] Young Min Cho,[3,4] Young Hwan Hwang,[4] Kook Whan Oh,[4] Seong Yeon Kim,[4] Hong Kyu Lee,[4] Curie Ahn,[4] and Kyong Soo Park[3,4]

Diabetic nephropathy is the most common cause of end-stage renal disease (ESRD). Genetic susceptibility plays an important role in the development and progression of diabetic nephropathy. Previous studies have revealed that polymorphisms in the *SLC12A3* (solute carrier family 12 member [sodium/chloride] 3) gene, which encodes solute carrier family 12 member 3, might contribute to genetic susceptibility to diabetic nephropathy and essential hypertension. In this study, we examined whether the *SLC12A3* gene locus is associated with ESRD resulting from diabetic nephropathy. We genotyped 11 common single nucleotide polymorphisms (SNPs) in the *SLC12A3* gene in 177 patients with ESRD due to type 2 diabetes and 184 patients with diabetic retinopathy but with no signs of renal involvement. Three SNPs (g.34372G>A [Arg913Gln], g.39143G>A, and g.41727C>T) were found to be associated with ESRD due to diabetic nephropathy. These three SNPs were in complete linkage disequilibrium. Haplotype 4 in block 2 (18806C, 21822C, 34372A, 39143A, 39240T, 39375C, and 41727T) showed a significant association with ESRD due to type 2 diabetes ($P = 0.0028$). These results suggest that the *SLC12A3* gene locus is associated with ESRD due to diabetic nephropathy. *Diabetes* 55:843–848, 2006

Diabetic nephropathy is the most common cause of end-stage renal disease (ESRD) (1,2). Strong evidence has been provided by epidemiological (3) and familial (4,5) studies suggesting that genetic susceptibility plays an important role in the pathogenesis of diabetic nephropathy; however, the causative genes remain elusive. The *SLC12A3* (solute carrier family 12 member [sodium/chloride] 3) gene, at chromosome 16q13, which encodes a thiazide-sensitive sodium chloride cotransporter (MIM600968), has been shown to be a new candidate gene for diabetic nephropathy (6,7) and hypertension (8–10). It was shown that loss-of-function mutations in the *SLC12A3* gene cause Gitelman's syndrome, which is inherited as an autosomal-recessive trait and is characterized by low blood pressure due to renal sodium wasting, hypokalemia, metabolic alkalosis, hypocalciuria, and volume depletion (11). On the other hand, substitution of Arg913 to Gln in the *SLC12A3* gene (Arg904Gln in the previous report), has been reported to predispose essential hypertension in the Swedish and Japanese (8,9). In addition, blood pressure reduction by thiazides was found to be dependent on genetic variations in the *SLC12A3* gene (10). Recently, a genome-wide association study using single nucleotide polymorphisms (SNPs) showed that the gene encoding *SLC12A3* (especially Arg913Gln) may contribute to genetic susceptibility to diabetic nephropathy in the Japanese (6). In this study, we investigated the associations between *SLC12A3* polymorphisms and ESRD due to type 2 diabetic nephropathy in the Korean population.

RESULTS

We studied 361 patients with type 2 diabetes, comprising 177 patients with ESRD resulting from diabetic nephropathy (the ESRD group) and 184 patients with diabetic retinopathy and duration of diabetes of >15 years but without evidence of renal disease (the control group). The clinical characteristics of these groups are summarized in Table 1. Among the identified 52 polymorphisms in the *SLC12A3* gene, as determined by sequencing of 48 Japanese DNA samples (12), we selected four SNPs (g.34372 G>A [Arg913Gln], g.39143 G>A, g.39240 C>T, and g.41727 C>T) that had been previously reported to be associated with diabetic nephropathy (6). We also selected an additional seven common SNPs based on frequency (frequency >0.05 in regulatory region, frequency >0.1 in introns) and linkage disequilibrium (LD) status (12). Two SNPs were in the promoter region, three in exons, and six in introns (Fig. 1*A*). Haplotype blocks were constructed by using LD patterns among the 11 SNPs genotyped. There was one break in the LD patterns; accordingly, two blocks were found in this study (Fig. 1*C*). We selected common haplotypes (frequency >0.05) from each block, which accounted for >95% of observed haplotypes (Fig. 1*B*). The

From the [1]Department of Internal Medicine, Seoul National University Boramae Hospital, Seoul, Korea; the [2]Genome Research Center for Diabetes and Endocrine Disease, Clinical Research Institute, Seoul National University Hospital, Seoul, Korea; the [3]Department of Genetic Epidemiology, SNP genetics, Seoul, Korea; and the [4]Department of Internal Medicine, Seoul National University College of Medicine, Seoul, Korea.

Address correspondence and reprint requests to Kyong Soo Park, MD, PhD, or Curie Ahn, MD, PhD, Department of Internal Medicine, Seoul National University College of Medicine, 28 Yongon-Dong Chongno-Gu, Seoul, 110-744, Korea. E-mail: kspark@snu.ac.kr or curie55@naver.com.

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ESRD, end-stage renal disease; LD, linkage disequilibrium; SNP, single nucleotide polymorphism.

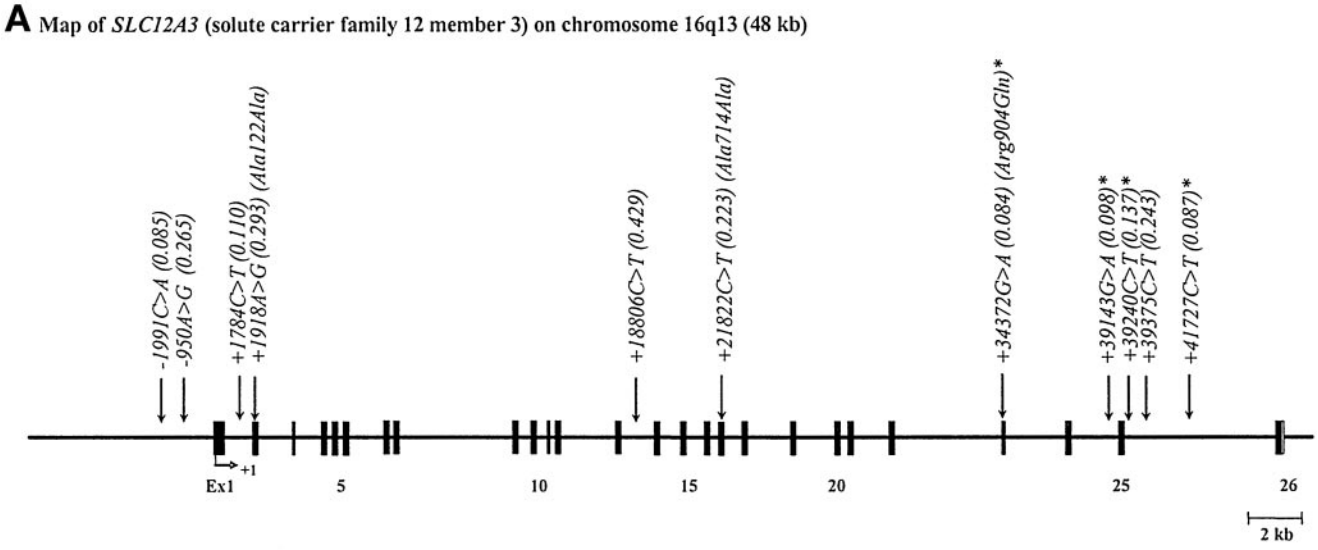
TABLE 1
Clinical characteristics of the study subjects

	Control	ESRD	*P*
n	184	177	
Sex (M/F)	70/114	105/72	<0.001
Age (years)	64 ± 7	61 ± 9	0.002
Duration of diabetes (years)	20 ± 5	18 ± 8	0.006
BMI (kg/m^2)	23.9 ± 2.6	23.2 ± 2.8	0.015
Systolic blood pressure (mmHg)	131 ± 16	150 ± 21	<0.001
Diastolic blood pressure (mmHg)	77 ± 9	83 ± 12	<0.001
Fasting plasma glucose (mmol/l)	8.1 ± 2.4	8.2 ± 3.2	NS
HbA_{1c} (%)	7.7 ± 1.2	7.2 ± 1.5	<0.001
Serum creatinine (μmol/l)	75 ± 12	622 ± 212	<0.001

Data are means ± SD.

genotype distributions of the 11 SNPs were in Hardy-Weinberg equilibrium.

Three individual polymorphisms (g.34372G>A [Arg913Gln], g.39143G>A, and g.41727C>T) of the 11 genotyped SNPs were found to be associated with ESRD caused by diabetic nephropathy (Table 2). Allele frequencies of g.34372G>A (Arg913Gln) and g.41727C>T were found to be significantly different between ESRD patients and control subjects after correction for multiple comparisons (corrected $P = 0.033$, odds ratio 2.30 [95% CI 1.32–4.00]; corrected $P = 0.044$, 2.20 [1.27–3.80], respectively; Table 2). These three SNPs were in complete LD ($|D'| = 1$) and were determinants of haplotype 4 in block 2 (Table 3). An analysis of the frequencies of common haplotypes from each block revealed a significant association between haplotype 4 (18806C, 21822C, 34372A, 39143A, 39240T, 39375C, and 41727T) in block 2 with ESRD due to diabetic nephropathy, even after Bonferroni correction (corrected $P = 0.031$; Table 3).



B Haplotype of *SLC12A3*

Block1

Hap.	-1991C>A	-950A>G	+1784C>T	+1918A>G	Freq.
BL1-ht1	C	G	C	A	0.422
BL1-ht2	C	G	C	G	0.226
BL1-ht3	C	A	T	A	0.110
BL1-ht4	C	A	C	A	0.088
BL1-ht5	A	G	C	A	0.087
BL1-ht6	C	A	C	G	0.068

Block2

Hap.	+18806C>T	+21822C>T	+34372G>A	+39143G>A	+39240C>T	+39375C>T	+41727C>T	Freq.
BL2-ht1	T	C	G	G	C	C	C	0.428
BL2-ht2	C	T	G	G	C	C	C	0.223
BL2-ht3	C	C	G	G	C	T	C	0.180
BL2-ht4	C	C	A	A	T	C	T	0.087
BL2-ht5	C	C	G	G	T	T	C	0.051
BL2-ht6	C	C	G	G	C	C	C	0.017

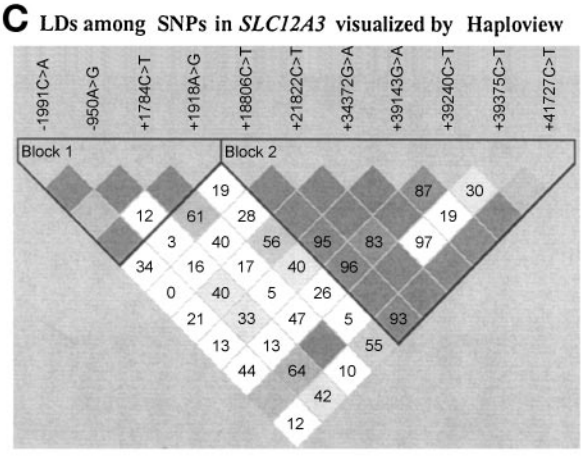


FIG. 1. Gene maps (*A*), haplotypes (*B*), and LD patterns (*C*) of the *SLC12A3* gene. *A*: Polymorphisms genotyped in the *SLC12A3* gene. Coding exons are marked by shaded blocks. *SNPs that have been previously associated with diabetic nephropathy (6). The first nucleotide of the transcription start site is denoted as nucleotide plus one (+1; reference sequence of *SLC12A3*: NM_000339). *B*: Common haplotypes of *SLC12A3* gene. Haplotypes and their frequencies were analyzed by the algorithm developed by Schaid et al. (haplo.score) (21). *C*: LD among SNPs in SLC12A3 visualized by Haploview. There was one break in the LD patterns; accordingly, two blocks were found in this study.

TABLE 2
Associations between *SLC12A3* SNPs and ESRD due to diabetic nephropathy

g.−1991C>A	CC	CA	AA	*P*	C	A	*P*	OR (95% CI)
ESRD	154 (87.0)	22 (12.4)	1 (0.5)	0.182	0.93	0.07	0.149	0.63 (0.37–1.08)
Control	147 (79.9)	36 (19.6)	1 (0.5)		0.90	0.10		
g.−950A>G	AA	AG	GG	*P*	A	G	*P*	OR (95% CI)
ESRD	85 (48.6)	74 (42.3)	16 (9.1)	0.077	0.70	0.30	0.023*	1.47 (1.05–2.06)
Control	108 (60.0)	62 (34.3)	10 (5.6)		0.77	0.23		
g.1784C>T	CC	CT	TT	*P*	C	T	*P*	OR (95% CI)
ESRD	139 (78.5)	34 (19.2)	4 (2.3)	0.825	0.88	0.12	0.527	1.16 (0.73–1.85)
Control	148 (80.9)	32 (17.5)	3 (1.6)		0.90	0.10		
g.1918A>G	AA	AG	GG	*P*	A	G	*P*	OR (95% CI)
ESRD	91 (51.7)	67 (38.1)	18 (10.2)	0.197	0.71	0.29	0.916	0.98 (0.71–1.35)
Control	87 (47.3)	85 (46.2)	12 (6.5)		0.70	0.30		
g.18806C>T	CC	CT	TT	*P*	C	T	*P*	OR (95% CI)
ESRD	69 (39.2)	76 (43.2)	31 (17.6)	0.129	0.61	0.39	0.042*	0.74 (0.55–0.99)
Control	54 (29.5)	87 (47.5)	42 (23.0)		0.53	0.47		
g.21822C>T	CC	CT	TT	*P*	C	T	*P*	OR (95% CI)
ESRD	98 (56.0)	71 (40.6)	6 (3.4)	0.464	0.76	0.24	0.369	1.18 (0.83–1.67)
Control	114 (62.0)	63 (34.2)	7 (3.8)		0.79	0.21		
g.34372G>A	GG	GA	AA	*P*	G	A	*P*	OR (95% CI)
ESRD	136 (77.7)	37 (21.1)	2 (1.1)	0.009*	0.88	0.12	0.003	2.30 (1.32–4.00)
Control	164 (89.6)	18 (9.8)	1 (0.5)		0.95	0.05		
g.39143G>A	GG	GA	AA	*P*	G	A	*P*	OR (95% CI)
ESRD	137 (77.4)	34 (19.2)	6 (3.4)	0.017*	0.87	0.13	0.005*	2.05 (1.23–3.41)
Control	163 (88.6)	17 (9.2)	4 (2.2)		0.93	0.07		
g.39240C>T	CC	CT	TT	*P*	C	T	*P*	OR (95% CI)
ESRD	124 (71.7)	47 (27.2)	2 (1.2)	0.350	0.85	0.15	0.462	1.17 (0.77–1.80)
Control	140 (76.5)	39 (21.3)	4 (2.2)		0.87	0.13		
g.39375C>T	CC	CT	TT	*P*	C	T	*P*	OR (95% CI)
ESRD	98 (56.3)	70 (40.2)	6 (3.4)	0.793	0.76	0.24	0.716	0.94 (0.67–1.32)
Control	102 (55.4)	73 (39.7)	9 (4.9)		0.75	0.25		
g.41727C>T	CC	CT	TT	*P*	C	T	*P*	OR (95% CI)
ESRD	137 (77.4)	38 (21.5)	2 (1.1)	0.013*	0.88	0.12	0.004	2.20 (1.27–3.80)
Control	162 (89.0)	19 (10.4)	1 (0.5)		0.94	0.06		

Genotype distributions are shown as *n* (%). *P* values, odds ratios (ORs), and 95% CIs were calculated using the χ^2 test. **P* values were not significant after correction for multiple comparisons (significant $P < 0.0045$).

DISCUSSION

In this study, we found that genetic variations of the *SLC12A3* gene are associated with ESRD resulting from diabetic nephropathy in the Korean population.

We found that three SNPs (g.34372G>A [Arg913Gln], g.39143G>A, and g.41727C>T) and haplotype 4 (18806C, 21822C, 34372A, 39143A, 39240T, 39375C, and 41727T) in block 2 were significantly associated with ESRD caused by diabetic nephropathy. The variants of three SNPs (34372A, 39143A, and 41727T) were in complete LD and were determinants of haplotype 4 in block 2 (Table 3). Association between haplotype 4 in block 2 and ESRD is more significant than each of three SNPs (g.34372G>A [Arg913Gln], g.39143G>A, and g.41727C>T).

Our results are consistent with the previous observation that the substitution of Arg913 to Gln in the *SLC12A3* gene was reported to increase the risk of developing essential hypertension (8,9), considering elevated blood pressure is one of the major risk factors for the development and progression of diabetic nephropathy (13).

However, our results are quite opposite to Japanese data showing that the substitution of Arg913 to Gln is associated with reduced risk of development of diabetic nephropathy (6,7). Although overall frequencies of four SNPs (g.34372 G>A [Arg913Gln], g.39143 G>A, g.39240 C>T, and g.41727 C>T), which had been previously reported to be associated with diabetic nephropathy, in our control group (5, 7, 13, and 6%, respectively) were similar to those of Japanese (8, 9, 12, and 8%, respectively), there were significant differences in the nephropathy group between two studies (12 vs. 3%, 13 vs. 4%, 15 vs. 7%, and 12 vs. 3%, respectively) (6). In addition, the frequency of haplotype 4 in block 2 in the control group (5.4%) was also similar to that of Japanese (6.0%, haplotype 7 + haplotype 9), whereas it was different in the nephropathy group between two studies (11.9 vs 0.9%) (6). At this time, the explanation for the conflicting results between the two studies is unclear, although we selected patients with ESRD due to diabetic nephropathy for nephropathy cases, whereas patients with overt proteinuria and patients undergoing renal replacement therapy were included in the previous report (6).

The use of prevalent ESRD patients as cases may lead to strong survival bias. However, after dividing the patients with

TABLE 3
Association between *SLC12A3* haplotype blocks and end-stage renal disease due to diabetic nephropathy

Block 1 Haplotype	Locus				Number (% Frequency [95% CI])		*P*	OR (95% CI)
	–1991	*–950*	*1784*	*1918*	ESRD	Control		
BL1-ht1	C	G	C	A	156 (44.1[38.9-49.3])	165 (44.8 [39.7-49.9])	0.835	0.97 (0.73–1.30)
BL1-ht2	C	G	C	G	66 (18.6 [14.5-22.7])	81 (22.0 [17.8-26.2])	0.352	0.84 (0.59–1.21)
BL1-ht3	C	A	T	A	42 (11.9 [8.5-15.3])	38 (10.3 [7.2-13.4])	0.51	1.17 (0.73–1.86)
BL1-ht4	C	A	C	G	36 (10.2 [7.0-13.4])	28 (7.6 [4.9-10.3])	0.226	1.38 (0.82–2.31)
BL1-ht5	A	G	C	A	24 (6.8 [4.2-9.4])	38 (10.3 [7.2-13.4])	0.089	0.63 (0.37–1.08)
BL1-ht6	C	A	C	A	28 (7.9 [5.1-10.7])	18 (4.9 [2.7-7.1])	0.097	1.67 (0.91–3.08)

Block 2 Haplotype	Locus							Number (% Frequency [95% CI])		*P*	OR (95% CI)
	18806	*21822*	*34372*	*39143*	*39240*	*39375*	*41727*	ESRD	Control		
BL2-ht1	T	C	G	G	C	C	C	139 (39.3 [34.2-44.4])	170 (46.2 [41.0-51.4])	0.06	0.75 (0.56–1.01)
BL2-ht2	C	T	G	G	C	C	C	86 (24.3 [19.8-28.8])	76 (20.7 [16.5-24.9])	0.241	1.23 (0.87–1.75)
BL2-ht3	C	C	G	G	C	T	C	67 (18.9 [14.8-23.0])	62 (16.8 [12.9-20.7])	0.466	1.15 (0.79–1.69)
BL2-ht4	C	C	A	A	T	C	T	42 (11.9 [8.5-15.3])	20 (5.4 [3.0-7.8])	**0.0028**	**2.34 (1.35–4.08)**
BL2-ht5	C	C	G	G	T	T	C	12 (3.4 [1.5-5.3])	25 (6.8 [4.2-9.4])	**0.05***	**0.48 (0.24–0.97)**

The haplotype frequencies are estimates made according to unphased data and are not based on actual haplotype counts. Each haplotype with a frequency >0.05 is shown. *P* values of haplotype associations were analyzed by the algorithm developed by Schaid et al. (haplo.score) (21). **P* values were not significant after correction for multiple comparisons (significant $P < 0.0045$). Shaded alleles revealed rare alleles in their loci.

ESRD into quartiles by duration of ESRD, we could not find any association of SLC12A3 with the duration of ESRD (data not shown). Therefore, it is less likely that there is a spurious association due to survival bias between SLC12A3 and ESRD attributed to diabetic nephropathy.

At the moment, functional significance of substitution of Arg913 to Gln is unclear, although there was a suggestion that the Arg913Gln in the *SLC12A3* gene might be a gain-of-function polymorphism and increase the risk of developing essential hypertension (9). Nevertheless, in this study, we found no association between Arg913Gln and blood pressure in either control subjects or ESRD cases (data not shown). Hence, further biological and/or functional evidence would be needed to confirm the suggestive association of *SLC12A3* polymorphisms with ESRD resulting from diabetic nephropathy that has been reported in this study.

In conclusion, we found that SNPs and haplotypes of the *SLC12A3* gene, especially Arg913Gln, are significantly associated with ESRD caused by diabetic nephropathy in the Korean population.

RESEARCH DESIGN AND METHODS

We studied 361 unrelated patients with type 2 diabetes comprising two groups according to the following criteria: *1*) the control group (n = 184): patients with diabetic retinopathy and a duration of diabetes >15 years but with no sign of renal involvement, i.e., a urinary albumin-to-creatinine ratio <30 mg/g and with a creatinine clearance (using the Cockroft equation [14]) of >60 ml/min per m^2; or *2*) the ESRD group (n = 177): patients with diabetic retinopathy and ESRD due to type 2 diabetes, as indicated by a creatinine clearance rate of <15 ml/min per m^2 or being under renal replacement therapy. We excluded subjects with ESRD with any one of the following criteria: *1*) no retinopathy, *2*) a consistent hematuria history before renal function deterioration, *3*) no history of proteinuria before renal function deterioration, *4*) reduced kidney size, and *5*) evidence of other systemic or primary glomerular diseases. The patients with ESRD were recruited from four university hospitals and three dialysis clinics, and the control subjects were recruited from two hospital clinics from June 2003 to July 2004. All subjects enrolled in this study were ethnic Koreans. Type 2 diabetes was diagnosed using World Health Organization criteria (15). Subjects with positive GAD antibodies and an age at diagnosis of type 2 diabetes of <30 years, as well as those who started insulin treatment within 1 year of diagnosis and were ketosis prone, were excluded. The institutional review board of the Clinical Research Institute at Seoul National University Hospital approved the study protocol, and informed consent for genetic analysis was obtained from each subject.

Genotyping for SNPs in the *SLC12A3* gene. Since there is a close genetic relationship between Koreans and Japanese (16,17), we used Japanese sequencing data to select common polymorphisms in the *SLC12A3* gene (12). We selected four SNPs (g.34372 G>A [Arg913Gln], g.39143 G>A, g.39240 C>T, and g.41727 C>T) that had been previously reported to be associated with diabetic nephropathy (6), and we also selected seven additional common SNPs based on frequency (frequency >0.05 in regulatory region, frequency >0.1 in introns) and LD status ($r^2 > 0.5$) among the identified polymorphisms in the *SLC12A3* gene, as determined by sequencing of 48 Japanese DNA samples (12) (Fig. 1*A*). The 11 polymorphisms were genotyped by fluorescence polarization detection or single-base extension (18). For genotyping polymorphic sites, amplifying primers and probes were designed for TaqMan (19). Primer sets for the amplification and sequencing analysis of *SLC12A3* gene were designed based on GenBank sequences (ref. genome seq. NC_000016, released on 29 December 2004). Information regarding the primers used is available on our website (http://www.snp-genetics.com/reference/SLC12A3.doc). The TaqMan method and single-base extension both achieved a >98% success rate for genotype.

Statistics. Differences in genotype frequencies were compared using the χ^2 test. We used Bonferroni correction for the adjustment of multiple comparisons (11 independent tests in genotype and 11 independent tests in haplotype). For association analyses between SNPs in *SLC12A3* and blood pressure, we adjusted for age, sex, and BMI using a general linear regression procedure. Haplotype structures were visualized by Haploview software (20). Haplotype frequencies and P values of haplotype associations were analyzed by the algorithm developed by Schaid et al. (haplo.score) (21). A P value <0.05 was considered statistically significant.

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