Gene interactions and stroke risk in children with sickle cell anemia

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Stroke is a devastating complication of sickle cell anemia (SCA), affecting up to 30% of children with the disease. Despite the relative frequency of stroke in SCA, few predictors of risk exist. Because stroke in SCA is likely a multifactorial disease, analysis of the combined effect of multiple genetic variants may prove more successful than evaluation of individual candidate genes. We genotyped 230 children with SCA for 104 polymorphisms among 65 candidate vascular genes to identify risk associations with stroke. Patients were phenotyped based on magnetic resonance imaging/angiography (MRI/MRA) findings into large-vessel (LV) versus small-vessel (SV) disease stroke subgroups. Specific polymorphisms in the IL4R 503, TNF (−308), and ADRB2 27 genes were independently associated with stroke susceptibility in the LV stroke subgroup, while variants in the VCAM1 (−1594) and LDLR NcoI genes were associated with SV stroke risk. The combination of TNF (−308)GG homozygosity and the IL4R 503P variant carrier status was associated with a particularly strong predisposition to LV stroke (odds ratio [OR] = 5.5; 95% confidence interval [CI] = 2.3-13.1). We show that several candidate genes may play a role in predisposition to specific stroke subtypes in children with SCA. If confirmed, these results provide a basis for population screening and targeted intervention to prevent stroke in SCA. (Blood. 2004;103:2391-2396)

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Introduction

Stroke is a devastating complication in children with sickle cell anemia (SCA), manifested clinically in 11%[1] and as silent cerebral infarction by magnetic resonance imaging (MRI) in another 17% to 22%.[2-4] Well-documented risk factors for ischemic stroke in SCA include prior transient ischemic attack (TIA), low steady-state hemoglobin (Hb) levels, high leukocyte counts, hypertension, and a history of acute chest syndrome.[1] However, the etiology of stroke in SCA remains unclear. Aside from the absence of lipid deposition and plaque formation, many of the histopathologic findings in cerebrovascular lesions in SCA resemble those found in stroke patients in the general population.[5,6] In fact, atherosclerotic stroke is now believed to be a result of chronic inflammation, involving pathways of immune regulation, thrombosis, and cellular adhesion.[7,8] These same pathways are likely to contribute to stroke in SCA.

Increasing evidence from epidemiologic, twin, and animal model studies suggest a genetic contribution to the development of stroke in the general population, and multiple genes have been investigated for stroke risk in SCA.[9] Driscoll et al recently documented an increased risk of stroke in siblings with SCA.[10] Specific β-globin haplotype associations have been shown in some studies, but not others.[11,12] Coinherited alpha thalassemia has also been reported to be a protective factor.[13,14]

We have documented HLA associations with specific stroke subtypes.[15] In a recent study of single nucleotide polymorphisms (SNPs) within the VCAM1 gene locus, Taylor et al identified a variant that appears to be protective.[16] Other studies have reported no association with prothrombotic polymorphisms.[17-22]

Because the etiology of stroke is most likely influenced by many genes, each with only modest effects, analysis of the combined effect of multiple genetic variants may prove more successful than evaluation of individual candidate genes. We previously examined the contribution of 36 candidate genes to stroke risk in SCA in a small pilot institutional study of children with and without MRI-documented cerebral infarction.[23] To extend these initial results, we surveyed 104 polymorphic markers among 65 candidate atherosclerotic, prothrombotic, and proinflammatory genes in a large, well-characterized population of children enrolled in the Cooperative Study of Sickle Cell Disease (CSSCD).

Patients, materials, and methods

Study patients and design

The CSSCD is a national, multicenter study designed to define the natural history of sickle cell disease (SCD) by following more than 4000 patients with SCD.[24] Objectives, eligibility requirements, enrollment procedures, and data collection methods for the CSSCD have been previously reported.[24-26] As part of this study, the CSSCD initiated a prospective newborn cohort trial in 1978 into which more than 400 infants were

enrolled at birth. These children were followed for more than 13 years and had an average age of 13.1 ± 2.9 years at the end of the study. Children in this cohort underwent brain MRI scanning at age 6 and then every 2 years thereafter, as part of phase 2 of the CSSCD study. Because many children were older than 6 years at the time that brain MRI studies were introduced as part of the study, the mean age for the first MRI study and during follow-up studies was 8.3 years and 12.1 years, respectively.[27] Complete MRI histories were available on 266 SCA children from this cohort. Of these, 230 had adequate DNA and were included in this study. Children with SCA (homozygous Hb S) and MRI-documented cerebral infarction (asymptomatic or symptomatic) were included as "case" subjects. Children with MRI evidence of atrophy or isolated cerebral hemorrhage without evidence of preexisting infarction were excluded. MRI-positive patients were subclassified as having large vessel (LV) or small vessel (SV) disease using an algorithm based on MRI/magnetic resonance angiography (MRA) findings, infarct size, and location.[15] Because LV disease is the primary cause of clinical stroke, patients with LV disease, with or without SV disease, were classified as LV stroke. Using the definition of cerebrovascular disease previously described by the CSSCD, MRI-positive patients were also characterized by a history of clinical stroke.[1] Children from the CSSCD newborn cohort who had a normal MRI at the age of 10 years or older were included as "control" subjects. This minimized the possibility of including in the control group a child who might subsequently develop stroke. Patient identities were blinded and patient samples were collected and stored with a study number previously assigned by the CSSCD. The present study was reviewed and approved by the institutional review board of Children's Hospital Oakland.

MRI scanning

The imaging systems and techniques used at each center for the brain MRI scan and the procedures for review of MRI images have been previously described.[2] In most cases, MRI scanning was performed on a 1.5-T MRI scanner. A few centers used a 0.6- or 1.0-T scanner. Criteria for acceptable images were established by the study neuroradiologists and included noncontrast spin-echo T1-weighted pulse sequence (short repetition time [TR], short echo time [TE]) and a T2-weighted axial spin-echo sequence (long TR, long TE) with an intermediate (long TR, short TE) axial and coronal T2 image. All MRIs were reviewed centrally by 3 neuroradiologists blinded to patient clinical history and genotyping results. There were 2 neuroradiologists who read each MRI scan independently and recorded interpretations on a standardized form. If the interpretations differed, consensus was reached in discussion with the third neuroradiologist. This study included results from all MRI scans performed on eligible patients.

Multilocus genotyping assay

A total of 104 polymorphisms among 65 genes were examined, representing various pathways implicated in the development of vascular disease (Table 1).

Genotyping was performed using multilocus polymerase chain reaction (PCR)–based assays, essentially as previously described.[28] Briefly, every sample was amplified using 3 "cocktails" of biotinylated primer pairs, each targeting between 24 and 50 genomic fragments. Amplified fragments within each PCR product pool were then detected colorimetrically with sequence-specific oligonucleotide probes immobilized in a linear array on nylon membranes.[29] Probe specificities were previously confirmed by sequencing, use of DNAs genotyped independently by other methods such as restriction length polymorphism analysis, or by confirming observed frequencies against published values. Interpretation of the alleles represented by positive probe signals was performed independently by 2 investigators blinded to case-control status. Ambiguous interpretations (< 1%) were resolved with repeat genotyping.

Statistical analysis

Given that all patients were homozygous for HbSS, we assumed that linkage equilibrium was present among all loci in the overall sample. Although we expect the overall study sample to meet Hardy-Weinberg

proportions, the 3 stroke subgroups examined may not meet these expectations if disease associations are present. Thus, any genotypic ratio distortion resulting from a strong disease association between a particular gene locus and stroke subgroup will be necessarily balanced by the other 2 stroke subgroups within the overall study population. In order to eliminate testing with low statistical power, the minimum allele frequency required for testing a particular gene locus was set at 10%. For those markers known to be in linkage disequilibrium, we chose a single marker for the analysis based on allelic frequency distributions (ie, those that were closest to an allelic frequency of 0.5). For each of the diallelic markers examined at a particular gene locus, the 2 genotypes with the less common allele were compared with the homozygous genotype of the more common allele. Univariate analyses were performed using contingency table testing to compute the χ^2. To reduce type 2 statistical error, the maximum associated P value for reporting individual marker effects was set at .10. Marker effects with associated P values less than .10 on univariate analysis were entered into a logistic regression model (STATA 6.0). As we had previously found independent HLA associations with stroke risk in the same study population,[15] we included those significant HLA-A, DPB1, and HLA homozygosity effects in the present multivariate model.

Interaction between specific markers was examined using log-linear modeling. This approach tests for rejection of a model containing each of 3 possible 2-way interactions between the 2-locus genotypes and stroke subgroup (LV, SV, and MRI(−)). Significantly deviant results imply the presence of 3-way interaction between the 2-loci and stroke subgroup. Within each subgroup, those markers identified by univariate analyses that share common physiologic pathways were examined. Tests of heterogeneity (2×2) for each of the 2-locus genotype categories within each stroke subgroup were also performed. Interactive effects due to specific genotypes were estimated with odds ratios.

Results

The clinical and laboratory features of the CSSCD newborn cohort have been previously described.[24,25] Of the 415 patients enrolled in the newborn cohort of the CSSCD, 266 were HbSS genotype and have been previously characterized.[27] Of these, 230 were eligible and included in the present study. The study population was composed of 115 females and 115 males with a mean age of 8.4 ± 1.7 years (median, 7.9 years; range, 5.0-14.6 years) at the time of baseline MRI.

Brain MRI results

Of the 230 SCA patients included in this study, 159 (69%) had normal brain MRI scans with a mean age of 12.4 ± 2.3 years at the time of the last follow-up MRI. These patients remained free of clinical stroke during the entire study period. At the time of a first positive MRI, 71 patients (31%) demonstrated infarctive lesions on MRI and had a mean age of 9.3 years (median, 9.0 years). Of these 71 patients, 36 (51%) had MRI evidence of LV involvement (with or without SV involvement), and 35 (49%) had exclusively SV involvement. A clinical history of cerebrovascular accidents (CVAs) was documented in 26 (37%) of these 71, with the majority (92%) due to large vessel (LV) and only 2 cases (8%) due to exclusively small vessel (SV) disease. There were 2 patients who demonstrated cerebral hemorrhage along with infarction on MRI.

Genotypic associations

Among 104 variant sites examined, 57 were sufficiently informative for statistical testing (Figure 1).

After classifying the MRI(+) patients by stroke subtype (LV vs SV), several markers were identified by univariate analysis

Table 1. Candidate gene polymorphisms assessed

Locus	Marker	rs no.
Lipid metabolism		
ADRB3	W64R	4994
APOA4	T347S	675
	Q360H	5110
APOB	T711	1367117
	R3500Q	5742904
APOC3	−641C>A	2542052
	−482C>T	2854117
	−455T>C	2854116
	1100C>T	4520
	3175C>G	5128
	3206T>G	4225
APOE	C112R	429358
	R158C	7412
CETP	−629C>A	1800775
	I405V	5882
	D442G	2303790
LDLR	NcoI+/−	5742911
LIPC	−480C>T	1800588
LPA	93C>T	1652503
	121G>A	1800769
LPL	−93T>G	1800590
	D9N	1801177
	N291S	268
	S447X	328
PON1	M55L	3202100
	Q192R	662
PON2	S311C	7493
PPARG	P12A	1801282
Cellular adhesion		
ICAM1	K56M	5491
	G241R	1799969
SELE	S128R	5361
	L554F	5355
SELP	S330N	6131
	V640L	6133
VCAM1	−1594T>C	1041163
Inflammation		
C3	R102G	2230199
C5	I802V	17611
CCR2	V62I	1799864
CCR3	P39L	5742906
CCR5	wt/Δ580-611	333
	−2454G>A	1799987
CD14	−260C>T	2569190
CSF2	I117T	25882
CTLA4	−318C>T	5742909
	T17A	231775
FCERB1	E237G	569108
GC	E416D	7041
	T420K	4588
IL1A	−889T>C	1800587
IL1B	−1418C>T	16944
	F105F	1143634
IL4	−590C>T	2243250
IL4R	I75V	1805010
	S503P	1805015
	Q576R	1801275
IL5RA	−80G>A	2290608
IL6	−572G>C	1800796
	−174G>C	1800795
IL9	T113M	2069885
IL10	−571C>A	1800872
IL13	4045C>T	1295686

Table 1. Candidate gene polymorphisms assessed (continued)

Locus	Marker	rs no.
Inflammation		
LTA	T26N	1041981
	252A>G	909253
LTC4S	−444A>C	730012
MMP3	−1171A$_5$>A$_6$	3025058
SCYA11	A23T	3744508
	−1328G>A	4795895
SDF1	+800 G>A	1801157
TCF7	P19T	5742913
TGFB1	−509C>T	1800469
TNF	−376G>A	1800750
	−308G>A	1800629
	−244G>A	673
	−238G>A	361525
UGB	+38G>A	3741240
VDR	M1T	2228570
	BsmI+/−	1544410
Homocysteine metabolism		
CBS	I278ins/T	5742905
MTHFR	677 C>T	1801133
Thrombosis		
F2	20210G>A	1799963
F5	R506Q	6025
F7	−323 ins/del	5742910
	R353Q	6046
FGB	−455G>A	1800790
ITGA2	873G>A	1062535
ITGB3	L33P	5918
PAI1	−675G$_5$>G$_4$	1799768
	11053G>T	7242
Blood pressure regulation		
ADD1	G460W	4961
ADRB2	R16G	1042713
	Q27E	1042714
	T164I	1800888
AGT	M235T	699
AGTR1	1166A>C	5186
ACE	intron 16 ins/del	1799752
GNB3	825C>T	5443
NOS2A	231C>T	1137933
NOS3	−922A>G	1800779
	−690C>T	3918226
	E298D	1799983
NPPA	664G>A	5063
	2238T>C	5065
SCNN1A	T493R	5742912
	A663T	2228756

All markers were screened using linear array technology from Roche Molecular Systems. See Appendix 2 for abbreviations of markers listed. All markers are referenced by reference SNP ID (rs) number in the National Center for Biotechnology Information (NCBI) SNP database.

(Table 2). These variants met criteria for inclusion into the logistic regression model. Among the 7 HLA effects previously identified in the same study population, 3 met criteria for inclusion in the model.

The multivariate model accounted for 19% of the overall variance ($P < .0001$). The IL4R 503*P*, ADRB2 27*E*, TNF (−308)*A*, VCAM1 (−1594)*C,* and LDLR *Nco*I variants revealed distinctive associations when analyzed by stroke subgroup (Table 2).

In the LV stroke subgroup, both the IL4R 503*P* variant and HLA-A variation predisposed to stroke, whereas ADRB2 27*E* and TNF(−308)*A* were associated with protection from stroke. The apoC3 3206*G* and NOS2A 231*T* variants showed a trend toward
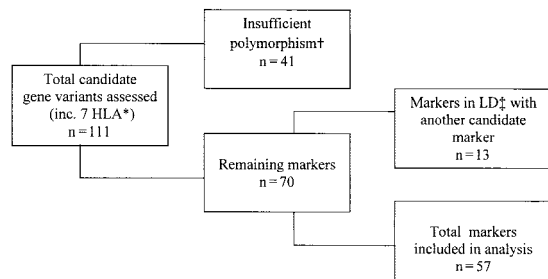
Figure 1. Process for inclusion of candidate gene variants in statistical analyses. *From previously published data.[15] †Insufficient polymorphism was defined as an allele frequency less than 10%. ‡LD denotes linkage disequilibrium.

Table 3. TNF and IL4R genotypic proportions (%) by stroke subgroup

Two-locus genotypes IL4R S503P − TNF G(−308)A	Large-vessel stroke	Small-vessel stroke	MRI (−)	Total
(SS) − (GG)	11.1	22.9	31.4	27.0
(SS) − (GA or AA)	5.6	8.6	6.2	6.5
(SP or PP) − (GG)	80.6	48.6	42.8	49.6
(SP or PP) − (GA or AA)	2.8	20.0	19.5	17.0
No. in group	36	35	159	230

A 3-way interaction test in 2 (IL4R) × 2 (TNF) × 3 (stroke subgroups) log-linear model: log likelihood ratio statistic = 6.719; degrees of freedom = 2; P = .035.

Alleles are abbreviated in parentheses. The less common polymorphisms are indicated in italics (eg, SP or PP − GG indicates patients carrying 1 or 2 copies of the less common IL4R 503 P allele and are homozygous for the TNF(−308) G allele).

IL4R indicates interleukin-4 receptor; TNF, tumor necrosis factor.

protection from LV stroke, but did not reach significance. In the SV stroke subgroup, the VCAM (−1594)C variant, HLA-DPB1, and HLA homozygosity effects predisposed to stroke, while the LDLR (exon18)NcoI variant appeared protective.

We further analyzed the data for interactive effects between these markers, focusing upon those involved in similar physiologic pathways (Table 1). Log-linear modeling for 2-locus interactions between NOS2A and ADRB2 in the LV stroke group and between HLA homozygosity and HLA-DPB1 in the SV stroke group failed to uncover significant interactive effects. However, the model revealed evidence for gene interaction at the 2 loci, IL4R and TNF, in the LV stroke group. In this unselected study population, the frequencies of the predisposing IL4R 503P and TNF G(−308) alleles were 0.39 and 0.87, respectively. A large excess of individuals with the combined IL4R 503P variant and wild-type TNF (−308)G allele in the LV stroke group (81%) compared with the MRI (−) control group (43%) was present (Table 3).

The test of heterogeneity revealed genotypic proportions in the LV stroke group that differed from the other 2 stroke subgroups (P = .01). This effect was significantly greater than expected from the individual effects of these 2 genotypes. SCA patients having this combined genotype (IL4R 503 SP or PP/TNF −308 GG) had a 5.5-fold increased risk of LV stroke, compared with the MRI-negative control group (Table 4).

Discussion

Although the natural history of stroke in SCA has been well characterized, the pathogenesis of stroke remains poorly defined. The development of cerebrovascular lesions in SCA begins with endothelial injury, perhaps influenced by abnormal adherence of sickle cells or leukocytes to endothelial cells,[30] followed by intimal damage and smooth muscle hypertrophy.[31]

Our previous pilot study was limited to a small sample size and a genotypically and phenotypically distinct group of patients,[23] so we could not justify inclusion of our pilot data in the present study. Our present findings on a larger, representative cohort of children participating in the CSSCD show unique associations with specific stroke subtypes and provide evidence that several candidate genes involved in pathways of thrombosis, inflammation, and cellular adhesion may confer stroke risk in children with SCA.

We found that the observed SV stroke risk was partly attributable to the VCAM (−1594)C variant. Adhesion molecules, such as VCAM-1, regulate the attachment and migration of leukocytes and play a dominant role in the development of vascular disease in the general population.[32] Furthermore, endothelial VCAM1 has been shown to be up-regulated in response to sickle erythrocytes and appears to be involved in the pathophysiology of microvascular occlusion in sickle cell disease.[33-35] Interestingly, the VCAM1 (−1594)C variant was associated exclusively with risk for small-vessel stroke in our study population. Taylor et al recently documented a protective association between the VCAM1 1238C variant and stroke, but this study was limited by a relatively small sample of SCA patients with clinically overt stroke.[16] Although we examined only the VCAM1 (−1594)C variant in our group, it is plausible that other SNPs in linkage disequilibrium at the VCAM1 locus may contribute as a "haplotype" to the development of stroke.

The IL4R 503P allelic variant was specifically associated with an increased stroke risk in the LV stroke subgroup.

Table 2. Logistic regression analysis of gene variant effects by stroke subtype

Genetic locus	Allele frequency*	Large-vessel stroke		Small-vessel stroke	
		OR ± SE	Z statistic, P	OR ± SE	Z statistic, P
IL4R 503P	0.39	2.50 ± 0.83	.006	1.30 ± 0.46	.45
HLA-A	0.06	7.71 ± 1.96	.013	1.98 ± 1.01	.18
ADRB2 27E	0.16	0.53 ± 0.16	.033	0.94 ± 0.21	.78
TNF (−308)A	0.13	0.52 ± 0.17	.048	0.97 ± 0.22	.90
VCAM1 (−1594)C	0.22	1.08 ± 0.24	.72	1.98 ± 0.43	.002
HLA-DPB1	0.16	1.77 ± 0.70	.16	3.50 ± 1.41	.002
HLA homozygosity	0.16	1.20 ± 0.25	.39	1.58 ± 0.32	.023
LDLR NcoI−	0.20	1.01 ± 0.05	.96	0.53 ± 0.139	.002

The MRI (−) group was used as the comparison group in the logistic regression model.
Likelihood ratio χ^2 = 73.1; probability greater than χ^2 = 0.000; pseudo R^2 = 0.19.
OR indicates odds ratio; SE, standard error.
*Variant allele frequencies are shown for the total study population (N = 230). For the HLA loci, carrier frequencies are given, as previously published.[15]

Table 4. Risk of combined IL4R/TNF genotype with LV stroke

| | No. with IL4R (S*P* or *PP*)/TNF (GG) genotype | | Total no. |
	+	−	
No. LV	29	7	36
No. MRI (−)	68	91	159
Total no.	97	98	195

OR = 5.54 (95% CI, 2.33-13.11); χ^2 = 16.77; P = .0000.

Polymorphisms in the *IL4R* gene have been studied extensively in immune-mediated diseases (including asthma and insulin-dependent diabetes mellitus), as the IL4 receptor is critical in T-cell development.[36,37] In addition, both *IL4R* and specific HLA genes have been reported to influence the risk of diabetes.[38] Our previous findings of HLA associations, along with our present findings revealing the IL4R variant contribution, suggest possible immune-mediated pathways by which stroke may occur (interleukin-dependent modulation of HLA expression on activated T cells). However, mechanistic studies are clearly required to determine the etiologic factors involved in the development of stroke in SCA. As TNF genes are known to be in linkage disequilibrium with the HLA locus, the TNF(−308) association with LV stroke may reflect linkage disequilibrium (LD) with HLA "risk" alleles previously identified in this study population. However, haplotype analyses on a larger population are needed to confirm whether these effects are due to linkage disequilibrium between TNF and HLA alleles.

Because specific cytokines, including IL4 and TNF, may have synergistic functions in proinflammatory pathways leading to the development of stroke, we investigated whether specific gene interactions could be contributing to stroke in our population. We detected a significant gene-gene interaction between the IL4R 503*P* variant and the common TNF G(−308) promoter allele. Individuals with the risk genotype for IL4R in combination with the common genotype for TNF had a 5.5-fold increased risk for the development of LV stroke compared with all other possible genotypes in the MRI-negative group (Table 4). Given a prevalence of 50% for the combined genotype in our study population of SCA patients (Table 3), the proportion of LV strokes attributable to this combined genotype may be as high as 69%.[39]

In our study, subclassification of MRI-positive patients by large- and small-vessel disease localized the observed candidate gene associations to a specific stroke subtype, suggesting that distinct pathogenetic mechanisms may underlie these 2 types of stroke. Determining which genes are responsible for severe phenotypic expression (eg, stroke due to LV disease) versus those that confer milder effects (eg, lacunar infarction) may lead to a better understanding of the distinctive pathophysiologies of stroke.

In conclusion, our results provide evidence for the involvement of multiple candidate genes predisposing to stroke in children with SCA. Our study is the first to investigate genetic associations with stroke in SCA by simultaneously screening many candidate gene variants in a large, representative cohort of patients. Both single gene effects and gene-gene interactions appear to influence the risk of specific vascular subtypes of stroke. Additional investigations in other larger SCA patient cohorts are indicated to corroborate our findings and determine the predictive value of a subset of alleles with the greatest combined impact on stroke risk in SCA. Adults with SCA also have an increased risk for stroke, distinct from that in children with SCA. Further studies investigating genetic modifiers of stroke in adults are also needed. The development of a genetic "risk profile" for primary stroke in SCA might thus be ultimately translated into clinical benefit through early identification and prevention of stroke in patients at greatest risk.

Acknowledgments

Appendix 1: senior investigators in the Cooperative Study of Sickle Cell Disease (CSSCD)

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Appendix 2: abbreviations for candidate gene polymorphisms assessed in Table 1

ADD1 indicates adducin alpha; ADRB, beta adrenergic receptor; AGT, angiotensinogen; AGTR1, angiotensin receptor 1; APO, apolipoprotein; CBS, cystathionine beta-synthase; CCR, chemokine receptor; CETP, cholesteryl ester transfer protein; CD14, monocyte differentiation antigen; CSF2, colony-stimulating factor 2; CTLA4, cytotoxic T-lymphocyte antigen 4; DCP1, dipeptidyl carboxypeptidase 1/angiotensin converting enzyme; F2, coagulation factor II; F5, factor V; F7, factor VII; FCER1B, immunoglobulin E receptor 1 beta; FGB, fibrinogen beta polypeptide; GC, human vitamin D-binding protein gene; GNB3, guanine nucleotide binding protein beta 3; ICAM1, intracellular adhesion molecule 1; IL, interleukin; IL1A, interleukin 1 alpha; IL1B, interleukin 1 beta; IL4R, interleukin 4 receptor; IL5RA, interleukin 5 receptor alpha; ITGA2, platelet glycoprotein 1a; ITGB3, platelet glycoprotein IIIa; LDLR, low density lipoprotein receptor; LIPC, hepatic lipase; LPA, apolipoprotein(a); LPL, lipoprotein lipase; LTA, tumor necrosis factor beta; LTC4S, leukotriene C4 synthase;

MMP3, matrix metalloproteinase 3; MTHFR-5, 10, methylenetetrahydrofo-late reductase; NOS2A, nitric oxide synthase 2A; NOS3, nitric oxide synthase 3 endothelial; NPPA, atrial natriuretic peptide; PAI1, plasminogen activator inhibitor type 1; PON, paraoxonase; PPARG, peroxisome prolif-erator activated receptor gamma; SCNN1A, sodium channel epithelial alpha subunit; SCYA11, eotaxin; SDF1, stromal-derived factor 1; SELE, E-selectin; SELP, P-selectin; TCF7, T-cell transcription factor 7; TGFB1, transforming growth factor-beta 1; TNF, tumor necrosis factor; UGB, uteroglobin; VCAM, vascular cell adhesion molecule; and VDR, vitamin D receptor.

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