Original Article

Association of Protein Tyrosine Phosphatase-N1 Polymorphisms With Coronary Calcified Plaque in the Diabetes Heart Study

Kathryn P. Burdon,[1,2] Jennifer L. Bento,[1,2] Carl D. Langefeld,[3] Joel K. Campbell,[3] J. Jeffery Carr,[4] Lynne M. Wagenknecht,[3] David M. Herrington,[5] Barry I. Freedman,[5] Stephen S. Rich,[3] and Donald W. Bowden[1,2,5]

Individuals with type 2 diabetes are at increased risk of cardiovascular disease (CVD) mortality and display increased levels of subclinical CVD. Genetic variation in *PTPN1*, a diabetes susceptibility gene, was investigated for a role in diabetic atherosclerosis. The *PTPN1* gene encodes protein tyrosine phosphatase-1B, which is ubiquitously expressed and plays a role in the regulation of several signaling pathways. Subclinical atherosclerosis was assessed in 590 Caucasian participants with type 2 diabetes in the Diabetes Heart Study using B-mode ultrasound measurement of carotid intima-media thickness (IMT) and computed tomography measurement of carotid calcified plaque (CarCP) and coronary calcified plaque (CorCP). Twenty-three single nucleotide polymorphisms (SNPs) in *PTPN1* were genotyped and assessed for association with IMT, CarCP, and CorCP. A total of 12 SNPs within a block of linkage disequilibrium encompassing the coding sequence of *PTPN1* were significantly associated with CorCP (*P* values from <0.0001 to 0.043) and 3 SNPs also within the block approached significance (*P* values from 0.058 to 0.066). In addition, a nine-SNP haplotype (GACTTCAGO) was also associated with increased CorCP under a dominant model ($P = 0.01$). No association was detected with IMT or CarCP. The associated SNPs and haplotype are the same as those observed to be associated with type 2 diabetes, insulin resistance, and fasting glucose in previous studies. With the inclusion of the most likely haplo-genotype for each individual, the heritability estimate of CorCP increased from 0.53 ± 0.1 to 0.57 ± 0.1 ($P = 8.1 \times 10^{-10}$), suggesting a modest but detectable effect of this gene on the phenotype of CorCP in type 2 diabetic patients. *Diabetes* 55:651–658, 2006

From the [1]Department of Biochemistry, Wake Forest University School of Medicine, Winston-Salem, North Carolina; the [2]Center for Human Genomics, Wake Forest University School of Medicine, Winston-Salem, North Carolina; the [3]Department of Public Health Sciences, Wake Forest University School of Medicine, Winston-Salem, North Carolina; the [4]Department of Radiology, Wake Forest University School of Medicine, Winston-Salem, North Carolina; and the [5]Department of Internal Medicine, Wake Forest University School of Medicine, Winston-Salem, North Carolina.

Address correspondence and reprint requests to Donald W. Bowden, PhD, Department of Biochemistry, Wake Forest University School of Medicine, Medical Center Boulevard, Winston-Salem, NC 27157. E-mail: dbowden@wfubmc.edu.

Received for publication 14 January 2005 and accepted in revised form 5 December 2005.

CarCP, carotid calcified plaque; CorCP, coronary calcified plaque; CVD, cardiovascular disease; DHS, Diabetes Heart Study; IMT, intima-media thickness; LD, linkage disequilibrium; SNP, single nucleotide polymorphism.

Type 2 diabetes is a complex disorder arising from interactions between predisposing genetic variants, lifestyle factors, and environmental exposures. Neither the genetic nor environmental exposures are well understood, but identification of genetic risk factors for type 2 diabetes is the focus of multiple investigations. We and others have identified protein tyrosine phosphatase, nonreceptor type 1 (*PTPN1*), the gene coding for the PTP1B protein, as a type 2 diabetes susceptibility gene that is associated with type 2 diabetes, measures of insulin resistance, and fasting glucose in a variety of populations (1–5). *PTPN1* has also been implicated in hypertension, lipid levels, and obesity (components of the metabolic syndrome) in Asian and French populations (6,7).

PTP1B is a ubiquitously expressed tyrosine phosphatase, playing an important role in the negative regulation of insulin signaling by dephosphorylating the insulin receptor tyrosine kinase, reducing its kinase activity, and thus inhibiting insulin signaling (8). Ablation of the gene in mice results in increased insulin sensitivity and resistance to diet-induced obesity (9,10). This second characteristic may be due to the role PTP1B plays in leptin signaling (11,12). In addition, PTP1B is known to be important in cell adhesion through both integrin and cadherin signaling (13,14).

Type 2 diabetes is characterized by insulin resistance, obesity, and an increased risk of cardiovascular disease (CVD) (15–17). It is unknown whether CVD and type 2 diabetes occur together because they share common predisposing causes or whether the metabolic dysregulation in diabetes leads to CVD. The Diabetes Heart Study (DHS) was designed to detect genes contributing to the cardiovascular complications of type 2 diabetes. In the DHS, subclinical atherosclerosis is assessed using B-mode ultrasound estimation of the common carotid artery intima-media thickness (IMT) and using fast-gated helical computed tomography estimation of coronary calcified plaque (CorCP) and carotid calcified plaque (CarCP). Both IMT and CorCP are commonly used surrogates for atherosclerosis and CVD (18–20), and much of the variation in IMT and CorCP can be attributed to genetic factors in the DHS (21,22). In addition, *PTPN1* polymorphisms are associated with type 2 diabetes in the DHS (1). In this study, we assessed whether subclinical atherosclerosis in type 2 diabetic patients is associated with *PTPN1* polymorphisms under the hypothesis that the common haplotype that is

TABLE 1
Clinical characteristics of participants in the DHS

Age (years)	61.9 ± 9.4	62.4 (34.2–83.7)
Sex (female)	314 (53.2)	—
Duration of diabetes (years)	10.7 ± 7.6	9.0 (1.0–59.0)
BMI (kg/m^2)	32.5 ± 7.0	31.3 (17.1–59.8)
Smoking		
Current	103 (17.6)	—
Past	252 (42.9)	—
Never	232 (39.5)	—
Lipid-lowering medication	245 (41.7)	—
Blood pressure–lowering medication	432 (73.2)	—
Laboratory		
Total cholesterol (mg/dl)	188.5 ± 44.8	184.5 (74–427)
HDL cholesterol (mg/dl)	42.4 ± 12.1	41.0 (18–90)
LDL cholesterol (mg/dl)	104.3 ± 32.5	101 (14–200)
Triglycerides (mg/dl)	217.0 ± 141.7	185.5 (38–1,310)
Fasting glucose (mg/dl)	150.5 ± 59.4	137 (16–463)
A1C (%)	7.8 ± 1.9	7.5 (4.3–18.3)
Albumin-to-creatinine ratio	145.9 ± 658.7	13.1 (0.8–9,449)
Prevalent CVD conditions		
Hypertension	492 (83.4)	—
Angina	112 (20.9)	—
Myocardial infarction	131 (22.5)	—
Stroke	64 (11.0)	—
Endarterectomy	13 (2.2)	—
Coronary artery bypass graft	93 (15.8)	—
IMT (mm)	0.691 ± 0.143	0.665 (0.46–1.57)
CorCP score	1,448 ± 2,704	366.8 (0–25,420)
CarCP score	390.6 ± 741.2	108 (0–6,122)

Data are means ± SD, *n* (%), or median (range). Only data on Caucasian subjects with type 2 diabetes are included.

known to increase the risk of type 2 diabetes and insulin resistance would also be associated with increased CVD.

RESEARCH DESIGN AND METHODS

Recruitment and phenotyping of DHS participants have been previously described (21,22). Briefly, siblings concordant for type 2 diabetes were recruited along with available unaffected siblings between 1999 and 2003 in Forsyth County, North Carolina. All protocols were approved by the institutional review board of Wake Forest University School of Medicine, and all participants gave informed consent. Participant examinations were conducted in the General Clinical Research Center of the Wake Forest University Baptist Medical Center and included interviews for medical history and health behaviors, anthropometric measures, resting blood pressure, fasting blood sampling, and spot urine collection (Table 1). IMT of the common carotid artery was available in 574 individuals, measured by high-resolution B-mode ultrasonography with a 7.5-MHz transducer and a Biosound Esaote (AU5) ultrasound machine as previously described (21). CorCP and CarCP scores were available in 454 and 523 individuals, respectively. The scores were determined using fast-gated helical computed tomography and were calculated using a modified Agatston unit method as described previously (23). Patients who had undergone vascular surgery (carotid endarterectomy or coronary artery bypass surgery or stent implantation) were excluded from analyses where relevant (e.g., endarterectomy in IMT analysis). All analyses were repeated excluding patients with angioplasty. The DHS also includes an African-American family collection (15%); however, because of the limited sample size in this ethnic group and our previous studies of *PTPN1* in another African-American sample that did not detect an association with diabetes-related traits, we investigated only the DHS Caucasian family collection.

Genetic analysis. Total genomic DNA was purified from whole-blood samples obtained from subjects using the Puregene DNA isolation kit (Gentra, Minneapolis, MN). DNA was quantitated using standardized fluorometric readings on a Hoefer DyNA Quant 200 fluorometer (Hoefer Pharmacia Biotech, San Francisco, CA). Each sample was diluted to a final concentration of 5 ng/μl.

Single nucleotide polymorphisms (SNPs) within *PTPN1* (Table 2) were genotyped using a MassARRAY SNP genotyping system (Sequenom, San Diego, CA) as previously described (24). This genotyping system uses single-base extension reactions to create allele-specific products that are separated and automatically scored in a MALDI-TOF mass spectrometer. Primers for PCR amplification and extension reactions were designed using MassARRAY assay design software (Sequenom). Primer sequences are available upon request.

Statistical analysis. Allele and genotype frequencies were calculated from unrelated subjects and tested for departure from Hardy-Weinberg proportions using a χ^2 goodness-of-fit test. Linkage disequilibrium (LD) was assessed by the calculation of pairwise D′ and R^2 values in the largest set of unrelated subjects. To test for an association among the polymorphisms of each individual SNP and each phenotype, a series of generalized estimating equations (GEE1) (25) was computed. The correlation between subjects within a family was adjusted for in the analyses by assuming exchangeable correlation among siblings within a pedigree and computing the sandwich estimator of the variance (26). The sandwich estimator is also denoted the robust or empirical estimator of the variance, as it is robust to misspecification of the correlation matrix because it estimates the within-pedigree correlation matrix from moments of the data. All tests of significance were computed after adjustment for age, sex, and smoking status (current, past, and never) as covariates. All analyses were repeated, including an adjustment for BMI. Within each trait, a sequential Bonferroni multiple comparison adjustment was computed (27). This conservative multiple testing adjustment rank orders the observed *P* values, divides the a priori threshold for statistical significance (e.g., 0.05) by the *P* value rank, and declares significance if the observed *P* value is less than the rank adjusted threshold for significance. We report the unadjusted *P* value and indicate which SNPs retain statistical significance after adjustment by the conservative sequential Bonferroni because of the strong a priori nature of our *PTPN1* and diabetes-related trait hypotheses. Significance levels are based on two-sided tests despite the a priori one-sided hypothesis.

Quantitative traits were log transformed to approximate the conditional normality, conditional on the covariates, and minimize heterogeneity of variance. Each SNP was initially assessed with the 2-df genotypic (three SNP genotypes) test of association, provided each genotype had sufficient counts. If the genotypic test of association was significant, individual genetic models were examined for significance. This is consistent with the Fisher's protected least significant difference multiple comparison procedure. Haplotype frequencies were estimated by the expectation-maximization algorithm implemented in the Zaplo program (28). Haplotype analysis was performed using data from eight SNPs chosen to "tag" all common haplotypes and to account for >85% of the haplotypic variation in the region of high LD (D′ >0.8) (1,2) and, in addition, includes a previously postulated functional variant (1484insG) (4). Genetic association was assessed overall and under three a priori genetic models (dominant, additive, and recessive) using GEE1 for the six haplotypes of >2% frequency.

SNP 1484insG is a modestly uncommon polymorphism with minor allele frequency of 0.07 and only three individuals homozygous for the minor allele "G." The statistical significance of the association was estimated using standard large-sample theory (Wald test) and empirically using gene dropping. Specifically, the 1484insG alleles were randomly assigned to pedigree founders assuming a 0.07 minor allele frequency and passed from parent to offspring consistent with simple Mendelian inheritance. Individuals without genotype data in the DHS study had their genotypes converted to missing such that the missing data pattern was consistent with the original data. The same GEE1 analyses were computed and the process was repeated 10,000 times. The empirical statistical significance was estimated as the proportion of the 10,000 replications with a Wald test χ^2 statistic greater than that observed in the original data.

Heritability was estimated using the variance component approach implemented in the software SOLAR (29). In these analyses, the overall phenotypic variation was partitioned into individual variance components due to polygenic effects (multiple unmeasured genes under an additive variance), covariates (e.g., age, sex, and haplotype), and random environmental effects. The estimated heritability (h^2) is defined as the ratio of the genetic variance component to the residual phenotypic variance and is an estimate of the familiality of the trait. To estimate the variation explained by the *PTPN1* haplotypes, the most likely haplo-genotypes were determined using the expectation-maximization algorithm implemented in the program Zaplo (28) and included as a covariate in the variance component model.

RESULTS

A total of 23 SNPs in a 160-kb interval encompassing the *PTPN1* gene were genotyped on DNA from 590 Caucasian-American individuals with type 2 diabetes from 267 fami-

TABLE 2
PTPN1 SNP alleles and frequency of allele 2, genomic location in base pairs relative to the start of translation, and *P* values for overall genotypic association with measures of atherosclerosis, adjusted for age, sex, and smoking status in type 2 diabetic subjects

					P values for genotypic association		
	SNP	Alleles 1/2	Frequency	Location (bp)	IMT	CorCP	CarCP
1	rs2904268	G/C	0.33	−76,977	0.617	0.620	0.822
2	rs803742*	C/T	0.47	−61,469	0.268	0.722	0.789
3	rs1967439	G/A	0.34	−45,782	0.726	0.356	0.738
4	rs718630	A/C	0.46	−15,809	0.145	0.289	0.959
5	rs4811078	C/T	0.13	−14,056	0.514	0.353	0.755
6	rs2206656*	G/C	0.45	3,054	0.690	**0.002**	0.916
7	rs932420	T/C	0.49	6,004	0.271	**0.004**	0.832
8	rs3787335	T/G	0.09	15,970	0.842	*0.066*	0.767
9	rs2426158	A/G	0.33	25,219	0.602	**0.007**	0.964
10	rs2904269	A/C	0.47	29,266	0.495	**0.023**	0.770
11	rs941798	A/G	0.39	36,170	0.846	0.129	0.818
12	rs1570179	C/T	0.42	39,646	0.663	*0.058*	0.829
13	rs3787345	T/C	0.46	58,358	0.310	**0.011**	0.856
14	rs1885177	A/C	0.46	64,163	0.481	**0.012**	0.892
15	rs754118	C/T	0.42	64,839	0.536	**0.032**	0.939
16	rs3215684	T/O†	0.42	67,802	0.711	**0.038**	0.933
17	rs968701	G/A	0.46	68,183	0.611	**0.011**	0.683
18	rs2282147	C/T	0.42	69,102	0.716	**0.043**	0.933
19	rs718049	T/C	0.44	69,534	0.534	**0.035**	0.991
20	rs718050	G/A	0.45	69,856	0.542	*0.060*	0.988
21	rs3787348	G/T	0.38	69,933	0.965	0.512	0.820
22	1484insG*	O†/G	0.07	72,292	0.311	**<0.0001**	**0.003**
23	rs914458*	C/G	0.36	83,888	0.191	0.304	0.890

*Variant not consistent with Hardy-Weinberg proportions. †O indicates deletion allele. *P* values in bold indicate $P < 0.05$; italics indicate $0.05 < P < 0.10$.

lies. Clinical characteristics of all diabetic subjects are given in Table 1. DHS subjects have a mean age of 61.9 ± 9.4 years. They are obese (mean BMI 32.5 ± 7.0 kg/m^2) and have significant evidence of subclinical CVD (mean IMT 0.691 ± 0.143 mm; mean CorCP 1,448 ± 2,704 Agatston units; 94% with CorCP >0).

Allele frequencies of the 23 *PTPN1* SNPs genotyped were calculated in 267 probands (Table 2). Four SNPs (rs803742 $P < 0.001$, rs2206656 $P = 0.048$, 1484insG $P = 0.011$, and rs914458 $P = 0.003$) were inconsistent with Hardy-Weinberg proportions. LD was assessed by calculation of pairwise D′ and r^2 values (Fig. 1). An extensive region of high LD (intermarker D′ >0.9) was detected, extending from rs4811078 to 1484insG, representing 70 kb and encompassing the entire coding region of the gene, consistent with previous investigations (1,2).

P values for genotypic association are summarized in Table 2 for Caucasian type 2 diabetic participants after adjustment for age, sex, and smoking status. Significant evidence of association with CorCP was detected for 12 of 18 SNPs in the *PTPN1* LD block with *P* values ranging from <0.0001 to 0.043. Of the six SNPs with $P > 0.05$, three SNPs had *P* values approaching statistical significance, ranging from 0.058 to 0.066. Even using a conservative adjustment for the number of uncorrelated CorCP comparisons such as the sequential Bonferroni (27), three SNPs remain statistically significant (rs2206656, rs932420, and 1484insG) and one approaches statistical significance (rs2426158). No significant associations were detected with IMT. Limiting the analyses to those individuals with complete information for both IMT and CAC did not substantially change the results or affect the conclusions (data not shown).

The 1484insG SNP was associated with CarCP (*P* value = 0.003); however, this was the only SNP associated with CarCP, it had little information (low heterozygosity), and the distribution of 1484insG genotypes was not consistent with Hardy-Weinberg expectations. The rarity of this SNP in the DHS population suggests that these results should be viewed with caution, because mean genotypic values are highly influenced by a few individuals. Simulation of empirical *P* values for this SNP indicated that the observed associations are likely to be spurious (logIMT $P = 0.794$, logCorCP $P = 0.475$, logCarCP $P = 0.610$).

Inclusion of BMI, HbA_{1c} (A1C), or fasting glucose as covariates in the SNP association analyses did not significantly affect the results (data not shown). The associations with CorCP remained significant in the same order of magnitude, and no association was detected with the other outcomes (IMT, CarCP). No significant associations were observed with BMI, waist circumference, waist-to-hip ratio, A1C, total cholesterol, LDL cholesterol, or triglycerides (data not shown). Three SNPs in the LD block were associated with HDL after additional adjustment for lipid-lowering medication use (rs941798 $P = 0.030$, rs1570179 $P = 0.039$, and rs3787348 $P = 0.030$, data not shown). These three SNPs were not significantly associated with CorCP. Analyses were also repeated excluding those individuals who had undergone any cardiovascular procedure including angioplasty because of the possible effect this procedure may have on arterial calcification (data not shown). These results were consistent with those presented in Table 2 and do not alter the conclusions, indicating that the procedure is not artificially affecting the observed associations.

Genotypic means and genetic model-specific *P* values for association with CorCP for all significantly associated SNPs are shown in Table 3. Overall, the dominant model

	SNP	1	2	3	4	5	6	7	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1	rs2904268	X	0.78	0.76	0.44	0.81	0.21	0.17	0.28	0.19	0.09	0.37	0.24	0.19	0.38	0.36	0.20	0.39	0.25	0.24	0.11	0.16	0.03
2	rs8038742	0.31	X	0.96	0.24	1.00	0.11	0.11	0.60	0.12	0.17	0.19	0.13	0.10	0.17	0.16	0.07	0.16	0.12	0.12	0.16	0.37	0.54
3	rs1967439	0.16	0.49	X	0.91	1.00	0.66	0.76	0.75	0.78	0.72	0.64	0.66	0.77	0.68	0.65	0.77	0.66	0.65	0.66	0.71	0.19	0.27
4	rs718630	0.09	0.06	0.48	X	1.00	0.84	0.80	0.95	0.80	0.76	0.97	0.86	0.82	0.98	0.98	0.82	0.98	0.90	0.90	0.74	0.35	0.03
5	rs4811078	0.20	0.15	0.08	0.15	X	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.54	0.32
6	rs2206656	0.02	0.01	0.28	0.62	0.13	X	0.98	0.98	0.98	0.97	0.98	1.00	0.98	1.00	1.00	0.98	1.00	0.98	1.00	0.97	1.00	0.01
7	rs932420	0.02	0.01	0.27	0.52	0.18	0.69	X	0.97	0.99	0.98	0.98	0.98	0.99	0.98	0.98	0.99	0.98	0.98	1.00	0.98	0.82	0.04
9	rs2426158	0.02	0.19	0.52	0.47	0.08	0.57	0.40	X	0.97	0.95	0.98	0.96	0.97	0.96	0.96	0.97	0.96	0.96	0.95	0.96	1.00	0.10
10	rs2904269	0.02	0.01	0.28	0.51	0.18	0.67	0.96	0.39	X	0.98	0.98	0.98	0.99	0.98	0.98	1.00	1.00	0.98	0.98	0.98	1.00	0.07
11	rs941798	0.01	0.02	0.16	0.31	0.27	0.45	0.64	0.26	0.64	X	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.04
12	rs1570179	0.05	0.03	0.31	0.72	0.11	0.83	0.60	0.65	0.58	0.41	X	0.98	1.00	1.00	1.00	1.00	1.00	0.99	0.99	1.00	1.00	0.02
13	rs3787345	0.02	0.01	0.29	0.64	0.13	0.98	0.68	0.56	0.67	0.48	0.85	X	1.00	1.00	1.00	1.00	1.00	0.99	1.00	1.00	1.00	0.01
14	rs1885177	0.02	0.01	0.27	0.53	0.19	0.67	0.96	0.38	0.97	0.68	0.60	0.69	X	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.10
15	rs754118	0.05	0.02	0.34	0.75	0.11	0.86	0.60	0.64	0.60	0.41	0.99	0.89	0.61	X	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00
16	rs3215684	0.05	0.02	0.32	0.74	0.11	0.86	0.60	0.65	0.59	0.42	0.99	0.87	0.61	1.00	X	1.00	1.00	1.00	1.00	1.00	1.00	0.02
17	rs968701	0.02	0.00	0.27	0.54	0.19	0.67	0.96	0.38	0.99	0.68	0.61	0.70	1.00	0.62	0.62	X	1.00	1.00	1.00	1.00	1.00	0.15
18	rs2282147	0.06	0.02	0.33	0.74	0.11	0.86	0.60	0.63	0.61	0.41	0.99	0.89	0.61	1.00	1.00	0.61	X	1.00	1.00	1.00	1.00	0.01
19	rs718049	0.02	0.01	0.29	0.70	0.12	0.92	0.66	0.57	0.65	0.46	0.88	0.95	0.67	0.91	0.90	0.69	0.89	X	1.00	1.00	1.00	0.01
20	rs718050	0.02	0.01	0.29	0.70	0.13	0.95	0.68	0.57	0.66	0.46	0.89	0.96	0.68	0.92	0.91	0.69	0.90	1.00	X	1.00	1.00	0.02
21	rs3787348	0.01	0.02	0.16	0.30	0.28	0.45	0.65	0.26	0.65	1.00	0.41	0.48	0.69	0.42	0.42	0.68	0.41	0.46	0.46	X	1.00	0.04
22	1484insG	0.00	0.01	0.00	0.01	0.00	0.07	0.05	0.04	0.07	0.05	0.06	0.07	0.07	0.06	0.06	0.07	0.06	0.06	0.06	0.05	X	0.38
23	rs914458	0.00	0.23	0.03	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.01	X

FIG. 1. LD structure of the *PTPN1* gene in unrelated probands of the DHS for SNPs with frequency >0.10 plus SNP 1484insG. The *upper triangle* contains pairwise D′ values, and the *lower triangle* contains pairwise r^2 values. The identified haplotype block is boxed.

provides the strongest evidence of association for most SNPs, with the exception of 1484insG, which is clearly recessive. The 1484insG result should be viewed with caution because the *P* value is strongly influenced by three homozygous individuals and does not retain significance when estimated empirically. The six SNPs (rs2206656, rs932420, rs2426158, rs2904269, rs1885177, and rs968701) remain statistically significant under the dominant model even after adjusting for multiple comparisons using the sequential Bonferroni.

All haplotypes observed in the probands and their frequencies are shown in Table 4. Haplotypes were estimated in the whole dataset using the same eight SNPs as Bento et al. (1) with the addition of rs24261588 (SNP 9), which tags an additional haplotype of ~7% frequency. The tag SNPs are indicated in bold in Table 4. The test of overall haplotype association was not significant (IMT $P = 0.931$, CorCP $P = 0.487$, and CarCP $P = 0.947$). Given previous research on this gene with a variety of phenotypes, there exists a strong a priori hypothesis for the association of the most frequent haplotype with increased risk of poor outcomes for diabetes, obesity, and hypertension (1,2,6). Therefore, tests of association under dominant, additive, and recessive models for the haplotypes with frequency >2% were performed (Table 5). The haplotype GACTTCAGO was associated with increased CorCP, under an additive model ($P = 0.010$), and is identical to the diabetes "risk" haplotype identified in previous studies (1,2). A second haplotype (AGTCCTGTO) differs from the "risk" haplotype at all SNPs except the 1484insG SNP and was not significantly associated with CorCP. This haplotype was associated with decreased HDL ($P = 0.015$, recessive model, data not shown). Decreased HDL would indicate a higher risk of CVD events, in contrast to the trend for association with CorCP and previously identified association with lower fasting glucose. The meaning of this result is unclear, given the weak evidence of individual SNPs and the high proportion of lipid-lowering medication use in this cohort. The other common haplotypes are less frequent and differ from each other at only the 1484insG SNP. Haplotype 3 (AATCCT GGO), showed evidence of association with decreased CorCP. The haplotype with the uncommon "insertion of G" allele (AATCCTGGG) showed evidence of association with increased CorCP; however, as shown in Table 4, this association was influenced by three homozygous individuals in the recessive model, and these results should be viewed with caution. Haplotype 5 was also associated with increased CorCP. This haplotype is a derivative of the common "risk" haplotype 1, differing only at SNP rs2426158. Haplotype 7 was not associated.

The heritability of CorCP in this dataset adjusting for the covariates age, sex, BMI, and smoking was 0.53 ± 0.10 ($P = 5.0 \times 10^{-9}$). The covariates explained 28% of the phenotypic variance. With the inclusion of the most likely haplo-genotype for each individual, the heritability increased to 0.57 ± 0.10 (*P* value = 8.1×10^{-10}) with 31% of the phenotypic variance explained by covariates. Therefore, while the effect is small, the most likely haplotype of *PTPN1* explains some of the variation in CorCP in type 2 diabetic patients.

TABLE 3
Unadjusted untransformed CorCP score by genotype and *P* values for association with CorCP under a priori genetic inheritance models, adjusted for age, sex, and smoking status in all significantly associated SNPs

	1/1			1/2			2/2			*P* values		
	n	Means± SD	Median	*n*	Means± SD	Median	*n*	Means± SD	Median	Dominant	Additive	Recessive
rs2206656	123	1,087 ± 1,833	268	155	1,325 ± 2,874	324	74	1,714 ± 3,766	499	0.004	**0.0005**	0.005
rs932420	91	1,806 ± 3,566	570	172	1,210 ± 2,890	202	92	1,181 ± 1,916	287	**0.0008**	0.012	0.461
rs2426158	169	1,016 ± 1,729	218	157	1,562 ± 3,568	330	40	1,503 ± 2,591	579	**0.002**	**0.002**	0.094
rs2904269	99	1,686 ± 3,445	494	169	1,284 ± 2,861	204	85	1,127 ± 1,754	264	**0.006**	0.031	0.471
rs3787345	122	1,230 ± 1,931	304	214	1,424 ± 2,784	291	95	1,781 ± 3,470	576	0.056	**0.004**	**0.006**
rs1885177	109	1,525 ± 3,298	448	196	1,168 ± 2,692	202	82	1,264 ± 2,003	313	**0.003**	0.023	0.603
rs754118	143	1,246 ± 2,145	265	219	1,495 ± 2,681	347	80	1,766 ± 3,673	541	**0.012**	**0.013**	0.163
rs3215684	118	1,014 ± 1,832	217	182	1,321 ± 2,814	242	65	2,648 ± 3,954	448	**0.015**	**0.013**	0.128
rs968701	108	1,543 ± 3,308	467	197	1,168 ± 2,684	206	77	1,274 ± 2,027	319	**0.003**	0.029	0.641
rs2282147	139	1,145 ± 1,853	268	222	1,556 ± 2,813	335	79	1,710 ± 3,631	565	**0.018**	**0.015**	0.128
rs718049	125	1,238 ± 1,938	302	213	1,432 ± 2,780	291	89	1,790 ± 3,560	570	0.049	**0.010**	**0.027**
1484insG*	401	1,549 ± 2,822	377	48	632 ± 1,166	129	3	2,951 ± 4,258	777	0.217	0.644	**<0.0001**

*Variant not consistent with Hardy-Weinberg proportions with a deficit of heterozygotes relative to estimated allele frequencies. Recessive model is based on three homozygote individuals. Results for additive and recessive models are exploratory and must be viewed with caution. *P* values in bold indicate $P < 0.05$.

DISCUSSION

This study reveals an association in the hypothesized direction of common *PTPN1* SNPs and haplotypes with

TABLE 4
Observed haplotypes in unrelated probands for the 17 SNPs in the linkage disequilibrium block

	SNPs																	
	06	07	08	**09**	10	**11**	12	**13**	14	**15**	16	17	**18**	**19**	**20**	**21**	**22**	Frequency
Haplotype																		
1	C	C	T	**G**	A	**A**	T	**C**	A	**T**	A	G	**T**	**C**	**A**	**G**	**O**	0.319
2	G	T	T	**A**	C	**G**	C	**T**	C	**C**	T	A	**C**	**T**	**G**	**T**	**O**	0.382
3	G	T	T	**A**	C	**A**	C	**T**	C	**C**	T	A	**C**	**T**	**G**	**G**	**O**	0.053
4	G	C	**T**	**A**	A	**A**	C	**T**	A	**C**	T	G	**C**	**T**	**G**	**G**	**G**	0.063
5	C	C	G	**A**	A	**A**	T	**C**	A	**T**	A	G	**T**	**C**	**A**	**G**	**O**	0.072
6	G	C	T	**A**	A	**A**	C	**T**	A	**C**	T	G	**C**	**T**	**G**	**G**	**O**	0.013
7	C	C	T	**A**	A	**A**	C	**C**	A	**C**	T	G	**C**	**C**	**A**	**G**	**O**	0.028
8	G	T	G	**A**	C	**A**	C	**T**	C	**C**	T	A	**C**	**T**	**G**	**G**	**O**	0.018
9	C	C	T	**A**	A	**A**	T	**C**	A	**T**	A	G	**T**	**C**	**A**	**G**	**O**	0.011

The SNPs used in the haplotype analysis are indicated in bold.

coronary artery calcification (CorCP), a surrogate measure of atherosclerosis and subclinical CVD in a sample of families with two or more members with type 2 diabetes. The pattern of association for single SNPs (Table 2) is similar to that observed with single SNP association to type 2 diabetes, insulin resistance, and fasting glucose (1,2). In the haplotype analysis, we observed that haplotype GACTTCAGO was significantly associated with increased CorCP. This same haplotype was associated with type 2 diabetes, increased insulin resistance, and increased fasting glucose in our prior studies (1,2). This is a common haplotype with a frequency of 41% in this study sample, consistent with the "common variant" hypothesis. The AGTCCTGTO haplotype was found to be associated with reduced diabetes risk, greater insulin sensitivity, and reduced fasting glucose in our other studies, (1,2), but these findings were not replicated for CorCP, suggesting that this haplotype may be neutral rather than protective. The 1484insG "insertion of G" allele described by Di Paola et al. (4) as being associated with diabetogenic traits is not present in the common risk haplotype for CorCP (Table 5), suggesting that it is no more likely to be a trait-determining SNP than any of the other *PTPN1* SNPs genotyped. This observation is consistent with results from Bento et al. (1) and Palmer et al. (2). Although there was some evidence of association with the 1484insG allele, this result should be viewed with caution given the small number of homozygous individuals.

The mechanism by which variation in this gene could contribute to variation in a variety of complex traits is not known. There are very few reported SNPs that would affect the amino acid sequence of the protein, and those that do are extremely rare. Given the frequency of the apparent risk haplotype, it seems unlikely that the effect is observed because of LD with a coding SNP. All the associated SNPs are intronic and not in any obvious regulatory region; however, the SNPs, or even the haplotype as a whole, may affect the regulation of the gene, either through interacting directly with transcription factors or affecting the folding of the DNA around the histone complex. There could also be some effects on the splicing of the mRNA or on the stability of the RNA strand before splicing. Some of these possibilities are under investigation in our laboratory.

The biological mechanisms underlying arterial calcification are complex, and many genes and environmental factors are likely to be involved. The mechanism by which *PTPN1* has an impact on CorCP is not clear. *PTPN1* is associated with type 2 diabetes and insulin resistance. Vascular calcification is correlated with insulin resistance (30,31), and the association may be due to insulin resistance in this diabetic sample. A relationship between calcium metabolism and insulin sensitivity has been shown in mice null for the *Ahsg* gene encoding α2-HS glycoprotein (32,33). The analyses undertaken in this study included only individuals affected with type 2 diabetes in order to improve the homogeneity of the sample. Fasting glucose and A1C levels were not correlated with CorCP in the DHS (22), suggesting independence of these traits, and therefore no adjustment was made for these factors.

The PTP1B protein is involved in several other pathways, which may contribute to risk of atherosclerosis. *PTPN1*-deficient mice display increased insulin sensitivity and a resistance to diet-induced obesity (9,10). The latter is believed to be due to the effect of PTP1B in leptin

TABLE 5
Haplotypic association with CorCP for haplotypes with frequency >2%, under a priori genetic inheritance models, adjusted for age, sex, and smoking status

			P values for inheritance models			
	Haplotype	Frequency	Dominant	Additive	Recessive	Direction
1	GACTTCAGO	0.319	**0.017**	**0.010**	0.106	IncreasedCorCP
2	AGTCCTGTO	0.382	0.094	0.137	0.582	NS
3	AATCCTGGO	0.053	0.060	**0.038**	0.089	DecreasedCorCP
4	AATCCTGGG*	0.063	0.613	0.729	**<0.001**	IncreasedCorCP
5	AACTTCAGO	0.072	0.192	0.502	**<0.001**	IncreasedCorCP
7	AACCCCAGO	0.028	0.678	0.678	—	NS

SNPs included in the haplotype are indicated in Table 4. *Analysis based in part on three homozygous individuals. *P* values in bold indicate $P < 0.05$.

signaling. PTP1B dephosphorylates and inactivates JAK2, the kinase responsible for tyrosine phosphorylation of the leptin receptor after leptin binding (11,12). In *PTPN1*-deficient mice, the receptor presumably remains activated, increasing leptin signaling and resulting in reduced obesity. Obesity is a well-known risk factor for CVD, and several studies suggest a possible role for *PTPN1* in determination of BMI (6,7). However, CorCP and BMI were not correlated in the DHS (22). Additional adjustments for BMI also did not alter the outcome, and no associations were detected with obesity in this study, suggesting that the association of *PTPN1* SNPs with CorCP occurs independently of obesity.

Cellular adhesion and migration is an important feature of atherosclerosis. Monocytes and T-cells migrate from the vasculature through the endothelium into the intima. As atherosclerosis progresses, vascular smooth muscle cells also migrate into the plaque (34). These processes are influenced by cell-matrix and cell-cell adhesion, mediated through integrins and calcium-dependent cadherins. PTP1B is present in its native form on the membrane of the endoplasmic reticulum. The protein is cleaved in a calcium-dependent fashion by calpain on engagement of integrins (35). The PTP1B cleavage product is translocated to the cytoskeleton, where it associates with the focal adhesion complexes. Active PTP1B appears to be necessary for integrin signaling, since the addition of a catalytically inactive form that retained substrate binding activity prevented integrin-mediated cell adhesion and spreading in vitro (14).

PTP1B also appears to be involved in regulation of cadherin function. Cadherins are present on the cell surface, and their activity depends on their association with the actin-containing cytoskeleton. This connection is made by α- and β-catenin and appears to be regulated by tyrosine phosphorylation of β-catenin. PTP1B is known to bind to the cytoplasmic domain of N-cadherin and dephosphorylate β-catenin, maintaining the link with the cytoskeleton. Again, the addition of catalytically inactive PTP1B protein resulted in disrupted N-cadherin–mediated cell-cell adhesion (13). N-cadherin is the primary cadherin expressed by smooth muscle cells, and its expression is upregulated in response to arterial injury. Inhibition of N-cadherin after arterial injury impaired migration of smooth muscle cells and wound healing (36). Additionally, E-cadherin was found to be expressed by macrophage foam cells in atherosclerotic plaque (37), and VE-cadherin appears to be important in the neovascularization of the intima in atherosclerotic plaque, which is an important entry route for inflammatory cells (38,39).

It is unclear why a consistent association with CorCP was detected, yet no association was observed with carotid IMT or CarCP. Restriction of the dataset to individuals with complete phenotype information did not change the results. Therefore, the slightly different sample for each outcome is not a contributing factor to the different association results. Weak (although significant) correlations are observed between IMT and CorCP ($r = 0.12$ when adjusted for covariates), suggesting that the two variables measure different aspects of atherosclerosis (40). For example, IMT is an indicator of plaque burden, whereas CorCP is thought to represent advanced complicated lesions, although the absence of calcification does not imply the absence of atherosclerosis. It is also possible that disease progression is regulated differently in the coronary versus carotid arteries.

This study consists of an evaluation of many *PTPN1* SNPs and haplotypes with three measures of subclinical atherosclerosis and therefore includes multiple statistical comparisons. This study was undertaken with a strong a priori hypothesis for the association of *PTPN1* SNPs and haplotypes with the reported phenotypes. It should be noted that there is no generally accepted method of how to apply corrections in studies of this kind where the SNPs are in high LD with each other and the traits are, in this case, also correlated. The associations for CorCP reported do remain statistically significant within a trait even after using a conservative multiple comparison procedure such as the sequential Bonferroni. Previous studies have implicated *PTPN1* polymorphisms in increasing the risk for diabetes, obesity, and hypertension, naturally leading to a candidate gene hypothesis for CVD-related traits. These results suggest an association also with CorCP, possibly mediated by insulin resistance or dysregulation of cellular patterns of adhesion and migration. *PTPN1* appears to contribute genetic risk of type 2 diabetes, obesity, hypertension, and subclinical CVD, suggesting a pleiotropic role for *PTPN1* in determination of these traits.

ACKNOWLEDGMENTS

This study was supported in part by the General Clinical Research Center of the Wake Forest University School of Medicine Grant M01 RR07122, and grants NHLBI R01 HL67348 and NIH NIDDK R01 DK56289 were awarded to D.W.B. and grant R01 AR48797 to J.J.C. K.P.B. was supported by an American Diabetes Association mentor-based fellowship.

REFERENCES

1. Bento JL, Palmer ND, Mychaleckyj JC, Lange LA, Langefeld CD, Rich SS, Freedman BI, Bowden DW: Association of protein tyrosine phosphatase 1B gene polymorphisms with type 2 diabetes. *Diabetes* 53:3007–3012, 2004
2. Palmer ND, Bento JL, Mychaleckyj JC, Langefeld CD, Campbell JK, Norris JM, Haffner SM, Bergman RN, Bowden DW: Association of protein tyrosine phosphatase 1B gene polymorphisms with measures of glucose homeostasis in Hispanic Americans: the Insulin Resistance Atherosclerosis Study (IRAS) Family Study. *Diabetes* 53:3013–3019, 2004
3. Mok A, Cao H, Zinman B, Hanley AJ, Harris SB, Kennedy BP, Hegele RA: A single nucleotide polymorphism in protein tyrosine phosphatase PTP-1B is associated with protection from diabetes or impaired glucose tolerance in Oji-Cree. *J Clin Endocrinol Metab* 87:724–727, 2002
4. Di Paola R, Frittitta L, Miscio G, Bozzali M, Baratta R, Centra M, Spampinato D, Santagati MG, Ercolino T, Cisternino C, Soccio T, Mastroianno S, Tassi V, Almgren P, Pizzuti A, Vigneri R, Trischitta V: A variation in 3′ UTR of hPTP1B increases specific gene expression and associates with insulin resistance. *Am J Hum Genet* 70:806–812, 2002
5. Echwald SM, Bach H, Vestergaard H, Richelsen B, Kristensen K, Drivsholm T, Borch-Johnsen K, Hansen T, Pedersen O: A P387L variant in protein tyrosine phosphatase-1B (PTP-1B) is associated with type 2 diabetes and impaired serine phosphorylation of PTP-1B in vitro. *Diabetes* 51:1–6, 2002
6. Olivier M, Hsiung CA, Chuang LM, Ho LT, Ting CT, Bustos VI, Lee TM, De Witte A, Chen YD, Olshen R, Rodriguez B, Wen CC, Cox DR: Single nucleotide polymorphisms in protein tyrosine phosphatase 1beta (PTPN1) are associated with essential hypertension and obesity. *Hum Mol Genet* 13:1885–1892, 2004
7. Kipfer-Coudreau S, Eberle D, Sahbatou M, Bonhomme A, Guy-Grand B, Froguel P, Galan P, Basdevant A, Clement K: Single nucleotide polymorphisms of protein tyrosine phosphatase 1B gene are associated with obesity in morbidly obese French subjects. *Diabetologia* 47:1278–1284, 2004
8. Byon JC, Kusari AB, Kusari J: Protein-tyrosine phosphatase-1B acts as a negative regulator of insulin signal transduction. *Mol Cell Biochem* 182: 101–108, 1998
9. Klaman LD, Boss O, Peroni OD, Kim JK, Martino JL, Zabolotny JM, Moghal N, Lubkin M, Kim YB, Sharpe AH, Stricker-Krongrad A, Shulman GI, Neel BG, Kahn BB: Increased energy expenditure, decreased adiposity, and

tissue-specific insulin sensitivity in protein-tyrosine phosphatase 1B-deficient mice. *Mol Cell Biol* 20:5479–5489, 2000
10. Elchebly M, Payette P, Michaliszyn E, Cromlish W, Collins S, Loy AL, Normandin D, Cheng A, Himms-Hagen J, Chan CC, Ramachandran C, Gresser MJ, Tremblay ML, Kennedy BP: Increased insulin sensitivity and obesity resistance in mice lacking the protein tyrosine phosphatase-1B gene. *Science* 283:1544–1548, 1999
11. Cheng A, Uetani N, Simoncic PD, Chaubey VP, Lee-Loy A, McGlade CJ, Kennedy BP, Tremblay ML: Attenuation of leptin action and regulation of obesity by protein tyrosine phosphatase 1B. *Dev Cell* 2:497–503, 2002
12. Zabolotny JM, Bence-Hanulec KK, Stricker-Krongrad A, Haj F, Wang Y, Minokoshi Y, Kim YB, Elmquist JK, Tartaglia LA, Kahn BB, Neel BG: PTP1B regulates leptin signal transduction in vivo. *Dev Cell* 2:489–495, 2002
13. Balsamo J, Arregui C, Leung T, Lilien J: The nonreceptor protein tyrosine phosphatase PTP1B binds to the cytoplasmic domain of N-cadherin and regulates the cadherin-actin linkage. *J Cell Biol* 143:523–532, 1998
14. Arregui CO, Balsamo J, Lilien J: Impaired integrin-mediated adhesion and signaling in fibroblasts expressing a dominant-negative mutant PTP1B. *J Cell Biol* 143:861–873, 1998
15. Kannel WB, McGee DL: Diabetes and glucose tolerance as risk factors for cardiovascular disease: the Framingham study. *Diabetes Care* 2:120–126, 1979
16. Pan WH, Cedres LB, Liu K, Dyer A, Schoenberger JA, Shekelle RB, Stamler R, Smith D, Collette P, Stamler J: Relationship of clinical diabetes and asymptomatic hyperglycemia to risk of coronary heart disease mortality in men and women. *Am J Epidemiol* 123:504–516, 1986
17. Haffner SM, Lehto S, Ronnemaa T, Pyorala K, Laakso M: Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. *N Engl J Med* 339:229–234, 1998
18. Bots ML, Hoes AW, Koudstaal PJ, Hofman A, Grobbee DE: Common carotid intima-media thickness and risk of stroke and myocardial infarction: the Rotterdam Study. *Circulation* 96:1432–1437, 1997
19. Chambless LE, Heiss G, Folsom AR, Rosamond W, Szklo M, Sharrett AR, Clegg LX: Association of coronary heart disease incidence with carotid arterial wall thickness and major risk factors: the Atherosclerosis Risk in Communities (ARIC) Study, 1987–1993. *Am J Epidemiol* 146:483–494, 1997
20. Frink RJ, Achor RW, Brown AL Jr, Kincaid OW, Brandenburg RO: Significance of calcification of the coronary arteries. *Am J Cardiol* 26:241–247, 1970
21. Lange LA, Bowden DW, Langefeld CD, Wagenknecht LE, Carr JJ, Rich SS, Riley WA, Freedman BI: Heritability of carotid artery intima-medial thickness in type 2 diabetes. *Stroke* 33:1876–1881, 2002
22. Wagenknecht LE, Bowden DW, Carr JJ, Langefeld CD, Freedman BI, Rich SS: Familial aggregation of coronary artery calcium in families with type 2 diabetes. *Diabetes* 50:861–866, 2001
23. Carr JJ, Crouse JR 3rd, Goff DC Jr, D'Agostino RB Jr, Peterson NP, Burke GL: Evaluation of subsecond gated helical CT for quantification of coronary artery calcium and comparison with electron beam CT. *AJR Am J Roentgenol* 174:915–921, 2000
24. Bensen JT, Langefeld CD, Hawkins GA, Green LE, Mychaleckyj JC, Brewer CS, Kiger DS, Binford SM, Colicigno CJ, Allred DC, Freedman BI, Bowden DW: Nucleotide variation, haplotype structure, and association with end-stage renal disease of the human interleukin-1 gene cluster. *Genomics* 82:194–217, 2003
25. Zeger SL, Liang KY: Longitudinal data analysis for discrete and continuous outcomes. *Biometrics* 42:121–130, 1986
26. Hardin JW, JM H: *Generalized Estimating Equations*. New York, Chapman & Hall/CRC, 2003
27. Hochberg Y: A sharper Bonferroni procedure for multiple tests of significance. *Biometrika* 75:800–802, 1988
28. O'Connell JR: Zero-recombinant haplotyping: applications to fine mapping using SNPs. *Genet Epidemiol* 19 (Suppl. 1):S64–S70, 2000
29. Almasy L, Blangero J: Multipoint quantitative-trait linkage analysis in general pedigrees. *Am J Hum Genet* 62:1198–1211, 1998
30. Meigs JB, Larson MG, D'Agostino RB, Levy D, Clouse ME, Nathan DM, Wilson PW, O'Donnell CJ: Coronary artery calcification in type 2 diabetes and insulin resistance: the Framingham Offspring Study. *Diabetes Care* 25:1313–1319, 2002
31. Arad Y, Newstein D, Cadet F, Roth M, Guerci AD: Association of multiple risk factors and insulin resistance with increased prevalence of asymptomatic coronary artery disease by an electron-beam computed tomographic study. *Arterioscler Thromb Vasc Biol* 21:2051–2058, 2001
32. Mathews ST, Singh GP, Ranalletta M, Cintron VJ, Qiang X, Goustin AS, Jen KL, Charron MJ, Jahnen-Dechent W, Grunberger G: Improved insulin sensitivity and resistance to weight gain in mice null for the Ahsg gene. *Diabetes* 51:2450–2458, 2002
33. Jahnen-Dechent W, Schinke T, Trindl A, Muller-Esterl W, Sablitzky F, Kaiser S, Blessing M: Cloning and targeted deletion of the mouse fetuin gene. *J Biol Chem* 272:31496–31503, 1997
34. Lusis AJ: Atherosclerosis. *Nature* 407:233–241, 2000
35. Rock MT, Brooks WH, Roszman TL: Calcium-dependent signaling pathways in T cells: potential role of calpain, protein tyrosine phosphatase 1b, and p130Cas in integrin-mediated signaling events. *J Biol Chem* 272:33377–33383, 1997
36. Jones M, Sabatini PJ, Lee FS, Bendeck MP, Langille BL: N-cadherin upregulation and function in response of smooth muscle cells to arterial injury. *Arterioscler Thromb Vasc Biol* 22:1972–1977, 2002
37. Bobryshev YV, Lord RS, Watanabe T, Ikezawa T: The cell adhesion molecule E-cadherin is widely expressed in human atherosclerotic lesions. *Cardiovasc Res* 40:191–205, 1998
38. Bobryshev YV, Cherian SM, Inder SJ, Lord RS: Neovascular expression of VE-cadherin in human atherosclerotic arteries and its relation to intimal inflammation. *Cardiovasc Res* 43:1003–1017, 1999
39. Sigala F, Vourliotakis G, Georgopoulos S, Kavantzas N, Papalambros E, Agapitos M, Bastounis E: Vascular endothelial cadherin expression in human carotid atherosclerotic plaque and its relationship with plaque morphology and clinical data. *Eur J Vasc Endovasc Surg* 26:523–528, 2003
40. Wagenknecht LE, Langefeld CD, Carr JJ, Riley W, Freedman BI, Moossavi S, Bowden DW: Race-specific relationships between coronary and carotid artery calcification and carotid intimal medial thickness. *Stroke* 35:e97–e99, 2004