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Association between *Fibrillin1* Polymorphisms (rs2118181, rs10519177) and Transforming Growth Factor β1 Concentration in Human Plasma

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Transforming growth factor (TGF)-β1 is a cytokine that participates in a broad range of cellular regulatory processes and is associated with various diseases including aortic aneurysm. Increased TGF-β1 levels are linked to Marfan syndrome (MFS) caused by *fibrillin1* (*FBN1*) mutations and subsequent defects in signaling system. *FBN1* single nucleotide polymorphisms (SNPs) rs2118181 and rs1059177 do not cause MFS but are associated with dilative pathology of aortic aneurysms (DPAAs). TGF-β1 and *FBN1* SNPs rs2118181 and rs1059177 are potential biomarkers for early diagnosis of DPAA. We investigated the relationship between TGF-β1 levels in human blood plasma and *FBN1* rs2118181 and rs1059177 in 269 individuals. The results showed a quantitative dependence of SNP genotype and TGF-β1 concentration. Presence of a single rs2118181 minor allele (G) increased the amount of TGF-β1 by roughly 1 ng/mL. Two copies of *FBN1* rs1059177 minor allele (G) were required to have an additive effect on TGF-β1 levels. We found higher TGF-β1 concentrations in men compared with women ($p = 0.001$). A strong correlation between TGF-β1 levels and *FBN1* SNPs suggests that a single nucleotide substitution in *FBN1* sequence might reduce bioavailability or binding properties of fibrillin-1 and have an effect on TGF-β1 activation and cytokine concentration in blood plasma. By establishing the relationship between TGF-β1 and *FBN1* SNPs rs2118181 and rs1059177, we provide evidence that their combination might be used as molecular biomarkers to identify patients at risk for sporadic ascending aortic aneurysm and aortic dissection.
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INTRODUCTION

FBN1 mutations are associated with the development of dilative pathology of ascending aorta (DPAA) (1). It has been shown that fibrillin-1 regulates the bioavailability of transforming growth factor (TGF)-β1 (2). TGF-β1 is a member of the TGF-β family of solubile proteins, cytokines, and, together with other TGF-β isoforms, works through the same signaling systems encompassing various cellular processes such as angiogenesis, proliferation, differentiation, apoptosis, wound healing and modification of extracellular matrix (3). Cellular and molecular mechanisms of aortic dilation have been investigated in the Marfan syndrome (MFS), where aortic aneurysm is one of the main features of a multisystem disorder. Increased TGF-β1 signaling has been associated with defective fibrillin-1 in the pathology of MFS (2). Genome-wide association study identified *FBN1* SNPs rs2118181 and rs10519177 to be associated with sporadic DPAA (4). The data were in part replicated by two independent studies (5,6), therefore strengthening the idea that these SNPs contribute to the molecular pathways leading to the sporadic thoracic disease. To uncover the mechanism of sporadic DPAA and to identify potential biomarkers, we investigated the association between TGF-β1 concentration in blood plasma and *FBN1* SNPs rs2118181 and rs10519177.

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MATERIALS AND METHODS

Study Subjects

A study group was recruited from three cluster samples of Kaunas population (n = 275). The first cluster consisted of student and staff volunteers from Lithuanian University of Health Sciences (LUHS) (n = 70, age range 18–55 years, mean 48, median 49); the second cluster was composed of volunteers (n = 151, age 55 years and older, mean 64, median 65) appointed to their physician in Dainavos outpatient's clinic

Table 1. Distribution of *FBN1* SNPs rs2118181 and rs10519177 genotypes in study samples.

	rs2118181			rs10519177		
Study subjects	AA	AG	GG	AA	AG	GG
I Cluster (n = 70)	53 (75.7%)	15 (21.4%)	2 (2.9%)	40 (57.2%)	25 (35.7%)	5 (7.1%)
II Cluster (n = 151)	125 (80.8%)	25 (16.5%)	1 (0.7%)	97 (64.2%)	45 (29.8%)	9 (6.0%)
χ^2, p		2.62, $p = 0.27$			1.02, $p = 0.60$	
Total (n = 221)	178 (80.5%)	40 (18.1%)	3 (1.4%)	137(62.0%)	70 (31.7%)	14 (6.3%)
Reference (n = 840)	663 (78.9%)	167 (19.9%)	10 (1.2%)	496 (59.0%)	297 (35.4%)	47 (5.6%)
χ^2, p		0.38, $p = 0.82$			1.11, $p = 0.58$	

(Kaunas). *FBN1* SNPs rs2118181 and rs10519177 genotype frequencies did not differ significantly between the two clusters. Because of the low number of homozygous minor allele carriers of *FBN1* SNPs rs2118181 (n = 3) and rs10519177 (n = 14), a third cluster (n = 54, age range 46–72 years, mean 62, median 64) was added that consisted of homozygous minor allele carriers (n = 10, rs2118181, and n = 44, rs10519177) (Table 1). It was selected from a reference sample of the population (n = 840, age range 45–73 years, mean and median 60) screened within the international HAPIEE (Health, Alcohol and Psychosocial factors In Eastern Europe) study (7). None of the study subject reported having aortic dilation.

Ethical approval was obtained from the Ethics Committee of the Lithuanian Health Science University (number BE-2-12), and all subjects provided written informed consent for participation in the study.

Determination of Genotypes

Genomic DNA was isolated from potassium EDTA blood as described (8). Genotyping of *FBN1* SNPs rs2118181 and rs10519177 was performed according to the manufacturer's instructions by using commercially available kits from Applied Biosystems: C_16234705_10 (rs2118181), and C_8932690_10 (rs10519177) and ABI 7900HT real-time PCR Thermocycler (Applied Biosystems). All testing procedures were performed at the laboratory of Molecular Cardiology of the Institute of Cardiology of the Lithuanian University of Health Sciences.

TGF-β1 Quantitative Detection

Blood samples were obtained by using 3-mL Venosafe™ blood collection tubes containing 5.9 mg K2EDTA (Terumo Europe, Belgium). Plasma was obtained by centrifuging blood samples for 15 min at 3,506*g* within 2 h after phlebotomy. Plasma samples were aliquoted and stored at –20°C. All samples were tested in duplicate with an eBioscience Platinum human TGF-β1 ELISA commercially available kit (Bender Med Systems) based on standard sandwich enzyme-linked immunosorbent assay technology according to the manufacturers' instructions. Absorbance was measured by using a Stat-fax 4200 microplate reader (Awareness Technology) at 450 nm OD (optical density). TGF-β1 concentration was automatically calculated by using a log-log linear regression. According to manufacturers' instructions, cross-reactivity with TGF-β2 and TGF-β3 was <0.01%.

Statistical Analysis

A nonparametric Kruskal-Wallis test was used for statistical analysis. The relationship between age and cytokine levels was calculated by using a two-tailed Spearman rank correlation coefficient (R). Multiple linear regression was used to estimate the effect of gender (male), age (years) and genotype on the logarithmically transformed TGF-β1 values.

RESULTS

Our study population consisted of 141 male and 128 female subjects. Six samples were rejected from further TGF-β1 testing because of the preanalytical errors. TGF-β1 concentrations differed significantly ($p = 0.001$) between men (median 8.32 ng/mL, range 1.00–33.10) and women (median 5.81 ng/mL, range 1.10–27.30) and showed dependency on age ($R = 0.236$, $p < 0.001$). Genotype distribution and corresponding TGF-β1 values are presented in Table 2. Median TGF-β1 concentrations for each genotype showed quantitative association. The presence of one and two rs2118181 G alleles led to a 1.24 and 3.21 ng/mL increase in median TGF-β1 concentrations, respectively. A single copy of rs10519177 G allele did not seem to cause an increase in TGF-β1, but the presence of two minor allele copies coincided with a 4.08 ng/mL increase in TGF-β1 levels.

A multiple linear regression analysis was adopted to investigate an association between gender, age, *FBN1* genotypes and TGF-β1 values (Table 3). Gender, age and rs2118181 AG + GG genotypes or a single-allele G presence were all significant variables to predict log-transformed TGF-β1 values ($p < 0.05$). An additive effect of G allele correlated to the observed TGF-β1 values in Table 2. We did the same testing for rs10519177 and found that the presence of GG genotype in addition to the gender and age was a significant variable for TGF-β1 values as well ($p < 0.05$).

DISCUSSION

This is the first report on the association between previously defined *FBN1* SNPs (rs2118181 and rs10519177) linked with sporadic thoracic aorta aneurysm and dissection (6) and TGF-β1

Table 2. Genotype frequencies and corresponding TGF-β1 values.

FBN1 SNPs	Genotype	Genotype frequency, n (%)	TGF-β1 concentration (ng/mL), minimum/medium/maximum
rs2118181			
	AA	192 (71.4)	1.00/6.57/33.12
	AG	64 (23.8)	2.71/7.81/28.08[a]
	GG	13 (4.8)	1.40/9.78/17.28[b]
	AG + GG	77 (28.6)	1.40/8.02/28.10
rs10519177			
	AA	138 (51.3)	1.00/6.40/33.12[c]
	AG	73 (27.1)	1.90/5.46/28.08[d]
	GG	58 (21.6)	1.40/10.48/27.29
	AG + GG	131 (48.7)	1.40/7.6/28.08

[a]TGF-β1 concentration compared with rs2118181 AG versus AA genotypes, $p = 0.024$.
[b]TGF-β1 concentration compared with rs2118181 GG versus AA genotypes, $p = 0.094$.
[c]TGF-β1 concentration compared with rs10519177 AA versus GG genotypes, $p < 0.0001$.
[d]TGF-β1 concentration compared with rs10519177 AG versus GG genotypes, $p < 0.0001$.

concentration in blood plasma of study subjects who were not aware of aortic dilation. Our data demonstrated that presence of rs2118181 minor allele significantly increased median TGF-β1 values, whereas such effect in rs10519177 was achieved only by homozygous carriers of two minor alleles. Moreover, these regularities correspond to the earlier reported associations between DPAA phenotypes and the genotyped *FBN1* SNPs (6). According to these data, an additive minor allele model fitted best in describing the association between rs2118181 and aneurysm (odd ratio [OR] 1.70, 95% confidence interval [CI] 1.17–2.46) or Stanford A dissection (OR 2.64, 95% CI 1.66–4.19), whereas a recessive model described the association between rs10519177 and Stanford A dissection (OR 4.31, 95% CI 2.06–9.01).

Chaudhry *et al.* (2) demonstrated that specific *FBN1* sequence encoded by exons 44–49 directly regulates endogenous TGF-β1 functionality without affecting cells or any other intermediate mechanisms, whereas abnormal fibrillin-1 with deficient functionality causes an excessive amount of active TGF-β1 to be released from the extracellular matrix (2). Mutations in *FBN1* domains increase proteolytic sensitivity to inflammatory enzymes (9) causing a degradation of microfibrills and subsequent changes in TGF-β1 bioavailability. It is yet unknown how *FBN1* SNPs (rs2118181, rs10519177) might affect the fibrillin-1 and TGF-β1 interaction. Both SNPs are in intronic regions of the *FBN1* (10). Thus, it might be speculated that they alter *FBN1* pre-mRNA thermodynamic and kinetic properties and affect splicing events. *FBN1* splicing errors have been shown to cause protein alteration: (a) elimination of an entire exon resulting in a loss of an entire protein domain (11) and (b) activation of alternative splice sites causing an addition of 11 amino acids to the protein (12).

It is obvious that the relationship between TGF-β1 and *FBN1* SNPs is quantitative. Unaltered fibrillin-1 activates TGF-β1 through interactions between latent TGF-β binding proteins (LTBPs 1–4) and the N terminus of fibrillin-1 (13). If this interaction is somehow disturbed because of changes in fibrillin-1 availability, the amount of LTBPs would increase. In our experiments, we used acid activation to release TGF-β1 from the LTBP complex to measure total TGF-β1 level in blood plasma. Therefore, we do not know whether TGF-β1 increase is due to higher levels of LTBP or increased levels of active TGF-β1. More LTBPs would indicate defective fibrillin-1 interaction with LTBPs and reduced activation of TGF-β1. Higher levels of active TGF-β1 would indicate an increase in the activation process through LTBPs and fibrillin-1 interactions.

The pathogenesis of DPAA remains unclear. It is unknown if a fibrillin-LTBPs junction is needed to protect the large

Table 3. Multiple linear regression analysis for logarithmically transformed TGF-β1 values.

FBN1 SNP	Dependent variables	Unstandardized coefficients B	*p*	Adjusted R^2
rs2118181	Constant	1.045	<0.001	0.103
	Gender (male)	0.185	0.015	
	Age	0.012	<0.001	
	Genotype AG + GG	0.222	0.008	
	Constant	1.035	<0.001	0.102
	Gender (male)	0.181	0.018	
	Age	0.012	<0.001	
	Allele G	0.172	0.01	
rs10519177	Constant	1.053	<0.001	0.146
	Gender (male)	0.158	0.034	
	Age	0.011	<0.001	
	Genotype GG	0.397	<0.001	

latent complex from proteolytic activation or whether *FBN1* functions more directly in controlling assembly or stability of latent TGF-β1 complexes (14). Second, the balance between the need for TGF-β1 in normal development and suppression of its activity in the treatment of disease should be considered (15).

CONCLUSION

Our study for the first time demonstrates the association between TGF-β1 levels in blood plasma and *FBN1* SNPs rs2118181 and rs10519177. We suggest that TGF-β1 levels in blood plasma in combination with *FBN1* SNPs might serve as a biomarker to identify patients at risk for sporadic ascending aortic aneurysm and aortic dissection.

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DISCLOSURE

The authors declare that they have no competing interests as defined by *Molecular Medicine*, or other interests that might be perceived to influence the results and discussion reported in this paper.

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