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Original Article

Hemodynamic fluid shear stress response genes and carotid intima-media thickness: a candidate gene association analysis in the cardiovascular health study

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Abstract: Objective: This study examined whether carotid artery intimal-medial thickness (cIMT) is associated with genetic variations (SNPs) in a hemodynamics-responsive gene pathway. Methods: Subjects were Cardiovascular Health Study participants free of cardiovascular events at baseline (N=3388). Genotype was measured using Illumina 370CNV HumanHap chip. Carotid IMT was measured using ultrasound. Estimated mean differences in cIMT per additional minor allele for 366 SNPs in MAP2K5, MAPK7, MEF2A/C, and KLF2 were adjusted for sex, age, clinic, and medication use. SNP-SNP interactions were examined using logic regression for 71 tagSNPs. Results: None of the associations was significant after correction for multiple comparisons; smallest P-value=0.065 for MAP2K5 and common cIMT. The best-performing logic regression tree combined two SNPs in MAP2K5—rs745212 and rs12905175—and common cIMT; this association was not significant, corrected P-value=0.062. Conclusion: There was not strong evidence of association between genetic variants in a hemodynamics-responsive gene pathway and atherosclerosis among older adults.

Keywords: Cardiovascular Health Study (CHS), candidate gene study, carotid intima-media thickness (cIMT), hemodynamics response, fluid shear stress, endothelium / endothelial cells, Kruppel-like factor 2 (KLF2), Mitogen-activated protein kinase 7 (MAPK7), Myocyte-specific enhancer factor 2 A/C (MEF2A/C), Mitogen-activated protein kinase kinase 5 (MAP2K5)

Introduction

Blood flow exerts biomechanical forces upon the vasculature and affects vessel physiology and function. A major flow-related force, fluid shear stress (FSS), functions parallel to the vessel wall and is a key factor in endothelial cell biology, affecting vascular development and morphology, leukocyte binding, and endothelial cell signaling [1]. Differentials in flow patterns elicit differential gene expression responses in the endothelium, affecting the process of atherogenesis [2]. Gene pathways responsive to such differentials have gained considerable interest in the molecular biology and pathology fields, and many have been characterized. The FSS-responsive pathway identified for this study includes five central genes: mitogen-activated protein kinase kinase 5 (MAP2K5, a.k.a. MEK5), mitogen-activated protein kinase 7 (MAPK7, a.k.a. ERK5), myocyte enhancer factor 2-A and -C (MEF2A; MEF2C), and Kruppel-like factor 2 (KLF2) [3, 4]. MAP2K5-MAPK7-MEF2A/C signaling results in increased expression of the transcription factor KLF2, which itself increases nitric oxide (NO) production, improves oxidative stress response, decreases inflammatory response, improves membrane function, and inhibits fibrinolysis [5, 6].

It is unclear whether genetic variation in this pathway is associated with presence or extent

of atherosclerosis in humans. Although genome-wide association studies (GWAS) conducted in patients with coronary artery disease (CAD) have not found any association between genetic variations in this pathway and disease risk, they have also been limited in statistical power to detect small effects or uncommon variants [7]. A recent GWAS study of carotid intima-media thickness (cIMT; OMIM #609338), conducted in a consortium setting, did not detect significant association with any of these candidate genes [8]. However, compared with a GWAS of similar sample size, a candidate gene approach can improve statistical power for small effects and rare variants.

Materials and methods

The study population included participants from the Cardiovascular Health Study (CHS), a longitudinal, population-based study of coronary heart disease and stroke in adults aged 65 years or more [9]. CHS subjects with a history of CVD events at baseline were excluded. African American participants were excluded because their genotyping data was not available at the time of analyses. Of the total 5,201 original cohort participants, 3,388 were included in the current analyses. Participants provided written, informed consent to participate in genetic studies.

The exposure in the primary analysis was the number of minor alleles corresponding to each single nucleotide polymorphism (SNP) representing variation in the five candidate genes (KLF2, MEF2C, MEF2C, MAPK7, MAP2K5). Exposure was modeled on an additive scale (0, 1, or 2 alleles). Genotyping was done using the Illumina 370CNV HumanHap chip. Unmeasured, autosomal SNPs were imputed with reference to genotypes from HapMap CEU, and defined as the expected number of copies of the minor allele—a fractional value between 0 and 2 [10].

SNPs were included in the analysis if they had been genotyped in the CHS GWAS; fell within the gene or its corresponding promoter region, based on data provided by Genome Variation Server (GVS); had a minor allele frequency (MAF)≥0.05; and had an imputation observed-expected variance ratio (OEvar)≥0.3, when relevant. The numbers of SNPs that met these inclusion criteria were 1 (KLF2), 53 (MEF2A), 95 (MEF2C), 1 (MAPK7), and 216 (MAP2K5). TagSNPs for logic regression were identified from bins with a linkage disequilibrium (LD) $r^2 \geq 0.8$, based on Genome Variation Server (http://gvs-p.Gs.Washington.Edu/gvs) data; the numbers of tagSNPs that met these criteria were 1 (KLF2), 10 (MEF2A), 25 (MEF2C), 1 (MAPK7), and 34 (MAP2K5).

The outcome was cIMT, a continuous measure of the presence and severity of atherosclerosis, which was measured by ultrasound at baseline (1989-90) for each of the common and internal carotid arteries, and calculated based on the mean of up to four (common carotid) or twelve (internal carotid) maximum wall thicknesses on the left, right, near, and far walls [11].

Linear regressions were conducted for each of the 366 SNPs. Adjustment variables included clinic site, sex, age, anti-hypertensive medications, statins, and diuretics. These three classes of drugs were hypothesized *a priori* to have causal pathway involvement. Multiple testing-adjusted statistical significance was assessed using an approximate permutation test which compared 10,000 chance-derived minimum P-values against the minimum P-value among all individual SNPs analyzed for each of the five genes. At the 0.05 level, gene-wide significance was defined by an original minimum P-value less than 95% of all permutation-based P-values [12].

As an exploratory analysis, logic regression was used to evaluate interactions among 71 tagSNPs within and among the five candidate genes, as combined predictors of the outcome, recoded to compare the fifth to the first quintiles of common cIMT (n = 1,340) [13, 14]. The best-scoring combination of SNPs found by the simulated annealing search algorithm was recorded for a model of every possible size, up to a maximum of three trees with ten leaves. Ten-fold cross-validation was used, and the model size with the best average lack-of-fit score was chosen. The strength of the evidence for interaction among the SNPs in the best-fit model of this size, and their association with the binary cIMT outcome, was evaluated using an approximate permutation test, which randomly permuted the binary phenotype data 10,000 times.

Statistical analyses were done using Stata SE v.8.2, R v.2.9.0, and R's LogicReg package v.1.4.7 [13, 15, 16].

Results

Table 1 summarizes selected characteristics of the 3,388 participants from the original CHS cohort.

Table 2 includes results from the primary linear regression analyses. The table provides the coefficients and 95% confidence intervals for each gene's single SNP with the smallest individual P-value, as well as the respective gene-wide permutation P-value. Once adjustments were made for multiple testing using permutations, none of the associations for the five genes with cIMT of either common or internal carotid arteries was statistically significant. The smallest genome-wide association's corrected, permutation P-value was 0.065 for MAP2K5, in the common carotid artery.

Table 1. Subject characteristics (N=3388)*

Female; N (%)	2043 (60.3)
Caucasian; N (%)	3362 (99.2)†
Age; mean (SD)	72.4 (5.4)
Statins; N (%)	49 (1.5)
Hypertension meds; N (%)	1223 (36.1)
Diuretics; N (%)	782 (23.1)
Common cIMT; mean (SD)	0.985 (0.19)
Internal cIMT; mean (SD)	1.441 (0.65)

* Adjustment variables identified a priori; † Non-white strata were too small to warrant adjustment for race

The secondary analyses comprising the logic regression-based tests for interaction among tagSNPs in the five genes produced similar results. The best lack-of-fit score computed by 10-fold cross-validation was for a single 2-leaf tree; this tree used an "and" link between rs12905175 (any copy of the C allele carrying the risk) and rs745212 (any copy of the A allele carrying the risk), indicating that subjects with both of these genotypes in the MAP2K5 gene (n=73) were more likely to have advanced atherosclerosis than subjects with neither of these genotypes. However, the association between this combination of alleles and binary cIMT, as described by this model, was not statistically significant with a corrected, permutation test P-value 0.062 (95% CI for P-value 0.057-0.067).

Discussion

After adjustment for multiple comparisons, our study examining genetic variations in this FSS-response pathway did not find statistically significant associations of individual SNPs (n=336) or tagSNP (n=76) interactions with atherogenesis in older adults.

In this study, SNPs with low call rates and unmeasured SNPs had to be imputed, which is an imperfect measure of genotype. The primary analysis used 44 directly-measured SNPs and 322 imputed SNPs. The incomplete coverage of

Table 2. Individual Linear Regression P-values*

Common carotid (N=3355)						
Gene	SNP with Smallest P-value	P-value un-corr	Beta	Beta 95%CI	Permutation P-value corr	Permutation 95%CI corr
MAP2K5	rs4776970	0.0038	-0.0141	-0.024, -0.005	0.065	0.060-0.070
MAPK7	rs2233072	0.6098	0.0028	-0.008, 0.014	0.610	0.600-0.619
MEF2C	rs11744850	0.0961	-0.0081	-0.018, 0.001	0.655	0.645-0.664
MEF2A	rs11247120	0.1745	0.0062	-0.003, 0.015	0.660	0.651-0.669
KLF2	rs8810	0.8423	-0.0012	-0.013, 0.010	0.839	0.831-0.846
Internal Carotid (N=3328)						
Gene	SNP with Smallest P-value	P-value un-corr	Beta	Beta 95%CI	Permutation P-value corr	Permutation 95%CI corr
MAP2K5	rs7172298	0.0245	0.035	0.004, 0.065	0.292	0.283-0.301
MAPK7	rs2233072	0.5591	0.011	-0.026, 0.048	0.566	0.556-0.576
MEF2C	rs4521516	0.0902	-0.052	-0.112, 0.008	0.629	0.620-0.639
MEF2A	rs8029326	0.0447	-0.031	-0.062, -0.001	0.271	0.263-0.280
KLF2	rs8810	0.7873	0.005	-0.034, 0.045	0.793	0.785-0.801

*Adjustment variables: site, sex, age, anti-hypertensive medications, statins, diuretics.

the variation in MAPK7 and KLF2 genes is another limitation. For the secondary analysis, 92 tagSNPs would have been necessary to adequately cover genomic variation for these five genes. The analytic frameworks used improve upon the statistical power available for a GWAS of similar sample size. However, larger sample sizes would be required to detect very small associations if they do exist.

The outcome measure, cIMT, is associated with the risk of cardiovascular disease events such as MI and stroke, but it is measured with error, due in part to the limited capacity of the imaging technology at the time the measurements were taken. Measurement error can attenuate detectable associations, especially those with small effect sizes.

This study is novel in the attempt to characterize association of FSS-responsive pathway genetic variation with the presence and extent of atherosclerosis in older adults. Since the pathway was identified *a priori* as a risk candidate based on evidence from the molecular biology literature, and both the individual and interaction analyses produced best-performing associations for the same gene (MAP2K5), further characterization of atherosclerosis and genetic variation in this pathway may be warranted. In addition, other mechanisms by which these and related genes influence endothelial health may merit further study.

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Disclosures

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