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Replication of Calpain-10 Genetic Association with Carotid Intima-Media Thickness

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Abstract

Objective—Diabetes and atherosclerosis may share common genetic determinants. A prior study in Hispanics found association of haplotypes in the diabetes gene calpain-10 (*CAPN10*) with carotid artery intima-media thickness (CMT). This study sought to replicate this association in an independent cohort.

Methods—Four *CAPN10* SNPs were genotyped and haplotypes determined in 487 Hispanic Americans from 143 families ascertained via an index case with hypertension. CMT was measured from B-mode ultrasound, and glycemic traits quantified from euglycemic clamps. Association of SNPs and haplotypes with CMT was determined.

Results—The minor alleles of SNP-56 and SNP-63 were associated with increased CMT in dominant and additive models. The association of haplotype 1112 with increased CMT was replicated. No associations with fasting insulin, insulin secretion, or insulin sensitivity were observed.

Conclusions—*CAPN10* association with CMT was replicated, further supporting its role as a common genetic determinant of diabetes and atherosclerosis in Hispanics.

1. Introduction

Diabetes mellitus and cardiovascular disease (CVD) often occur together. Family history of diabetes is an independent predictor of increased carotid intima-media thickness (CMT) [1].

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This and other epidemiologic evidence suggest that diabetes and CVD may share common genetic underpinnings [2]. Calpain-10 (*CAPN10*) was the first diabetes gene identified by positional cloning [3]. We previously reported that *CAPN10* haplotypes were associated with CIMT, providing an example of a common genetic determinant of diabetes and subclinical atherosclerosis [4]. In the current study, we sought to replicate the association of *CAPN10* variants with CIMT.

2. Methods

2.1. Subjects and phenotyping

The subjects in this study consisted of 487 Los Angeles Hispanic Americans from 143 families ascertained via a proband with essential hypertension. The recruitment and phenotyping of this cohort have been described previously and included euglycemic clamp, and CIMT by B-mode ultrasound [5]. Only non-diabetic offspring were included in the current analysis.

B-mode carotid artery images were obtained at the University of Southern California Atherosclerosis Research Unit. Right distal common carotid IMT was measured by using an automated computerized edge detection algorithm [6,7]. The measure of CIMT represented the average of ~80 to 100 IMT measurements made over 1 cm. The coefficient of variation of IMT measurement was 3% [6].

All studies were approved by the Institutional Review Boards at participating institutions. All subjects gave informed consent before participation.

2.2. Genotyping

We genotyped SNPs 44, 43, 56, and 63 of *CAPN10*. (rs2975760, rs3792267, rs2975762, rs5030952, respectively). SNP-56 is in near-perfect linkage disequilibrium with Indel-19 and thus served as a surrogate for Indel-19 that was compatible with our genotyping technology [3,4]. To maintain consistency with the literature in terms of haplotype designations, we represented SNP-56 A (minor) as allele 1 and SNP-56 G (major) as allele 2; for the other SNPs, 1 = major allele, 2 = minor allele. Genotyping was performed using the 5'-exonuclease assay (Taqman™ MGB, Applied Biosystems, Foster City, CA); primer and probe sequences are listed in Supplementary Table 1. All four markers were in Hardy-Weinberg equilibrium.

2.3. Data Analysis

Haplotypes were determined by the maximum likelihood method, using a simulated annealing algorithm [8]. Haplotype (comprised of SNPs 44-43-56-63) frequencies were 32.4% for 1121, 24.0% for 1221, 18.5% for 1112, 10.7% for 1111, and 8.2% for 2111.

Log-transformed or square-root-transformed trait values were used to reduce skewness for all analyses. T tests were used to compare trait values between men and women. Quantitative trait values are given as median (interquartile range).

We evaluated association using a robust variance estimation approach, employing the generalized estimating equation (GEE1 [9]) to test hypothesized associations between phenotypes and haplotypes while accounting for familial correlations present in the data. The PROC GENMOD procedure in SAS (version 8.0, SAS Institute, Cary, NC) was used for the analysis utilizing the GEE1 model. Family was taken as the cluster factor. Age, gender, and BMI were specified as covariates in all analyses. SNPs were analyzed in additive and dominant models; haplotypes were analyzed in dominant models.

The primary analysis evaluated association of *CAPN10* genetic variation with CIMT. Subsequent secondary analyses focused on elucidating the relationship of these genetic variants with diabetes-related traits (fasting insulin; insulin sensitivity index, SI, from euglycemic clamp; and insulin secretion, HOMA-%B [10], based on fasting glucose and insulin). To minimize multiple testing, only SNPs and haplotypes showing association with primary traits were analyzed for association with secondary traits.

3. Results

Table 1 lists clinical characteristics of the phenotyped offspring generation. Men had significantly higher CIMT as well as less favorable glycemic, blood pressure, and lipid traits.

Table 2 displays median CIMT values by genotype. SNP-56 and SNP-63 were associated with increased CIMT in dominant and additive models ($P=0.047$ and 0.036 for SNP-56; $P=0.023$ and 0.048 for SNP-63, respectively). Carriers of haplotype 1112 had a significantly increased carotid IMT ($P=0.01$). All of these associations with CIMT remained statistically significant in additional analyses adjusting for systolic blood pressure, diastolic blood pressure, total cholesterol or LDL-cholesterol (data not shown).

Given prior reports of association of the 43-19-63 haplotype combination 112/121 with diabetes [3,11] and CIMT [4], we analyzed the equivalent 44-43-56-63 haplotype combination 1112/1121, but found no association with CIMT.

CAPN10 SNPs or haplotypes were not associated with fasting insulin, HOMA-%B, or SI in this cohort (Supplemental Tables 2–4).

4. Discussion

This is the second report connecting *CAPN10* to CIMT in a Hispanic cohort, providing further evidence that *CAPN10* genetic variation predisposes to subclinical atherosclerosis and may be a common determinant of diabetes and CVD. In the current study, haplotype 1112 was associated with increased CIMT, replicating the association observed in the original study and providing clear evidence that this haplotype affects CIMT [4].

Replication of genetic association is critical to establishing the validity of a finding. Key factors that facilitated the successful replication herein include using a replication cohort of the same ethnicity, recruited from the same geographic area, and phenotyped for CIMT using the same techniques. There was no overlap between the two cohorts in subjects. A meta-analysis of the covariate-adjusted P values of association of haplotype 1112 with CIMT from the two studies produced a P value of 0.0077.

The first study also found association of haplotype 1112 with decreased insulin sensitivity and insulin secretion [4], associations not observed in the current study. We believe this was related to statistical chance, as effect sizes are typically overestimated in initial reports of association, rendering them more difficult to replicate in subsequent cohorts [12]. Also, while both studies included euglycemic clamps for insulin sensitivity assessment, they differed in how they assessed insulin secretion. Given the wealth of evidence associating *CAPN10* variation with insulin-related traits, searching for such associations was a secondary goal. Our main objective was to replicate the unique finding of association with CIMT.

No physiologic study has specifically implicated calpain-10 in atherogenic processes; however, calpains as a group influence such processes including nitric oxide production, adhesion molecular expression, leukocyte adherence to vascular endothelium, vascular smooth muscle cell proliferation and migration, and platelet function [13–15]. This evidence, along with

studies implicating calpains in insulin sensitivity and insulin secretion [16–18], lend support that the product of a single gene (*CAPN10*) may pleiotropically affect both atherosclerosis and diabetes.

A few genes have been associated with diabetes or glycemic traits, and clinical or subclinical CVD [4,19]. Recently, *CDKN2A/2B*, a type 2 diabetes locus, was also observed to be associated with coronary heart disease and ischemic stroke [20,21]. The discovery of common factors underlying both diabetes and atherosclerosis may provide key targets for diagnostic or therapeutic measures.

This report confirms, in an independent cohort, that the diabetes gene *CAPN10* may also influence atherosclerosis. Because both the initial and the replication studies were conducted in Hispanics, further work in non-Hispanic cohorts will be needed to establish whether this finding is generalizable to other populations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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