



LITHUANIAN UNIVERSITY
OF HEALTH SCIENCES

**ASSOCIATION BETWEEN *FBN1* POLYMORPHISMS
AND *TGF-β1* CONCENTRATION WITHIN ANEURYSMS
AND DISSECTIONS OF ASCENDING THORACIC AORTA**



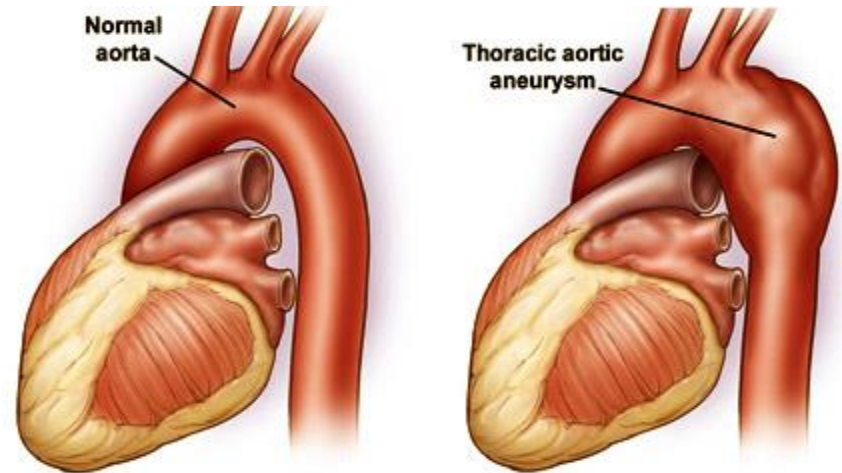
Ramune Sepetiene

**Laboratory of Molecular Cardiology,
Institute of Cardiology ,
Medicine Academy, LUHS**

26-27 October, Chicago

- A high mortality is determined by Dilatative Pathology of Ascending Aorta (DPAA).
15 000 people die every year due to the complications of DPAA in USA.

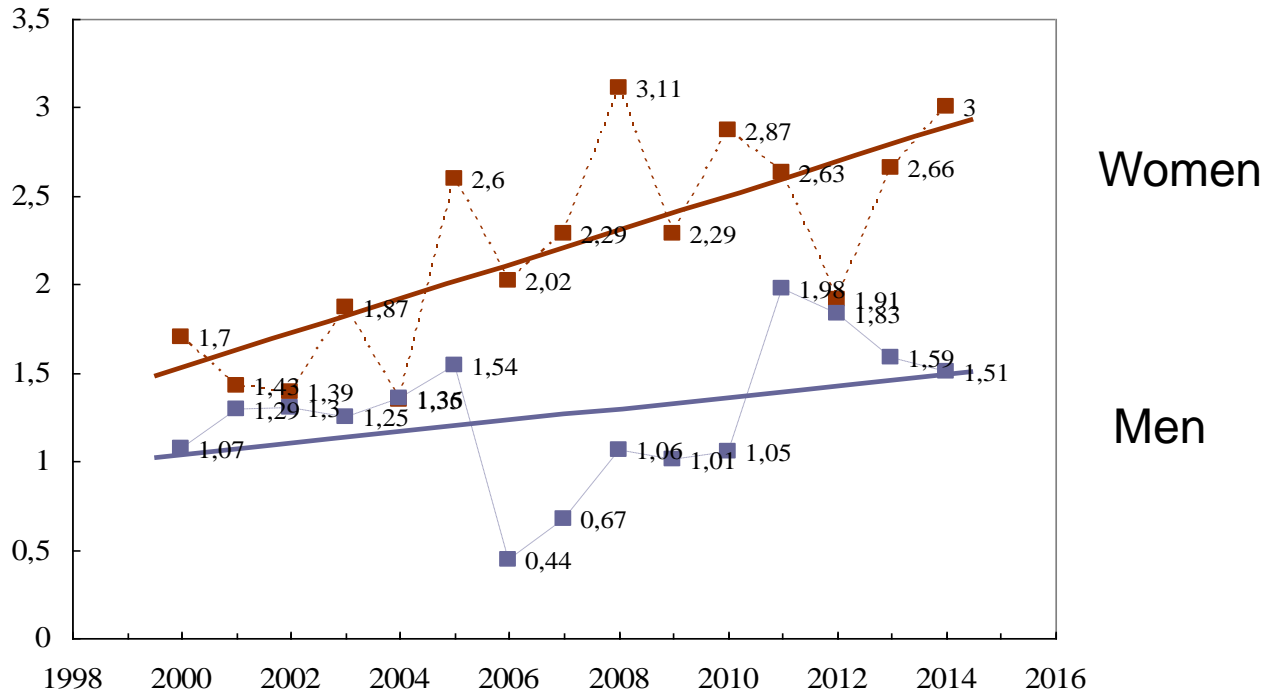
- DPAA takes **14th** place according to the reasons of mortality among 55 years people and older
(National Centre of Health Statistics in USA, 2012)



- DPAA is diagnosed **5,9 : 100 000 worldwide**
(Anderson CA et al. Ascending aortic aneurysms. Cardiac surgery in adult. New York: McGraw-Hill; 2009; 1123-1148)

Morbidity by DPAA in Lithuania

(cases per/100 000 population)



ICD-10 Version:2016 - World HealthOrganization

I71.01 – Dissection of Thoracic Aorta

I71.1 – Aneurysm of Thoracic Aorta, dissected

I71.2 – Aneurysm of Thoracic Aorta, dissection not indicated

Data from Health Information Centre at Lithuanian Hygiene Institute, 2014

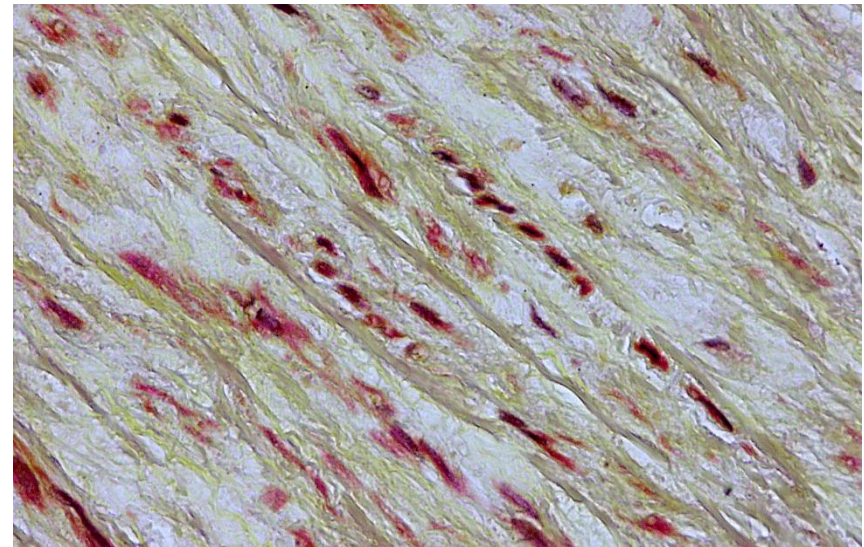
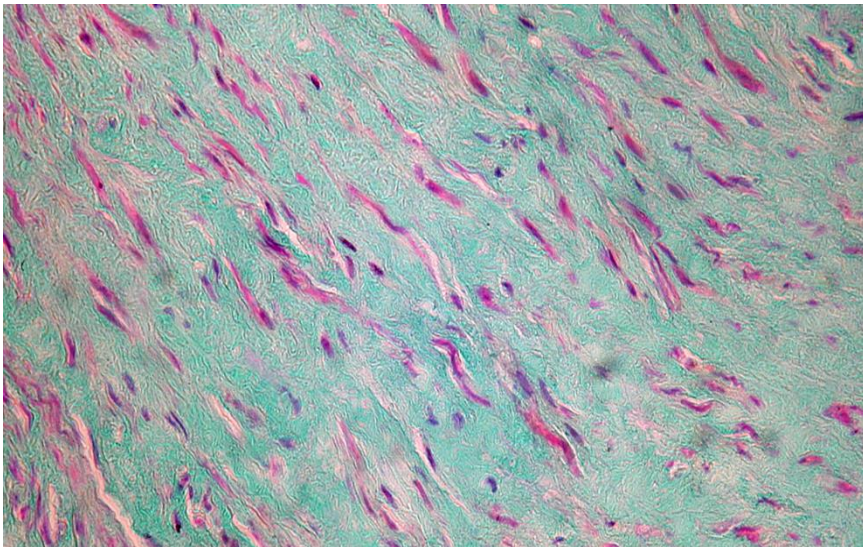
Morphogenesis of aneurysm

- A contractive phenotype of smooth muscle cells, responsible for vascular resistance, to be converted to synthetic phenotype.

(Lesauskaite et al "Smooth muscle cells of the media in the dilatative pathology of ascending thoracic aorta: morphology, immunoreactivity for osteopontin, matrix metalloproteinases, and their inhibitors." *Human pathology* 32.9 (2001): 1003-1011)

- Primary remodeling of pathological structural proteins: collagen and elastin, with extracellular proteases involved

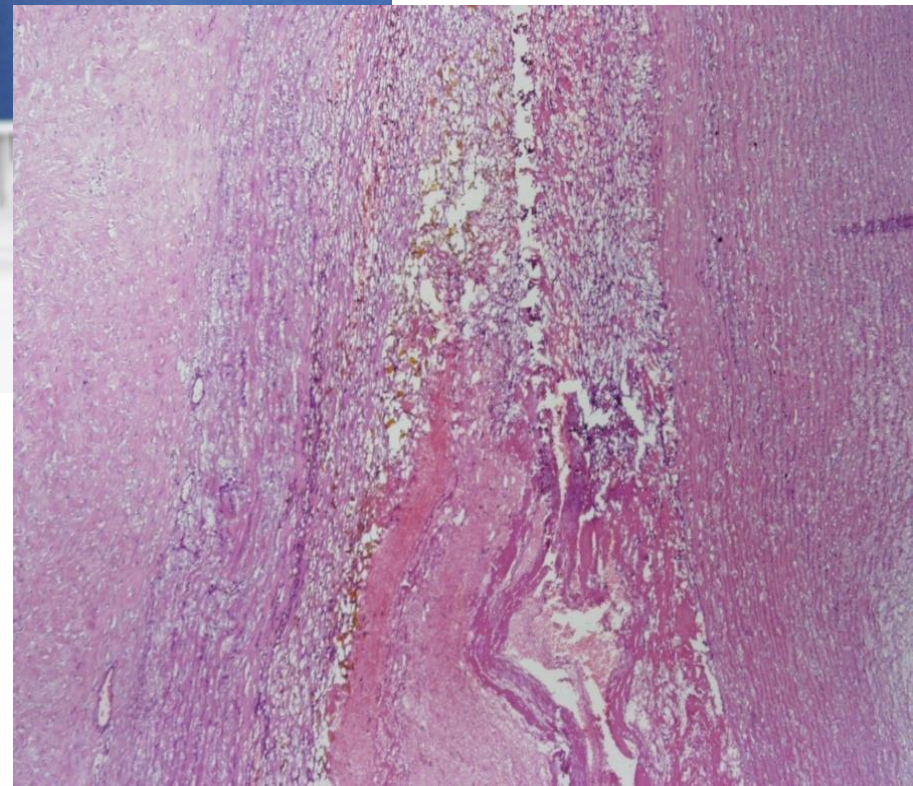
Aortic media





Aortic dissection

Lesauskaite V, Dilatative pathology of ascending aorta, a new attitude, Oral presentation , 2012.



Mutations of *Fibrillin-1* gene

Elevated concentration of TGF- β 1 in blood is found for patients with diagnose of Marfan syndrome (MFS), determined by mutations of *Fibrillin-1* (*FBN1*) gene.

Milewicz DM, Michael K, Fisher N, Coselli JS, Markello T and Biddinger A. (1996) Fibrillin-1 (*FBN1*) mutations in patients with thoracic aortic aneurysms. *Circulation* 94:2708-2711

Chaudhry SS, et al (2007) Fibrillin-1 regulates the bioavailability of TGF β 1. *J Cell Biol.* 176:355-367 .



[El Greco \(Domenikos Theotokopoulos\)](#)
[\(1541–1614\)](#)



The Great Virtuoso Violinists/Composers of the 18th Century: Nicola Paganini

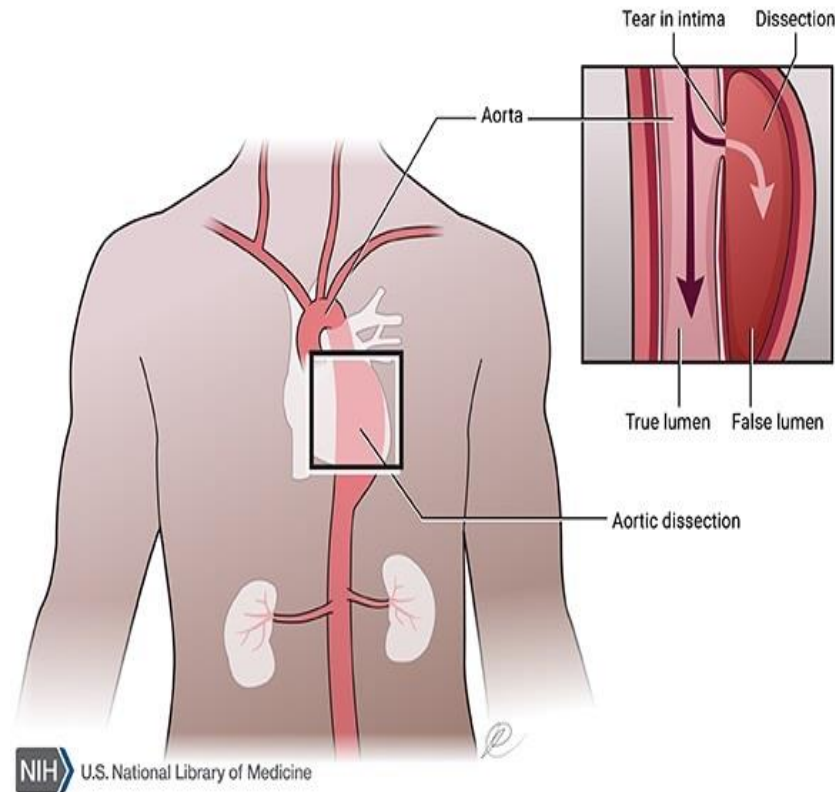
It is now thought that Paganini's genetic condition was Marfan Syndrome, which would explain his bouts of ill health, especially in his later life. Paganini suffered with joint pain, poor vision, breathlessness, chest pains and fatigue. These less desirable symptoms meant that he frequently had to cancel public performances and he died at the relatively young age of 58.

It's established already, that *FBN1* SNP's (rs2118181, rs1059177) even did not cause MFS, but are significantly associated with DPAA formation.

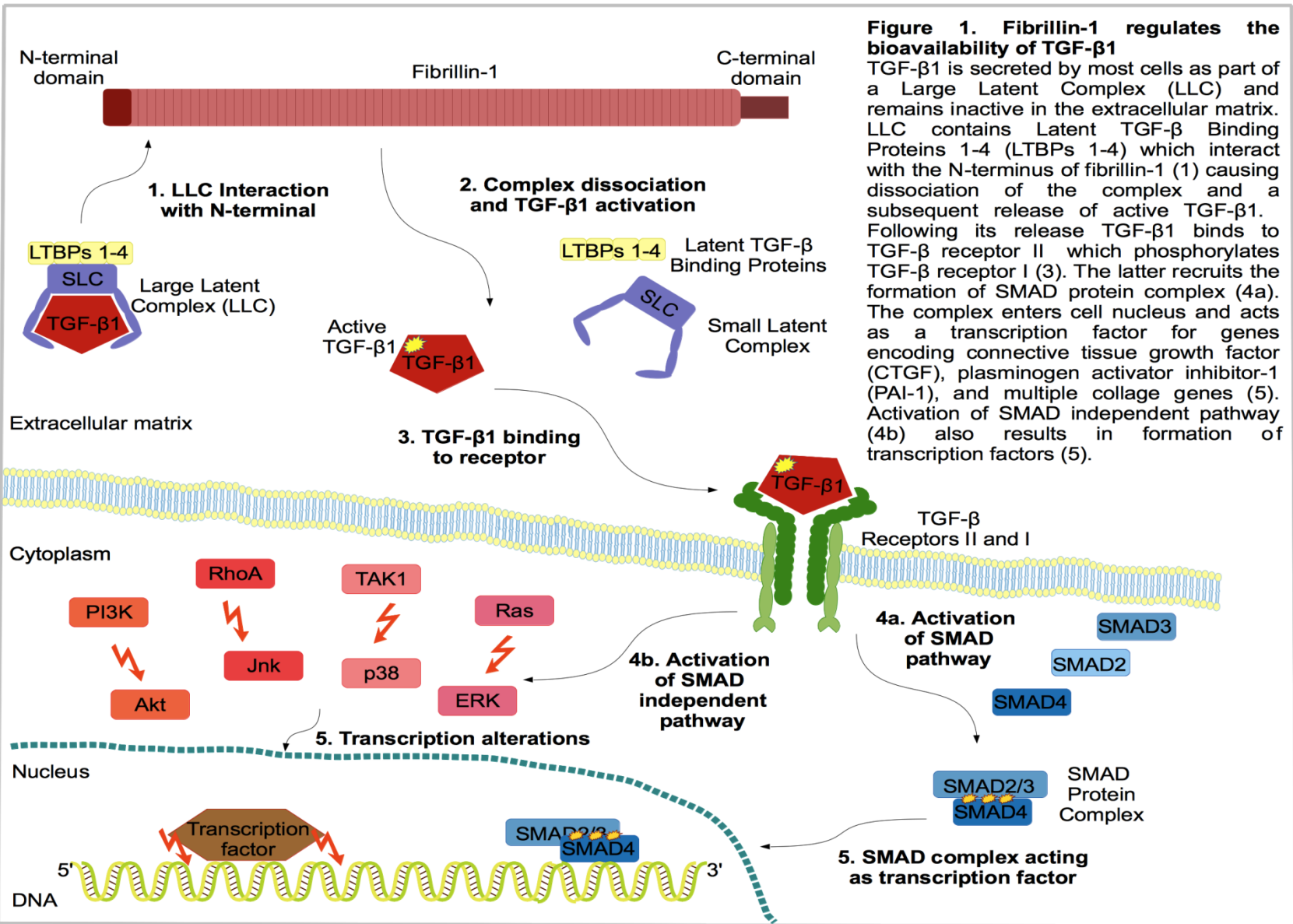
LeMaire SA et al (2011) Genome-wide association study identifies a susceptibility locus for thoracic aortic aneurysms and aortic dissections spanning *FBN1* at 15q21. 1. *Nat Genet.* 43:996-1000

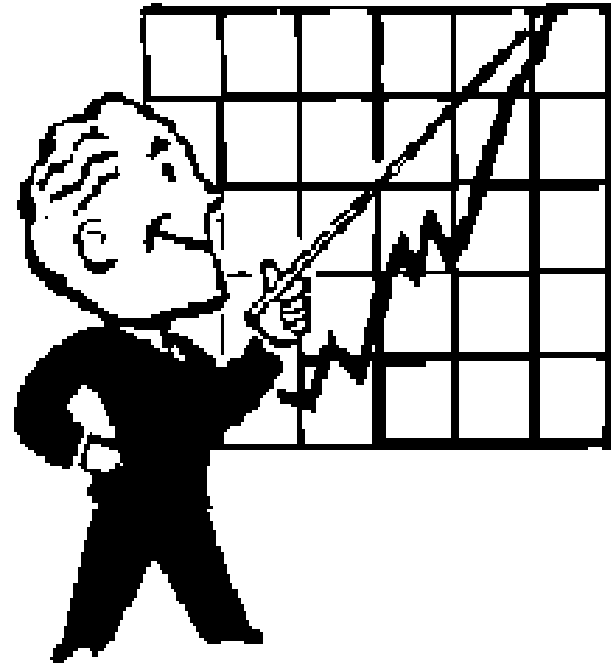
With changes in *FBN1* protein, TGF-beta1 concentration of active form becomes elevated in blood

Milewicz DM, Michael K, Fisher N, Coselli JS, Markello T and Biddinger A. (1996) Fibrillin-1 (*FBN1*) mutations in patients with thoracic aortic aneurysms. *Circulation* 94:2708-2711



Interaction of FBN1 and TGFβ1 mechanisms in molecular level





A purpose of the research

To check a hypothesis-

does the correlation between *FBN1* SNP's (rs2118181, rs1059177) and sporadic DPAA formation exist with elevated concentration of TGF- beta1 in blood plasma

Materials and methods

1. A sample of patients, operated due to the DPAA conditions, $n= 312$.

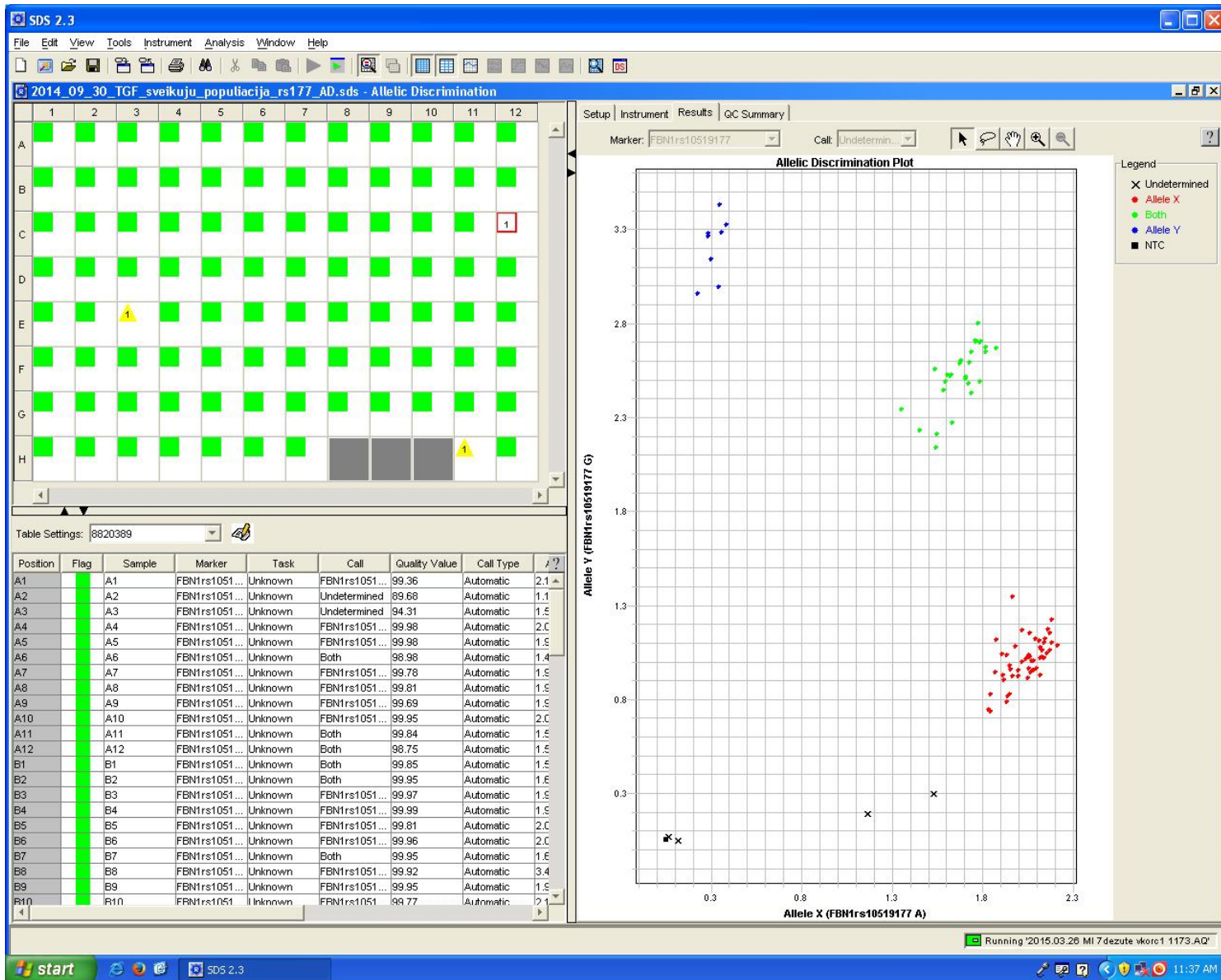
2. A sample of Reference group, $n=472$, consisted from random Lithuanian population, collected during epidemiologic studies.

3. A sample from random clusters, $n=269$, consisted from Kaunas city population, without complains of CVD, except AH.

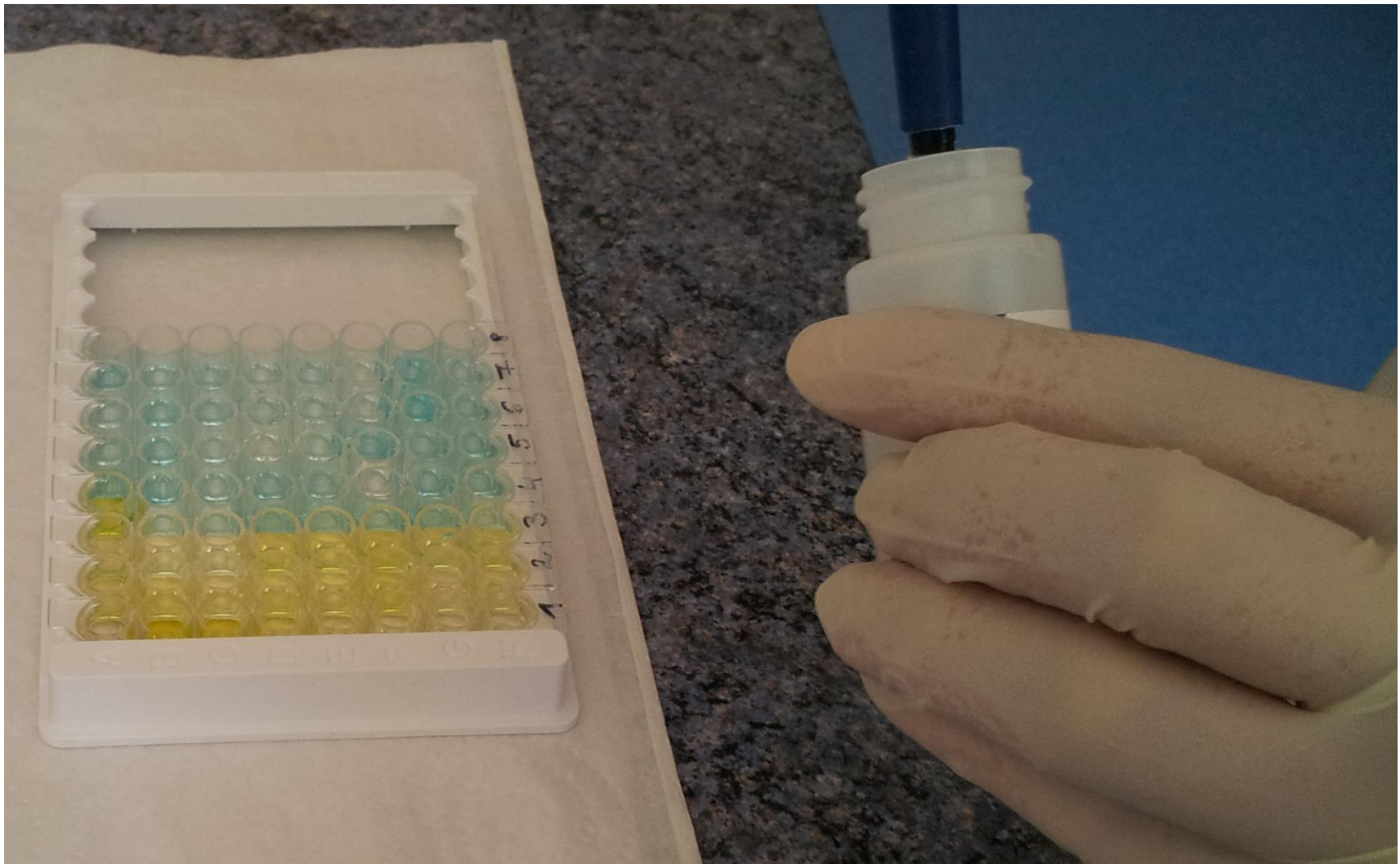




Blood samples from *cubital vein* were collected in two tubes with EDTA conservating material. Blood plasma was separated and freezed at -20°C



Genomic DNA, extracted from blood, and genotyping analysis was carried out using the real-time polymerase chain reaction (method (7900HT Applied Biosystem, USA).



Plasma was obtained within 2 hours by spinning for 15 min at 2500 rpm, aliquoted and stored frozen at -20°C. Plasma samples were tested in duplicate using eBioscience Platinum (Bender Med Systems GmbH, Austria) human TGF- β 1 ELISA kit based on standard sandwich enzyme-linked immune-sorbent assay technology according to manufacturers' instructions .

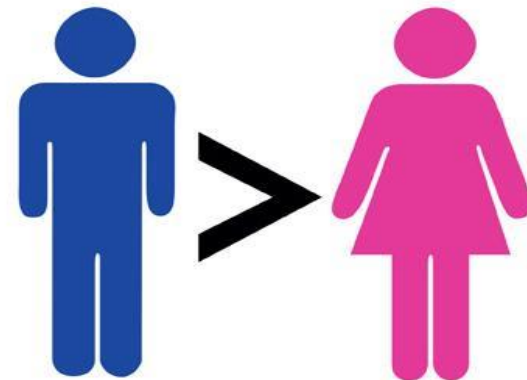
Statistics done by SPSS package(version 16.0) with

- non-parametric Kruskal-Wallis test,
- two-tailed Spearman's 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- β 1 values.



Distribution of samples between Reference group and Patients with DPAA according to the age and gender

	<i>Aneurysms</i> <i>n = 160</i>	<i>Postenotic dilatation</i> <i>n = 79</i>	<i>Stanford A dissection</i> <i>n = 73</i>	<i>Total</i> <i>n=312</i>	<i>Reference group</i> <i>n = 472</i>
<i>Age (years)</i> Median (range)	60 (18-83)	65 (27-81)	62 (24-84)	62 (18-84)	61 (25-83)
<i>Gender</i> Males, n (%)	135 (84.4)	57 (72.2)	54 (74.0)	246 (78.8)	372 (78.8)



Genotyping results for subgroups tested (1)

<i>Investigated group, n</i>	<i>Genotype</i>	<i>Genotype frequency, n (%)</i>	
		<i>rs2118181</i>	<i>rs10519177</i>
Aneurysms n = 160 p	AA	112, (70.4)	83, (52.5)
	GA	42, (26.4)	61, (38.6)
	GG	5, (3.2)	14, (8.9)
		0.019	0.254
Postenotic dilatations n = 79 p	AA	56, (74.7)	47, (59.5)
	GA	17, (22.7)	26, (32.9)
	GG	2, (2.6)	6, (7.6)
		0.362	0.657
Stanford A dissections n = 73 p	AA	44, (60.3)	28, (39.4)
	GA	24, (32.9)	29, (40.9)
	GG	5, (6.8) ⁺	14, (19.7) ⁺⁺
		<0.001	<0.001
Reference group n = 472	AA	378, (80.1)	272, (57.6)
	GA	89, (18.9)	174, (36.9)
	GG	5, (1.0)	26, (5.5)

Lesauskaite V, Sepetiene R, Jariene G, Patamsyte V, Zukovas G *et al.* (2015) FBN1 polymorphisms in patients with the dilatative pathology of the ascending thoracic aorta. *Eur J Cardiothorac Surg.* 47:e124-30.

Genotyping results for subgroups tested (2)

<i>SNP</i>	<i>Aneurysms</i>		<i>Postenotic dilatations</i>		<i>Dissections (Stanford A)</i>		<i>Reference group</i>	
	<i>MAF</i>	<i>OR (CI, 95%)</i>	<i>MAF</i>	<i>OR (CI, 95%)</i>	<i>MAF</i>	<i>OR (CI, 95%)</i>	<i>MAF</i>	<i>OR (CI, 95%)</i>
<i>rs2118181</i>	0.16	1.67 (1.61-2.40) p=0.005	0.14	1.39 (0.84-2.31) p=0.20	0.23	2.59 (1.67-4.01) p<0.001	0.10	1.00 - -
<i>rs10519177</i>	0.28	1.25 (0.94-1.66) p=0.13	0.24	1.01 (0.68-1.49) p=0.98	0.40	2.13 (1.48-3.08) p<0.001	0.24	1.00 - -

MAF-Minor allel frequency

OR- odd ratio

CI- Confidence interval

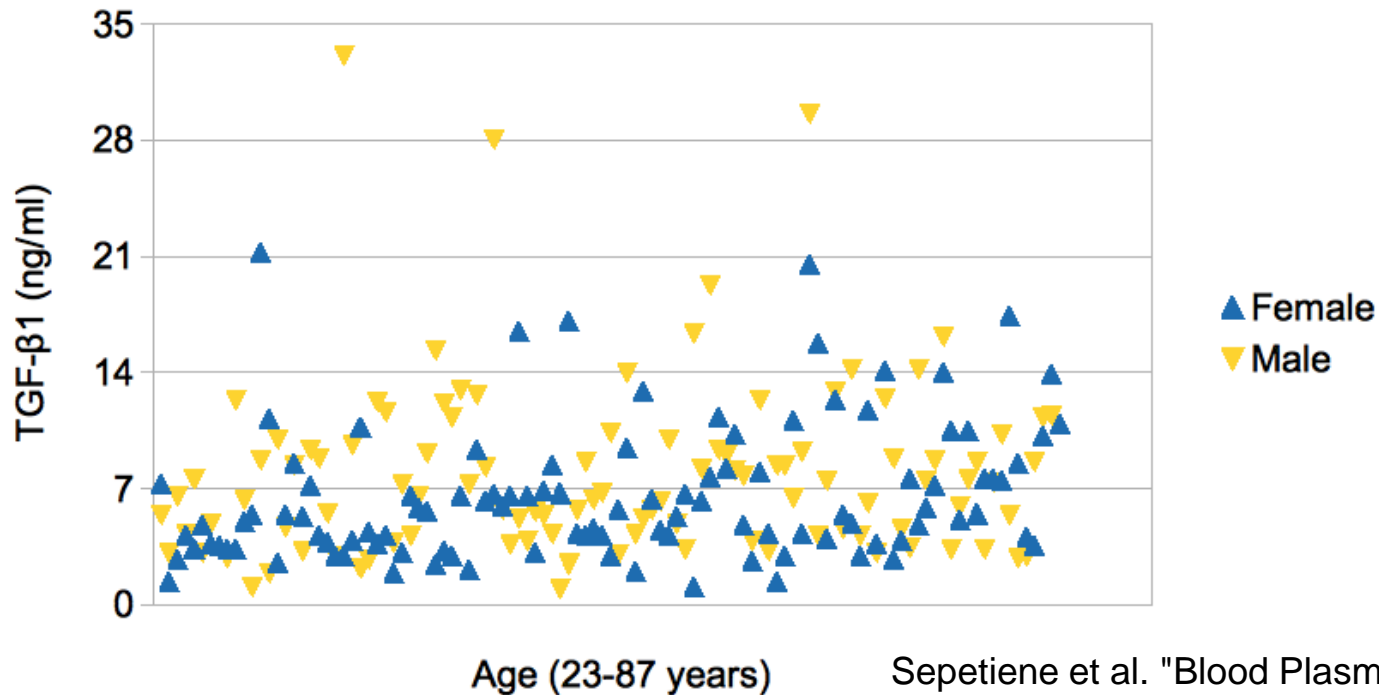
Lesauskaite V, Sepetiene R, Jariene G, Patamsyte V, Zukovas G *et al.* (2015) FBN1 polymorphisms in patients with the dilatative pathology of the ascending thoracic aorta. *Eur J Cardiothorac Surg.* 47:e124-30.

Concentration of TGF- β 1 ng/ml for DPAA and Reference group

TGF- β 1	Aneurysms n=35	Postenotic dilatation, n=21	Stanford A dissections, n=6	Total n=62	Reference group, n=212
Median (range) ng/ml	7.8 (2.3-25.3)	7.7 (2.1-22.0)	10.1 (6.58-17.6)	7.7 (2.1-5.3)	6.2 (1.0-33.1)

Sepetiene, Ramune, et al. "Blood Plasma TGF- β 1 Concentration in Sporadic Dilatative Pathology of Ascending Aorta: More Questions than Answers." *PloS one* 10.6 (2015): e0129353.

TGF β -1 concentration in plasma for males (mediana 8,32 ng/ml) was significantly ($p=0,001$) different from females (mediana 5,81 ng/ml) and was growing up depending on age ($p<0,001$)



Sepetiene et al. "Blood Plasma TGF- β 1 Concentration in Sporadic Dilatative Pathology of Ascending Aorta: More Questions than Answers." *PloS one* 10.6 (2015) e0129353.

Association between *FBNI* SNPs genotypes and TGF- β 1 concentration

<i>FBNI</i> SNP genotype	Genotype frequency n (%)	TGF- β 1 concentration (ng/ml) min/ med/ max
rs2118181		
AA	198 (72.0)	1.00/ 6.57 / 33.12
AG	64 (23.3)	2.71/ 7.81 / 28.08 ^a
GG	13 (4.7)	1.40/ 9.78 / 17.28 ^b
rs10519177		
AA	144 (52.4)	1.00/ 6.40 / 33.12 ^c
AG	73 (26.5)	1.90/ 5.46 / 28.08 ^d
GG	58 (21.1)	1.40/ 10.48 / 27.29

People with rs2118181 AA genotype had TGF- β 1 concentration with median of 6,57 ng/ml, one minor allele **G rised TGF- β 1 median of concentration by 1,97 ng/ml, **GG** genotype -3,21 ng/ml**

Higher TGF- β 1 concentration median was detected for people with **rs10519177 GG** genotype, comparing with **AA** genotype (10,48ng/ml and 6,40 ng/ml with $p < 0,0001$, respectively).

One rs10519177 minor allele G had no effect on TGF- β 1 concentration.

a, TGF- β 1 concentration, in compare rs2118181 AG and AA genotypes, $p=0.024$; Sepetiene, et al. "Association between Fibrillin1 Polymorphisms (rs2118181, rs10519177) and TGF β 1 Concentration in Human Plasma." *Molecular Medicine* 21.1 (2015): 735.
b, TGF- β 1 concentration, in compare rs2118181 GG and AA genotypes, $p=0.094$;
c, TGF- β 1 concentration, in compare rs10519177 AA and GG genotypes, $p < 0.0001$;
d, TGF- β 1 concentration, in compare rs10519177 AG and GG genotypes, $p < 0.0001$;

Multiple linear regression for different models of *FBN1* rs2118181 and rs10519177

<i>FBN1</i> SNP	Dependent variables	Unstandardised Coefficients B	p value	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	
rs10519177	Constant	1.035	<0.001	0.102
	Gender (male)	0.181	0.018	
	Age	0.012	<0.001	
	Allele G	0.172	0.010	
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	

Elevation of TGF-β1 concentration with statistically confident manner is effected by **male gender, older age and rs2118181 G allele**

When *FBN1* rs2118181 was replaced by rs10519177 in this modeling, TGF-β1 concentration with statistically confident manner is effected by **male gender, older age and rs10519177 GG genotype**

Sepetiene et al. "Association between Fibrillin1 Polymorphisms (rs2118181, rs10519177) and Transforming Growth Factor β1 Concentration in Human Plasma." *Molecular Medicine* 21.1 (2015): 735.

Conclusions



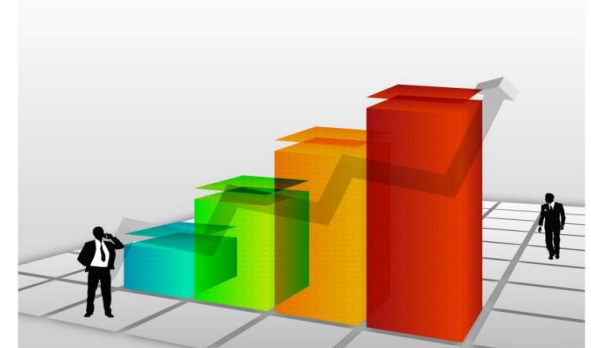
FBN1 SNP rs2118181 and rs10519177 may rise a risk for dissection of ascending aorta, with **OR 2.59 (CI 1.67-4.01), OR 2.13 (1.48-3.08)**, respectively



FBN1 SNP rs10519177 rises a risk for aneurysms of ascending aorta formation, with **OR 1.67 (CI 1.61-2.40)**



FBN1 SNP rs2118181 minor allele or a pair of minor alleles for rs1059177 elevate the **TGF- β 1** concentration in blood plasma, but the mechanism of this correlation is still unknown



A Genotype determination of *FBN1* SNPs rs2118181 rs10519177 and detection of TGF-β1 concentration in blood plasma may be the potential biomarkers in clinical practice, for early diagnostics establish a risk for DPAA formation, to avoid a development of complications.



UNCORRECTED PROOF

Surgical Treatment

Tadashi INOUE, Koza KAWA, Yoshiya ISHIKURA, Yasuki

Abstract: For 93 cases of thoracic aneurysms, the over-all operative mortality rate was 24.7 per cent, recent advances in surgical treatment so that since 1975 no death occurred. Cardiopulmonary bypass with or without temporary external bypass were used.

Keywords: aortic aneurysm, dissecting aneurysm, graft replacement, temporary external bypass.

Association between Transforming Growth Factor-β1 Concentration and Aortic Dissection

Ramune Sepetiene¹, Zita Stanioniene¹, Rimas Sukeleliu¹, Rimas Sukeleliu², Ramune Sepetiene¹, Rimas Sukeleliu²

Abstract: The aim of this study was to determine the association between plasma TGF-β1 concentration and the risk of aortic dissection. We studied 100 patients with aortic dissection and 100 healthy controls. Plasma TGF-β1 concentration was significantly higher in patients with aortic dissection compared to healthy controls (p < 0.001).

Keywords: aortic dissection, TGF-β1, biomarkers.

Corresponding author: Ramune Sepetiene, Rimas Sukeleliu

Address: Institute of Cardiology of Lithuanian University of Health Sciences, Sukileliu 17, Kaunas, Lithuania

Phone: +370 612 31784

Journal: Japanese Journal of Surgery, Vol. 8, No. 10, October 1995

PLOS ONE

European Journal of Cardio-Thoracic Surgery Advance Access published January 12, 2015

ORIGINAL ARTICLE

***FBN1* polymorphisms in patients with the dilatative pathology of the ascending thoracic aorta**

Vaiva Lesauskaite*, Ramune Sepetiene, Giedre Jariene, Vaiva Patamsyte, Giedrius Zukovas, Ingrida Grabauskyte, Zita Stanioniene, Raimondas Sirmenis and Rimas Sukeleliu

Abstract

OBJECTIVES: To investigate polymorphisms of the fibrillin-1 (*FBN1*) gene (namely, rs2118181, rs1036477, rs10519177, rs747517, rs4774517) in a case-control study for dilatative pathology of the ascending thoracic aorta (DPATA) from Lithuanians.

METHODS: We studied 312 patients who had undergone aortic reconstructive surgery for DPATA. These patients were subdivided into three groups according to the phenotype of their DPATA: (i) ascending aortic aneurysm (n = 160), (ii) post-stenotic dilatation of the ascending aorta (n = 79) and (iii) Stanford A dissection (n = 73). The reference group (n = 472) was recruited from a sample screened within epidemiological studies of the Lithuanian population. *FBN1* polymorphisms were studied by real-time PCR using TaqMan probe and genotyping.

RESULTS: Patients within the aortic dissection sub-group had significantly higher minor allele frequencies in all five *FBN1* single nucleotide polymorphisms (SNPs) studied versus reference group subjects (P < 0.0001). Minor allele frequencies in SNPs rs2118181, rs1036477, rs10519177, rs747517, rs4774517 were significantly higher in those with aortic aneurysm when compared with the reference group (P < 0.007). Thus, minor alleles of *FBN1* polymorphisms were significantly associated with aortic dissection with odds ratios (ORs) 2.59–2.13, P < 0.001, while SNPs rs2118181, rs1036477 with an increased risk of ascending aortic aneurysm [OR 1.67, confidence interval (CI) 95% 1.61–2.40]. The association of *FBN1* polymorphisms with aortic dissection was assessed using logistic regression models adjusted for gender, age and hypertension. The best fit model for ascending aortic aneurysm sub-group (OR 1.70, 95% CI 1.17–2.46) or the Stanford A dissection sub-group (OR 2.64, CI 95% 1.66–4.19). A recessive model fitted best the association between rs10519177, rs747517, rs4774517 and Stanford A dissection (OR 4.31, CI 95% 2.06–9.01). There were no significant associations between *FBN1* polymorphisms and post-stenotic or bicuspid aortic dilatation.

CONCLUSIONS: Our study provides evidence for the following: (i) *FBN1* SNPs rs2118181, rs1036477, rs10519177, rs747517, rs4774517 may increase susceptibility to aortic dissections and (ii) *FBN1* SNPs rs2118181, rs1036477 to the formation of aortic aneurysms. These SNPs might be considered as biomarkers for identifying patients at risk for ascending aortic aneurysm and aortic dissection.

Keywords: Thoracic aorta • Aneurysm • Dissection • Fibrillin-1 • Polymorphisms

INTRODUCTION

The aetiology and pathogenesis of dilatative pathology within the ascending thoracic aorta (DPATA) remain under discussion. In most cases, the causes leading to such pathology of the aortic wall are unknown. Only in certain cases is the condition caused by aortic aneurysm or inherited as a single gene mutation. In these cases, the mutations are in the fibrillin (*FBN1*) genes (e.g. Marfan syndrome [1]; inherited collagen mutations (e.g. Ehlers-Danlos syndrome [2]); by mutations of the transforming growth factor-beta gene causing Loeys-Dietz syndrome [3] or by gene mutations [4]. Evidence has shown that *FBN1* mutations predispose one to DPATA in the absence of phenotypic characteristics of Marfan syndrome [5, 6]. Recently, published data from genome-wide association study (GWAS) identified novel associations of *FBN1* SNPs at chromosome 15q21.1: namely, rs10519177, rs2118181, rs10519177, rs7474517, rs755251, with sporadic aortic dissection [7]. These data extend knowledge of the molecular path leading to sporadic thoracic disease, and may connect it to the model of Marfan syndrome.

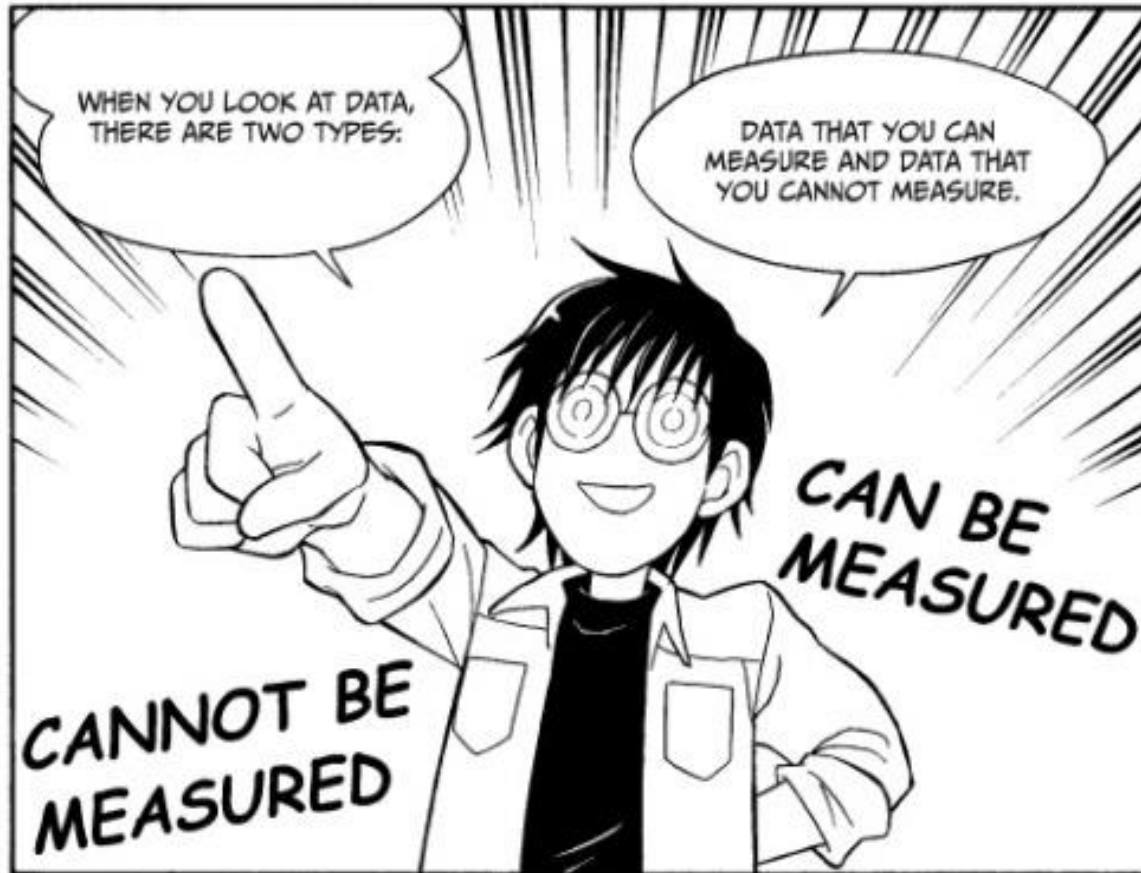
Introduction

Marfan syndrome was first discovered by J. Marfan in 1896 [1]. The genetic defect was discovered by V. V. Blot in 1949 [2]. The gene for Marfan syndrome was identified by G. S. Pastan et al. in 1990 [3]. The gene for Marfan syndrome is located on chromosome 15q21.1 [4]. The gene for Marfan syndrome is the fibrillin-1 (*FBN1*) gene [5]. The fibrillin-1 gene is a large gene with 33 exons and 32 introns. The fibrillin-1 gene is located on chromosome 15q21.1 [6]. The fibrillin-1 gene is a large gene with 33 exons and 32 introns. The fibrillin-1 gene is located on chromosome 15q21.1 [6].

References

- Lesauskaite, Vaiva, et al. FBN1 polymorphisms in patients with the dilatative pathology of the ascending thoracic aorta, 2015, *European Journal of Cardio-Thoracic Surgery*: ezu520
- Allaire E et al, New insight in aetiopathogenesis of aortic diseases, 2009, *Eur J Vasc Endovasc Surg* 37, 531-537
- Kaartinen V, Warburton D, Fibrillin controls TGF- β activation, 2003, *Nat Genet* 33, 644-647
- Jones J A et al, Transforming Growth Factor- β signaling in thoracic aortic aneurysm development: a paradox in Pathogenesis, 2009, *J Vasc Research* 46, 119-137
- Pannu H et al, Genetic basis of thoracic aortic aneurysms and aortic dissection, 2005, *American J Med Genet* 139, 10-16
- Toma I et al, Transforming growth factor- β and atherosclerosis: interwoven atherogenic and atheroprotective aspects, 2012, *Cell Tissue Res* 347, 155-175
- Pardali E et al, Signaling by members of the TGF- β family in vascular morphogenesis and disease, 2010, *Trends in cell Biol* 20, 556-567
- Lesauskaite V, Dilatative pathology of ascending aorta, a new attitude, Oral presentation , 2012.

Thank you!



From "Manga Guide to Statistics", Shin Takahashi, 2008

Thank's to our team for the all hard work!



Head of Laboratory of
Molecular Cardiology,
Prof. habil. dr. Vaiva
Lesauskaite



Prof. habil.dr. Jolanta
Justina Vaskelyte



Prof. habil.dr. Rimantas
Benetis



Prof. habil.dr. Abdonas
Tamosiunas



PhD student, MD
Giedrius Zukovas



PhD student Vaiva
Patamsyte



Dr. Giedre Jariene



Dr. Giedre Marsalkiene