Gene polymorphisms of fibronectin rs2289202 and fibrillin 2 rs331069 associate with vascular disease, the TAMRISK study

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Abstract. Cell surface heparan sulfate (HS) proteoglycans interact with other extracellular matrix (ECM) components, and HS-binding regions are present in ECM proteins such as fibronectin and fibrillin. Because of their previously established role in susceptibility to intracranial aneurysms, the authors sought to determine whether polymorphisms of fibronectin (FN1, rs2289202) and fibrillin 2 (FBN2, rs331069) associate with selected cardiovascular risk factors and events in the TAMRISK study. A 50-year-old Finnish cohort of 810 subjects of whom 340 had diagnosed hypertension was analyzed. Samples were genotyped for FN1 rs2289202 and FBN2 rs331069 polymorphisms. Incidence of myocardial infarction (I21-I22), transient cerebral ischemic attacks (TIA, G45) and cerebrovascular diseases (I60-I69) were followed up until the subjects were on the average 60 years old. Subjects with FN1 rs2289202 (G>A) minor genotype AA had significantly more cerebrovascular disease than those with the G allele [P<0.001, odds ratio (OR), 8.73; confidence index (CI), 2.79-27.31], although those with the A allele had lower body mass index (P=0.008). Subjects with fibrillin rs331069 (T>C) minor genotype CC had more atherothrombotic disease (P=0.012, OR, 3.16; CI, 1.29-7.71), as measured by combined myocardial infarction and TIA, than those with the T allele. The gene polymorphisms for fibronectin and fibrillin 2 appear to associate with vascular disease.

Introduction

The extracellular matrix (ECM) consists of a network of proteins, glycoproteins, and proteoglycans. Mutations in genes coding for ECM are known to lead to numerous diseases (1). Heparan sulfate (HS) glycosaminoglycan chains are components of HS proteoglycans (HSPG) that mediate interactions between the cell and the ECM. HS chains mediate signaling by binding a multitude of growth factors, but also cell adhesion proteins such as fibronectin and fibrillin 2, which are unique in having cell surface integrin- and HS-binding regions (2). An alteration in cross talk between ECM and cells is most likely linked to development of diseases (1).

Fibronectin is a major glycoprotein component of the ECM. It plays an important part in cell adhesion, migration and differentiation (3). The single pre-mRNA is alternatively spliced, and fibronectin has at least 20 different isoforms in humans (4). Fibronectin is upregulated in atherosclerosis (5). Mutations in the fibronectin gene are known to be associated with human glomerulopathy (6). Fibrillin 2 is a widely distributed major component of extracellular microfibrils throughout the body. Mutations in the fibrillin 2 (FBN2) gene cause congenital contractural arachnodactyly (CCA), which is phenotypically similar to the Marfan syndrome (MFS) (7). However, in contrast to MFS, CCA does not affect the aorta or the eyes. All the identified disease-causing mutations in FBN2 cluster in a limited region between exons 23 and 34 (8).

The authors have previously observed in the present study population an association between the HSPG gene syndecan-4 SNP rs1981429 variant with BMI and coronary artery disease (9). Previous findings have indicated that variation in genes involved in the maintenance of the integrity of the ECM of the arterial wall, including serpine1, transforming growth factor β induced, perlecan, fibronectin, fibrillin 2 and alpha 1 type IV collagen, serve a role in susceptibility to intracranial aneurysms (10). For these reasons, the authors sought to assess whether there was an association between clinical characteristics of the subjects and the gene polymorphisms of HS-binding ECM proteins fibronectin and fibrillin 2.

Patients and methods

Subjects. Tampere adult population cardiovascular risk (TAMRISK) study data derive from periodic health examinations (PHE) done for 50-year-old men and women living in Tampere, Finland with 220,000 inhabitants (11). A public health nurse conducted basic examination in 2003 with an interview using a structured questionnaire about health and health-related behavior. Height (cm) and weight (kg) were recorded, and

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Table I. Clinical characteristics of the study population stratified according to gene polymorphisms fibronectin rs2289202 and fibrillin rs331069.

A, Fibronectin rs2289202

	GG	GA	AA	P-value[a] (AA+GA) vs. GG	P-value[a] AA vs. (GA+GG)	P-value[b] AA vs. (GA+GG)	OR (95% CI)[b]
Frequency (n)	0.595 (474)	0.338 (269)	0.067 (53)				
Characteristics							
Cerebrovascular disease, % (n)	1.9 (9)	0.4 (1)	9.4 (5)	1.000	0.002	<0.001	8.73 (2.79-27.31)
Myocardial infarction and TIA, % (n)	4.8 (23)	4.3 (12)	1.9 (1)	0.608	0.724		
Hypertension, % (n)	42.7 (199)	46.6 (124)	37.3 (19)	0.510	0.383		
Body mass index, kg/m^2 (SD)	27.3 (5.0)	26.5 (4.2)	26.0 (3.6)	0.008	0.128		
Glucose, mmol/l (SD)	5.0 (1.1)	5.0 (1.2)	5.2 (1.4)	0.637	0.381		
Cholesterol, mmol/l (SD)	5.43 (1.03)	5.32 (0.92)	5.32 (0.82)	0.139	0.604		
HDL-cholesterol, mmol/l (SD)	1.61 (0.45)	1.59 (0.44)	1.61 (0.50)	0.529	0.890		
Triglycerides, mmol/l (SD)	1.42 (1.16)	1.47 (1.75)	1.28 (0.79)	0.812	0.439		
LDL-cholesterol, mmol/l (SD)	3.19 (0.91)	3.11 (0.75)	3.13 (0.75)	0.213	0.808		
Systolic blood pressure, mmHg (SD)	134.8 (16.5)	135.5 (17.6)	136.1 (14.5)	0.486	0.646		
Diastolic blood pressure, mmHg (SD)	87.7 (9.6)	88.3 (10.3)	88.7 (7.8)	0.408	0.494		

B, Fibrillin 2 rs331069

	TT	TC	CC	P-value[a] (TC+CC) vs. TT	P-value[a] CC vs. (TT+TC)	P-value[b] CC vs. (TT+TC)	OR (95% CI)[b]
Frequency (n)	0.487 (379)	0.414 (322)	0.099 (77)				
Characteristics							
Cerebrovascular disease, % (n)	2.3 (9)	1.5 (5)	1.2 (1)	0.438	1.000		
Myocardial infarction and TIA, % (n)	3.6 (14)	3.9 (13)	9.8 (8)	0.388	0.021	0.012	3.16 (1.29-7.71)
Hypertension, % (SD)	43.0 (159)	44.5 (141)	42.3 (33)	0.771	0.904		
Body mass index, kg/m^2 (SD)	26.9 (4.7)	27.1 (4.5)	26.0 (4.6)	0.937	0.078		
Glucose, mmol/l (SD)	5.0 (1.1)	5.1 (1.2)	4.9 (0.7)	0.835	0.223		
Cholesterol, mmol/l (SD)	5.35 (1.02)	5.41 (0.88)	5.41 (1.14)	0.395	0.805		
HDL-cholesterol, mmol/l (SD)	1.61 (0.46)	1.59 (0.42)	1.65 (0.50)	0.795	0.309		
Triglycerides mmol/l (SD)	1.43 (1.58)	1.43 (0.97)	1.44 (1.80)	0.999	0.919		
LDL-cholesterol, mmol/l (SD)	3.13 (0.89)	3.20 (0.79)	3.10 (0.87)	0.435	0.520		
Systolic blood pressure, mmHg (SD)	135.3 (17.2)	135.4 (17.1)	133.0 (13.8)	0.723	0.233		
Diastolic blood pressure, mmHg (SD)	88.3 (9.6)	88.0 (10.1)	85.9 (9.2)	0.301	0.050		

[a]Chi-square test or Fisher's exact test for categorical variables and t-test for continuous variables. [b]Logistic regression adjusted for sex and body mass index. TIA, transient ischemic attacks; SD, standard deviation; HDL, high-density lipoprotein; LDL, low-density lipoprotein; OR, odds ratio; CI, confidence interval.

the body mass index (BMI) was calculated. Blood pressure measurement (mmHg) was done using a calibrated mercury sphygmomanometer. Following an overnight fast, cholesterol (mmol/l), high-density lipoprotein (HDL)-cholesterol (mmol/l) and triglycerides (mmol/l) (from which low-density lipoprotein (LDL)-cholesterol was calculated) and serum glucose (mmol/l) were measured by standard techniques. Buccal swabs for DNA extraction, and a permission form to use PHE information and national registry data were collected by mail separately of the physical examination during years 2006-2010. The Ethics Committees of the Tampere University Hospital and the City of Tampere approved the study. Informed consent was obtained from all participants.

Data on hospitalizations including ICD-10 codes for discharge diagnoses were obtained from the National Hospital Discharge Registry (HILMO) maintained by the National Institute of Health and Welfare (Helsinki, Finland). Incidence of cerebrovascular diseases (I60-I69), myocardial infarction (I21-I22) and transient cerebral ischemic attacks (TIA; G45) were followed up from 2005 to 2014 until the subjects were, on average, 60 years old.

Cases (n=340) were subjects who had hypertension at the age of 50 years (as diagnosed by a physician) and for each case, at least one normotensive control subject (n=470) with the same sex and similar smoking habits was chosen in order of admission from the PHE cohort (n=6,000). Therefore,

the present study population at the age of 50 years included 810 subjects. Genotyping was successful in 796 subjects for fibronectin and in 778 for fibrillin.

Genotyping. DNA was extracted from buccal swabs (Qiagen Inc., Valencia, CA, USA). The DNA samples were genotyped at the KBioscience Institute (Hoddesdon, UK) using Competitive Allele Specific PCR (KASP) technique. Details of this method can be obtained from https://www.lgcgroup.com/genotyping/.

Statistical analysis. Associations of the genotyped SNPs with clinical characteristics were analyzed using dominant and recessive models for genotype groups, according to the major and minor alleles, respectively (12). T-test for continuous variables and Chi-square test or Fisher's exact test for categorical variables were applied for the comparison of genotype groups. If the distribution was skewed, the analysis was performed using transformed values to approximately normalize the distribution. Logistic regression was used to obtain odds ratio (OR) and 95% confidence interval (CI) for association analyses of fibronectin and fibrillin genotypes with cardiovascular events. Analyses were carried out using SPSS 20.0 for Windows software (version, 20.0; IBM SPSS, Armonk, NY, USA).

Results

Clinical characteristics of patients. The study population of hypertensive subjects and controls at the age of 50 years has been previously described (11). The frequencies of fibronectin rs2289202 (G>A) and FBN2 rs331069 (T>C) were in Hardy-Weinberg equilibrium in the whole study population (P=0.0821 and P=0.479, respectively). In follow-up of the genotyped subjects, there were 15 who had a diagnosis of cerebrovascular disease, 28 with MI and 9 with TIA. Since MI and TIA may be considered as thrombotic complications of atherosclerosis (12), subjects with MI and TIA were combined.

Association of fibronectin and fibrillin 2 genotypes with clinical characteristics. The statistical analysis of clinical characteristics between different genotype groups for fibronectin rs2289202 and FBN2 rs331069 are provided in Table I. Subjects with fibronectin rs2289202 (G>A) minor genotype AA had significantly more cerebrovascular disease than those with the G allele. This difference remained after adjusting for BMI and sex. Those with rs2289202 genotype GG had higher BMI than those with the A allele.

Subjects with fibrillin rs331069 (T>C) minor genotype CC had more myocardial infarction and TIA than those with the T allele, even when adjusted for BMI and sex. The other measured clinical characteristics did not differ between genotype groups.

Discussion

The authors previously observed in the present TAMRISK study population an association between a HSPG gene syndecan-4 SNP rs1981429 variant with BMI and coronary artery disease (9). Cell surface HSPGs such as syndecans serve an important role in the organization of the ECM. Fibrillin and fibronectin are ECM proteins that bind to HS chains in HSPGs and consequently also convey information to the cell (2). Therefore, they wanted to assess whether these HS-binding ECM proteins are associated with clinical characteristics such as BMI and cardiovascular diseases in this middle-aged cohort followed up to 60 years of age.

Subjects with fibronectin rs2289202 (G>A) minor genotype AA had significantly more cerebrovascular disease than those with the G allele. This difference remained following adjustments for BMI and sex. This was in accordance with the previous study addressing the association of this polymorphism with intracranial aneurysms, where the OR was 1.35 for the minor allele (10). Fibronectin (FN1) is an ECM glycoprotein that is important for cell adhesion and also migration. As the ECM affects cell growth, motility and differentiation, its modifications in the arterial wall may lead to cerebrovascular complications (10).

The authors identified that subjects with fibrillin rs331069 (T>C) minor genotype CC had more myocardial infarction and TIA than those with the T allele. Patients younger than 60 years old at the time of a TIA may have an especially high age-adjusted risk for future MI (13). The minor allele has previously been associated with intracranial aneurysm with an OR of 1.29 (10). However, there was no association of this variation with cerebrovascular disease as such. The fibrillins FBN1 and FBN2 polymerize to a scaffold for the assembly of elastin that provides architectural framework for tissues (14). Fibrillin is present in blood vessels to convey elasticity and flexibility to resist stretch and pressure forces. Disease-causing mutations in FBN2 result in CCA, which unlike the MFA does not affect the aorta (7). However, the results for thrombotic complications such as MI and TIA, together with the earlier observation for intracranial aneurysm indicate that the FBN2 gene may be involved in cardiovascular pathology as well.

FBN2 rs331069 is an intron variant and FN1 rs2289202 is a synonymous codon. Up to date there is only one report on their association with intracranial aneurysms, and the functional importance of these variations has not been elucidated (10). On the other hand, it is known that few SNPs are exonic and cause non-synonymous missense substitutions that would alter the amino acid protein sequence. Most of the genetic risk variants for cardiovascular disease are located in DNA sequence that do not code for protein. In fact, >85% of SNPs for CAD/AMI are located either in introns or intergenic regions (15).

FBN2 rs331069 and FN1 rs2289202 appear to have biological relevance in human pathophysiology, possibly through interference with vascular ECM composition.

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