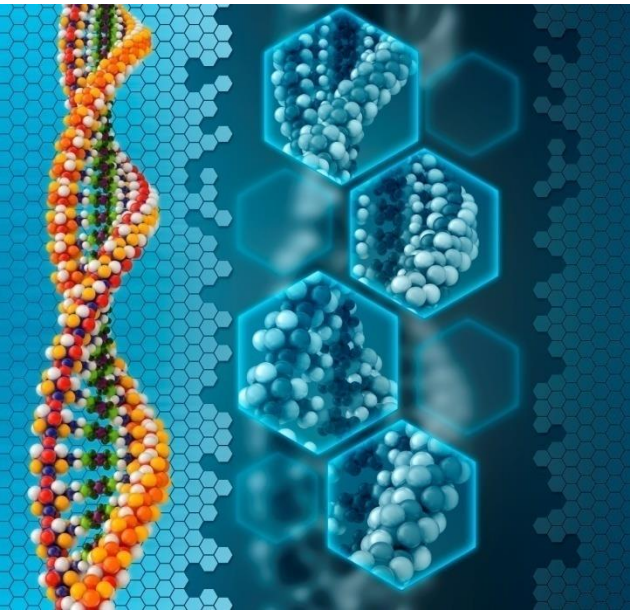
LITHUANIAN UNIVERSITY OF HEALTH SCIENCES

ASSOCIATION BETWEEN *FBN1 POLYMORPHISMS AND TGF- ß1 CONCENTRATION WITHIN ANEURYSMS AND DISSECTIONS OF ASCENDING THORACIC AORTA*



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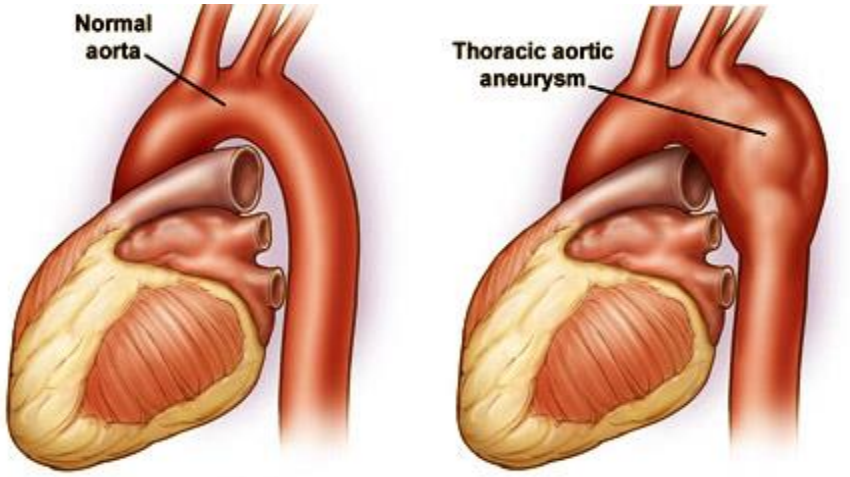
26-27 October, Chicago

- 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.

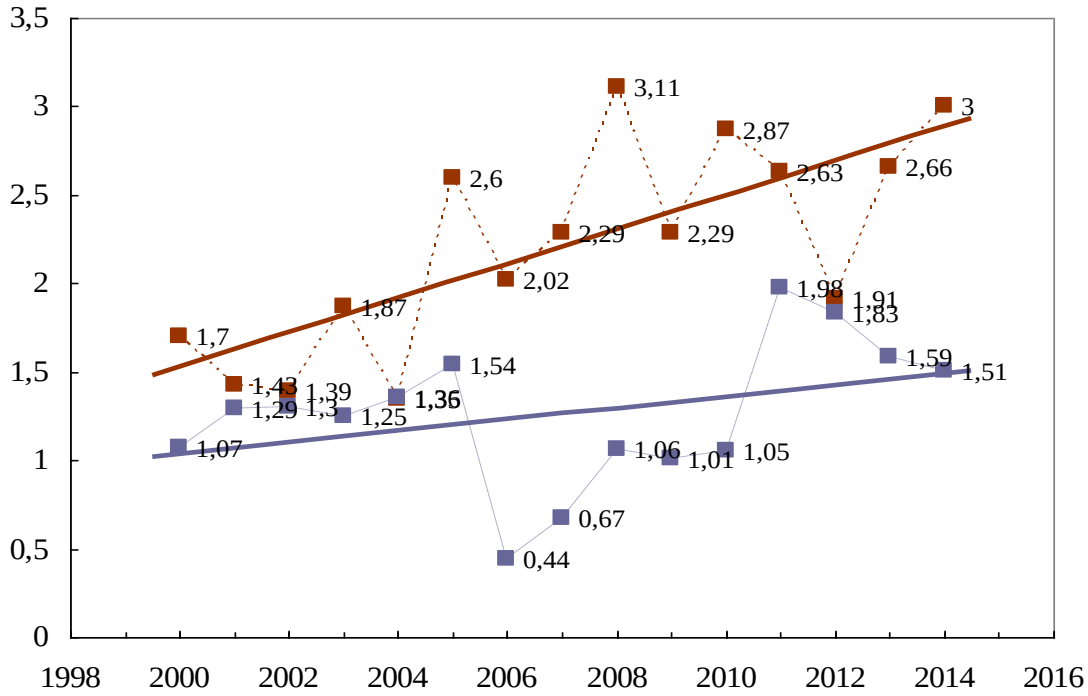
- DPAA takes **14th** place according to the reasons of mortality among 55 years people and older (National Centre of Health Statistics in USA, 2012)



- 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

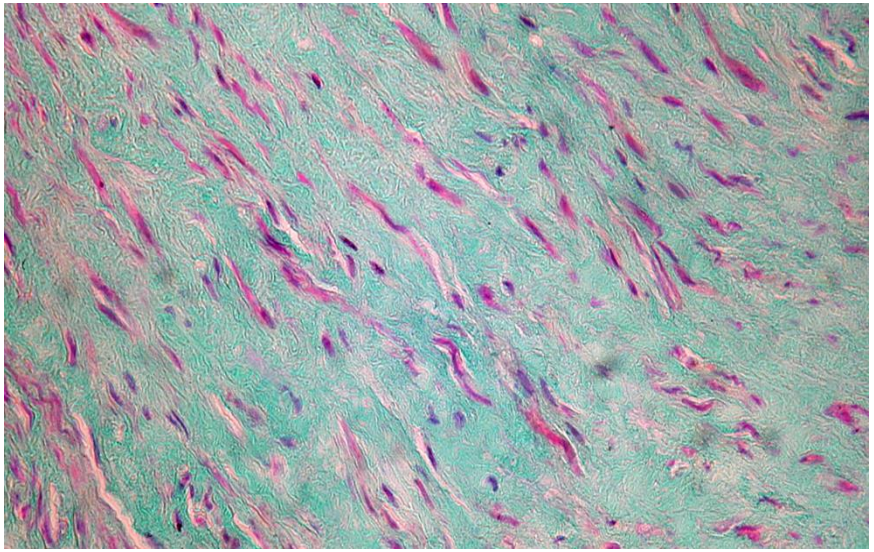
Morphogenesis of aneurysm

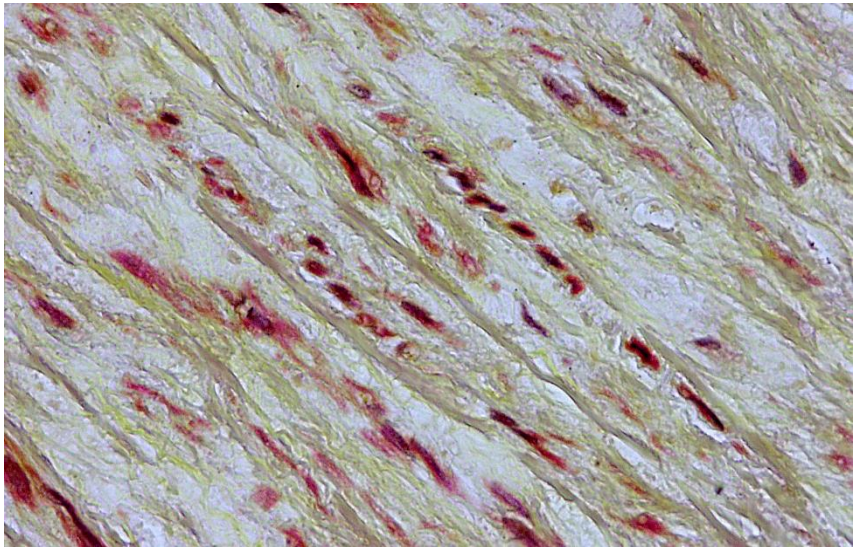
- A contractive phenotype of smooth muscle cells, responsable for vascular resistance, to be conversed to synthetic phenotype.

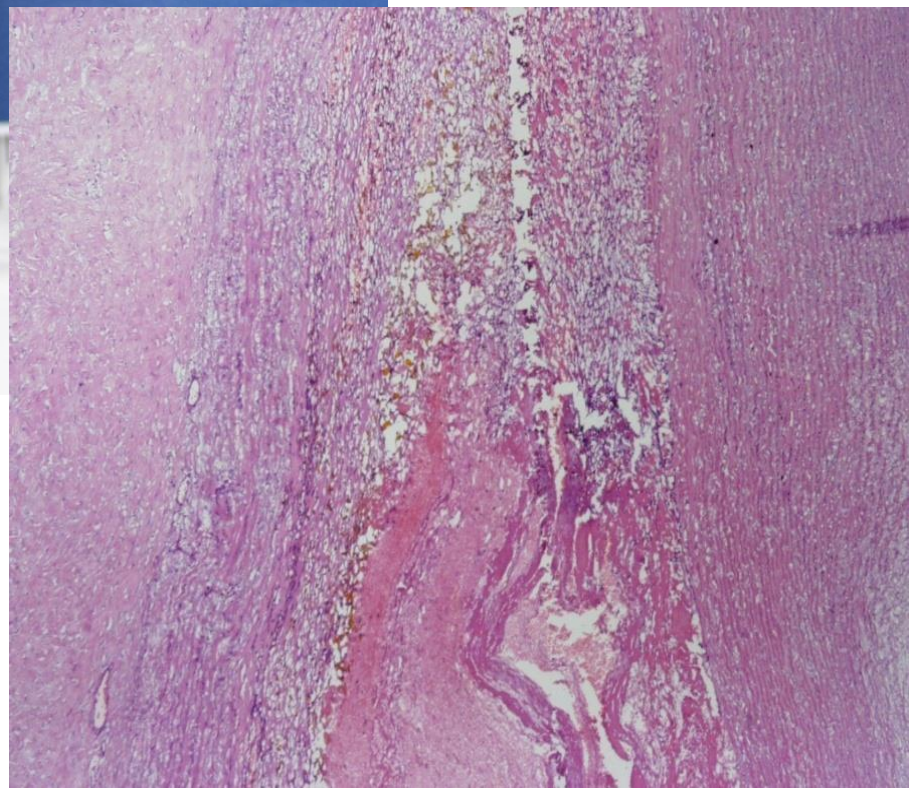
  (Lesauskaite et al "Smooth muscle cells of the media in the dilatative pathology of ascending thoracic aorta: morphology, immunoreactivity for osteopontin, matrix metalloproteinases, and their inhibitors." *Human pathology* 32.9 (2001): 1003-1011)

- 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*-1gene

Elevated concentration of TGF- β1 in blood is found for patients with diagnose of Marfan syndrome (MFS), determined by mutations of *Fibrillin-1 (FBN1) gene*.

El Greco (Domenikos Theotokopoulos) (1541–1614)

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

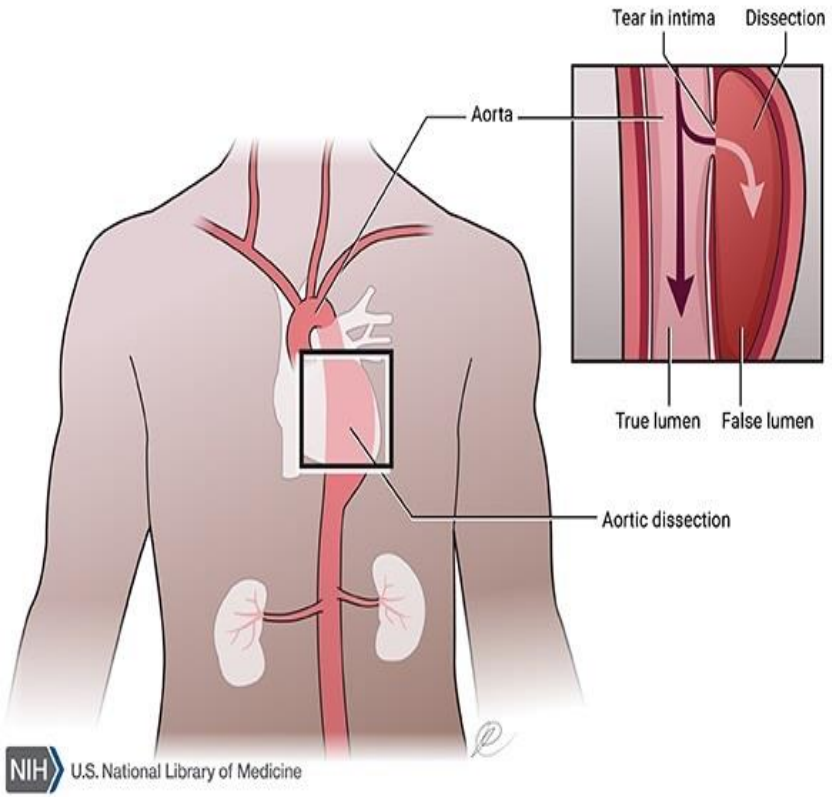
Chaudhry SS, et al (2007) Fibrillin-1 regulates the bioavailability of TGFβ1. J Cell Biol. 176:355-367 .

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, MarkelloT and Biddinger A. (1996) Fibrillin-1 (FBN1) mutations in patients with thoracic aortic aneurysms. *Circulation* 94:2708-2711

Interaction of FBN1 and TGFß1 mechanisms in molecular level

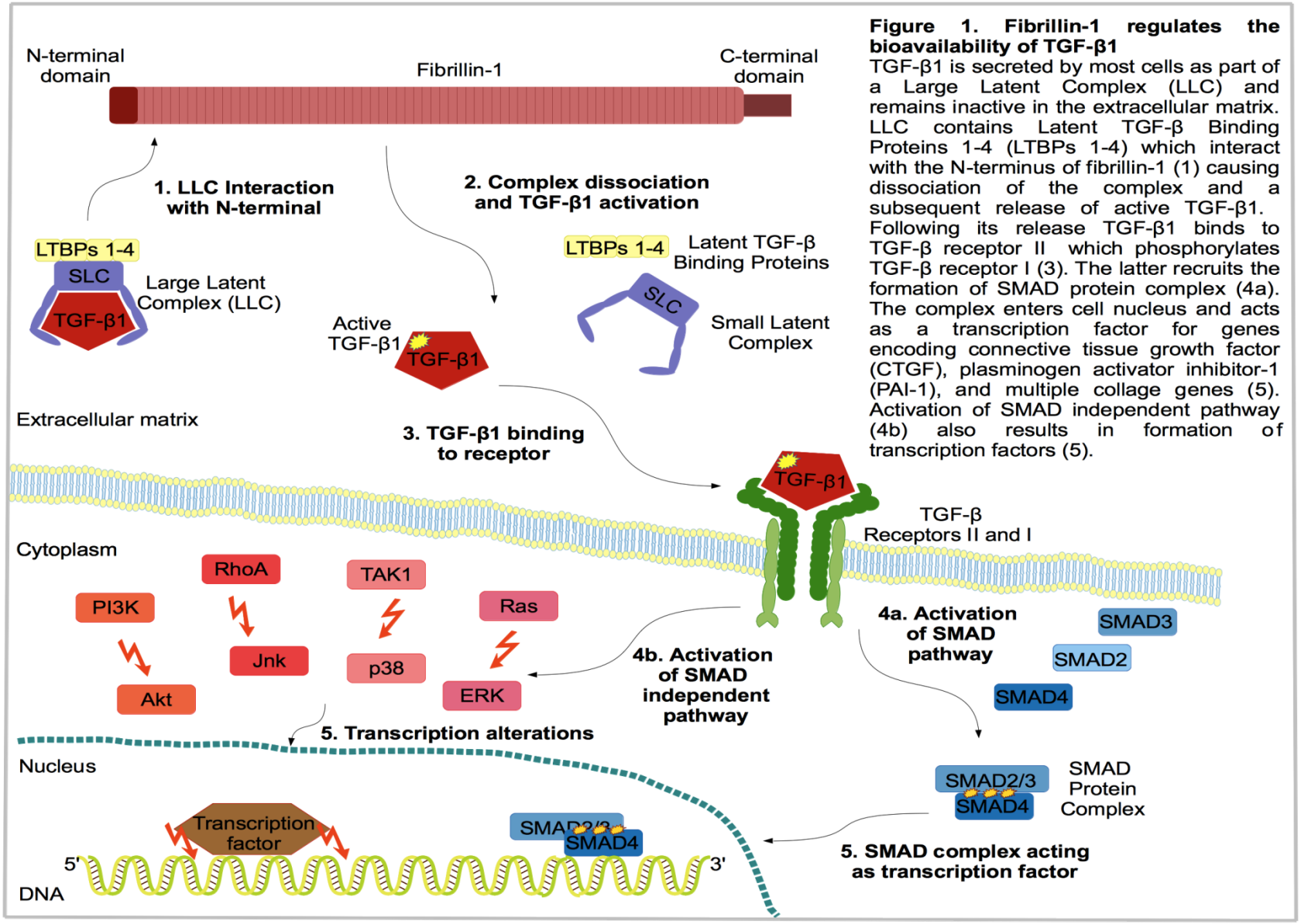
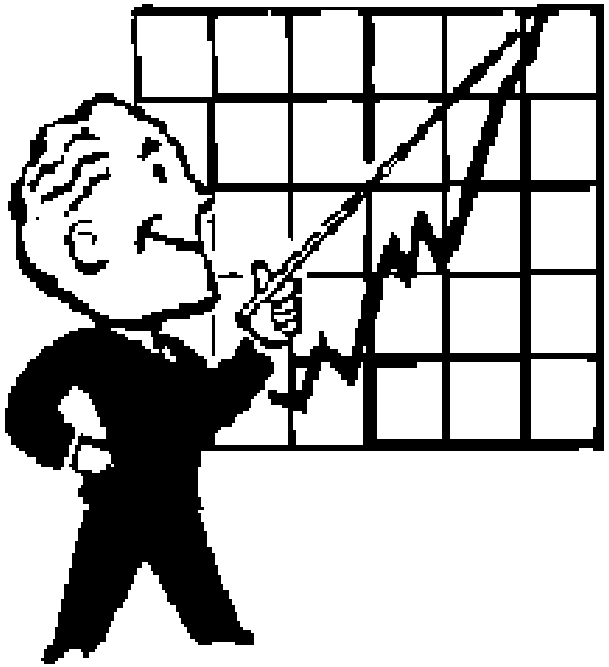


Figure 1. Fibrillin-1 regulates the bioavailability of TGF-β1
TGF-β1 is secreted by most cells as part of a Large Latent Complex (LLC) and remains inactive in the extracellular matrix. LLC contains Latent TGF-β Binding Proteins 1-4 (LTBPs 1-4) which interact with the N-terminus of fibrillin-1 (1) causing dissociation of the complex and a subsequent release of active TGF-β1. Following its release TGF-β1 binds to TGF-β receptor II which phosphorylates TGF-β receptor I (3). The latter recruits the formation of SMAD protein complex (4a). The complex enters cell nucleus and acts as a transcription factor for genes encoding connective tissue growth factor (CTGF), plasminogen activator inhibitor-1 (PAI-1), and multiple collage genes (5). Activation of SMAD independent pathway (4b) also results in formation of transcription factors (5).



A purpose of the research

To chech a hypothesis-

does the correlation between *FBN1* SNP's (rs2118181, rs1059177) and sporadic DPAA formation exist with elevated concentration of TGF- beta1in blood plasma

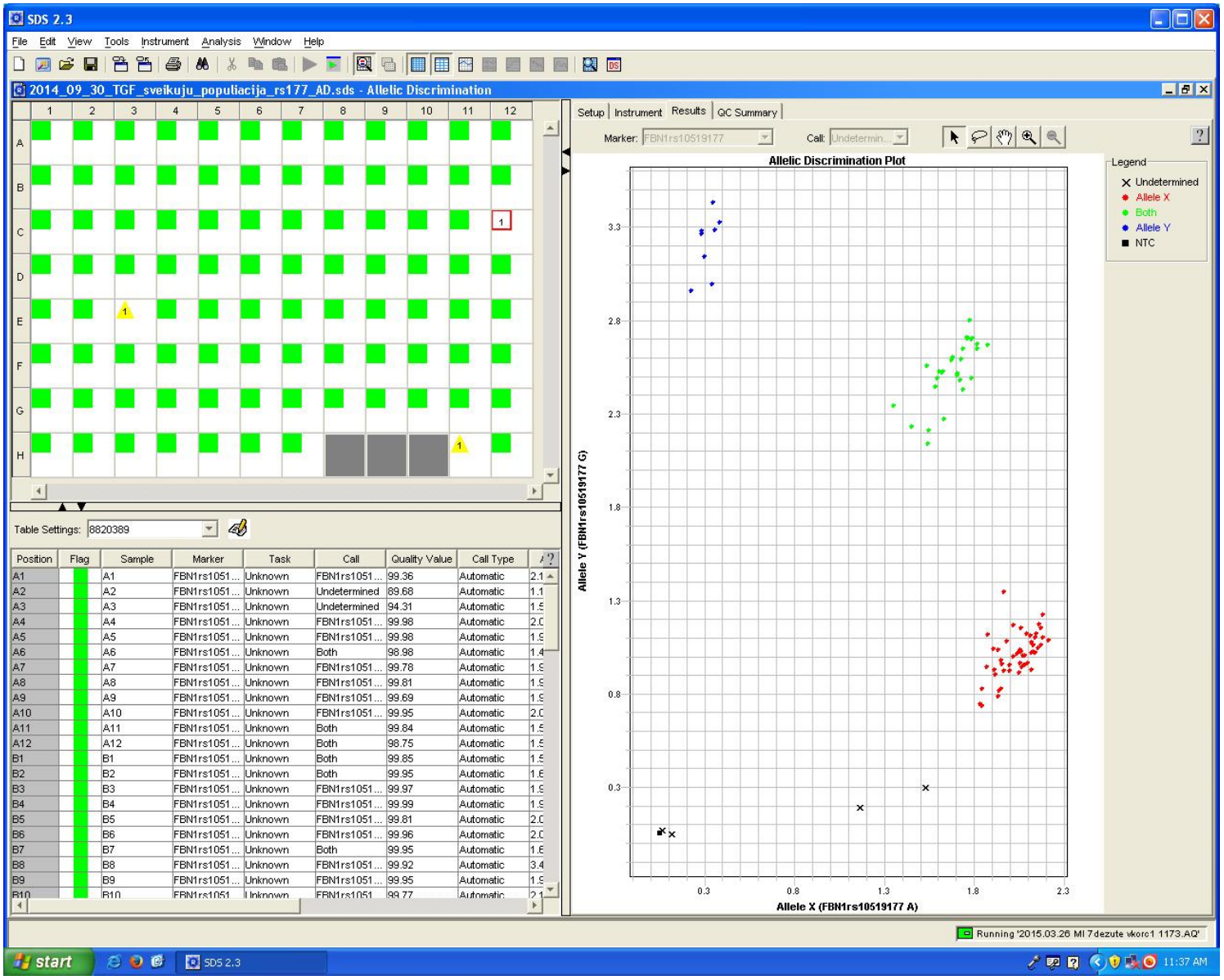
Materials and methods

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

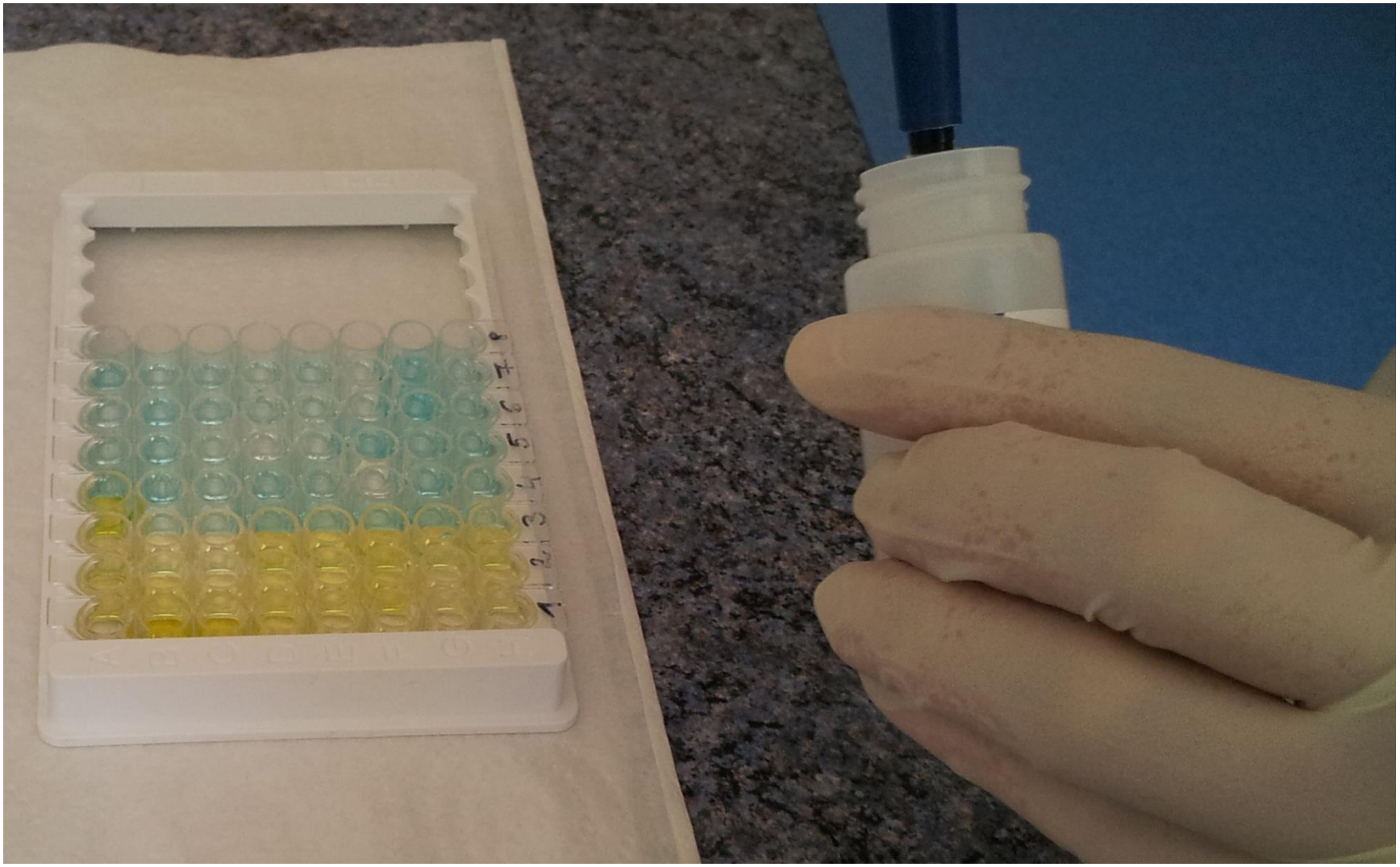
2. A sample of Reference group, **n=472**, consisted from random Lithuanian population, collected during epidemiologic studies.

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).

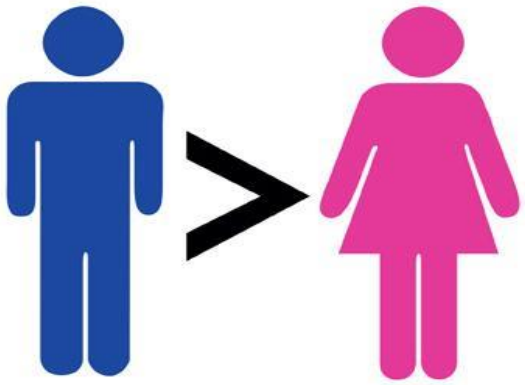


Plasma was obtained within 2 hours by spinning for 15 min at 2500 rpm, aliquoted and stored frozen at -20°C. Plasma samples were tested in duplicate using eBioscience Platinum (Bender Med Systems GmbH, Austria) human TGF-β1 ELISA kit based on standard sandwich enzyme-linked immune-sorbent assay technology 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-β1 values.

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

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



Genotyping results for subgroups tested (1)

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

SNP	*Aneurysms*		*Postenotic dilatations*		*Dissections (Stanford A)*		*Reference group*	
	MAF	*OR (CI, 95%)*	*MAF*	*OR (CI, 95%)*	*MAF*	*OR (CI, 95%)*	*MAF*	*OR (CI, 95%)*
rs2118181	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 - -
rs10519177	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 alell frequency OR- odd ratio CI- Confidence interval

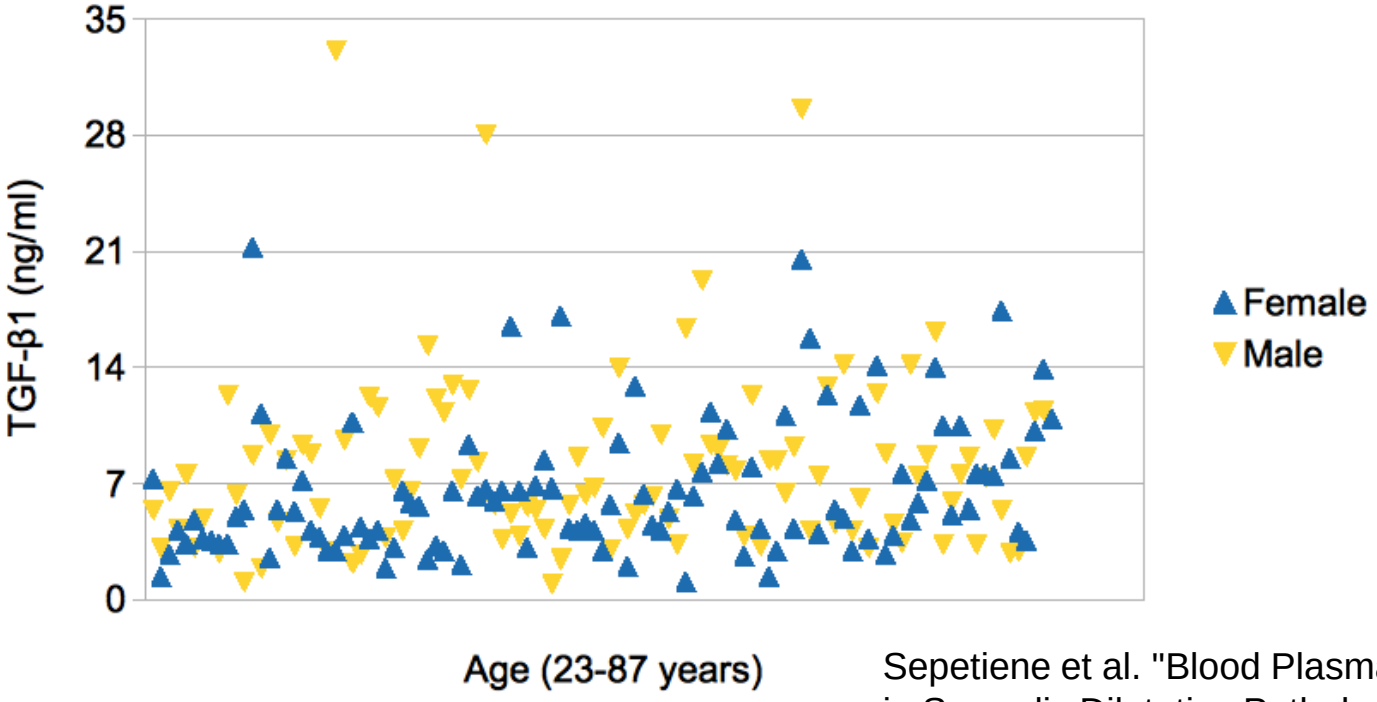
Lesauskaite V, Sepetiene R, Jariene G, Patamsyte V, Zukovas G *et al*. (2015) FBN1 polymorphisms in patients with the dilatative pathology of the ascending thoracic aorta. Eur J Cardiothorac Surg. 47:e124-30.

Concentration of TGF-β1 ng/ml for DPAA and Reference group

TGF-β1	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-β1 Concentration in Sporadic Dilatative Pathology of Ascending Aorta: More Questions than Answers." *PloS one* 10.6 (2015): e0129353.

TGFß-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-β1 Concentration in Sporadic Dilatative Pathology of Ascending Aorta: More Questions than Answers." *PloS one* 10.6 (2015): e0129353.

Association between *FBN1* SNPs genotypes and TGF-β1 concentration

***FBN1* SNP genotype**	**Genotype frequency n (%)**	**TGF-β1 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-β1 concentration with median of 6,57 ng/ml,
one minor allele **G** rised TGF-β1 median of concentration by 1,97 ng/ml, **GG** genotype -3,21 ng/ml

Higher TGF-β1 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 alelle **G** had no effect on TGF-β1 concentration.

a, TGF-β1 concentration, in compare rs2118181 AG and AA genotypes, p=0.024;
b, TGF-β1 concentration, in compare rs2118181 GG and AA genotypes, p=0.094;
c, TGF-β1 concentration, in compare rs10519177 AA and GG genotypes, p<0.0001;
d, TGF-β1 concentration, in compare rs10519177 AG and GG genotypes, p<0.0001;

Sepetiene,et al. "Association between Fibrillin1 Polymorphisms (rs2118181, rs10519177) and TGFβ1 Concentration in Human Plasma. " *Molecular Medicine* 21.1 (2015): 735.

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

***FBN1* 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	
	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 mannier 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 mannier is effected by **male gender**, **older age and rs10519177 GG** genotype

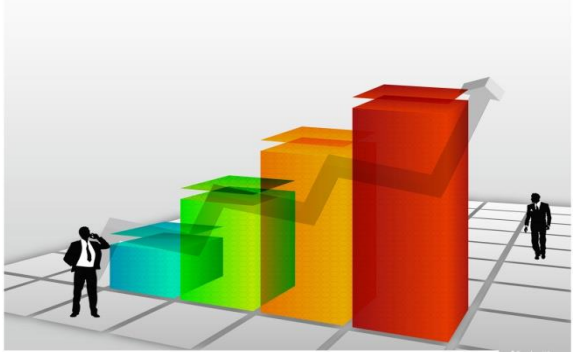
Sepetiene et al. "Association between Fibrillin1 Polymorphisms (rs2118181, rs10519177) and Transforming Growth Factor β1 Concentration in Human Plasma." *Molecular Medicine* 21.1 (2015): 735.

Conclusions

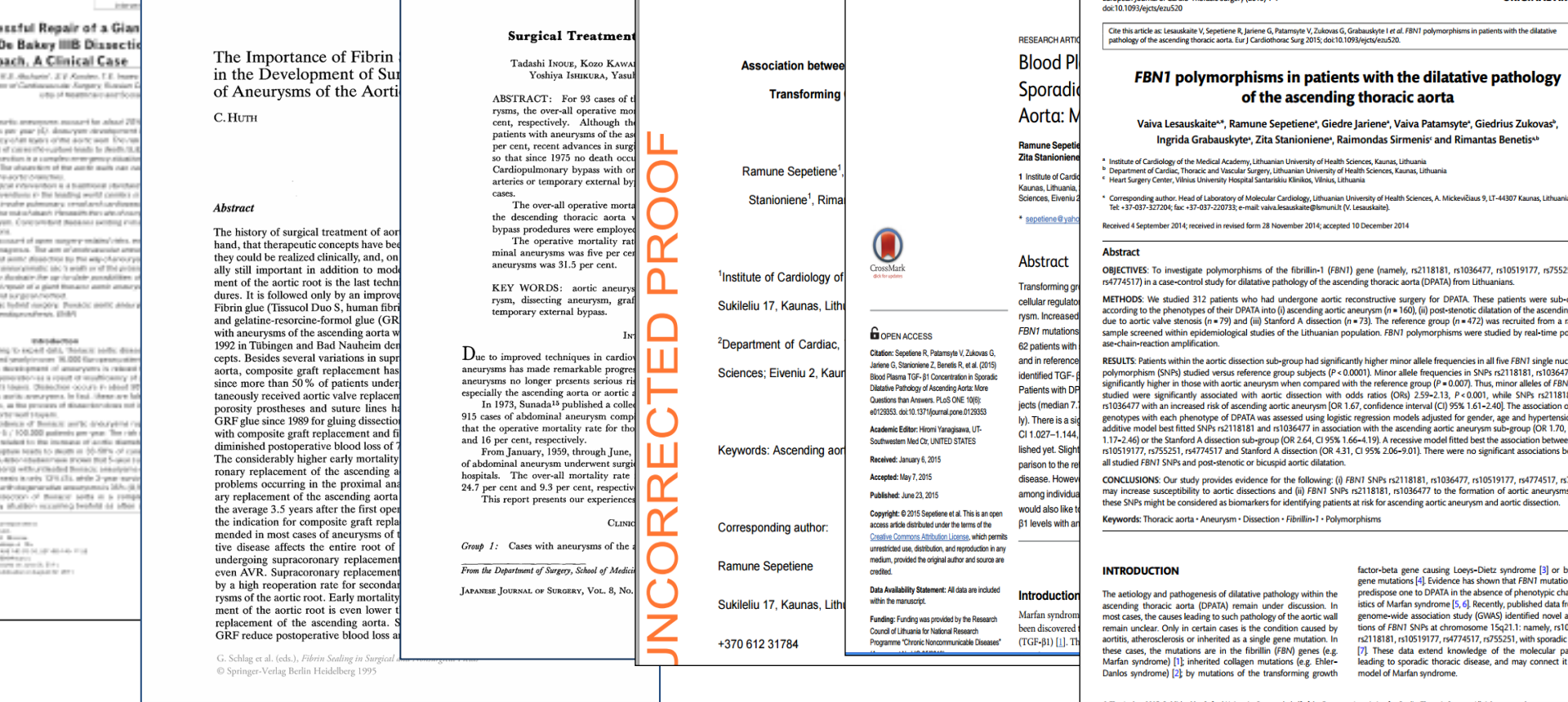
- *FBN1* SNP rs2118181 and rs10519177 may rise a risk for dissection of ascending aorta, with **OR 2.59 (CI 1.67-4.01), OR2.13 (1.48-3.08),** respectively

- *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 alelle or a pair of minor alleles for rs1059177 elevate the **TGF- β1** 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.



References

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- **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**
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- **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**
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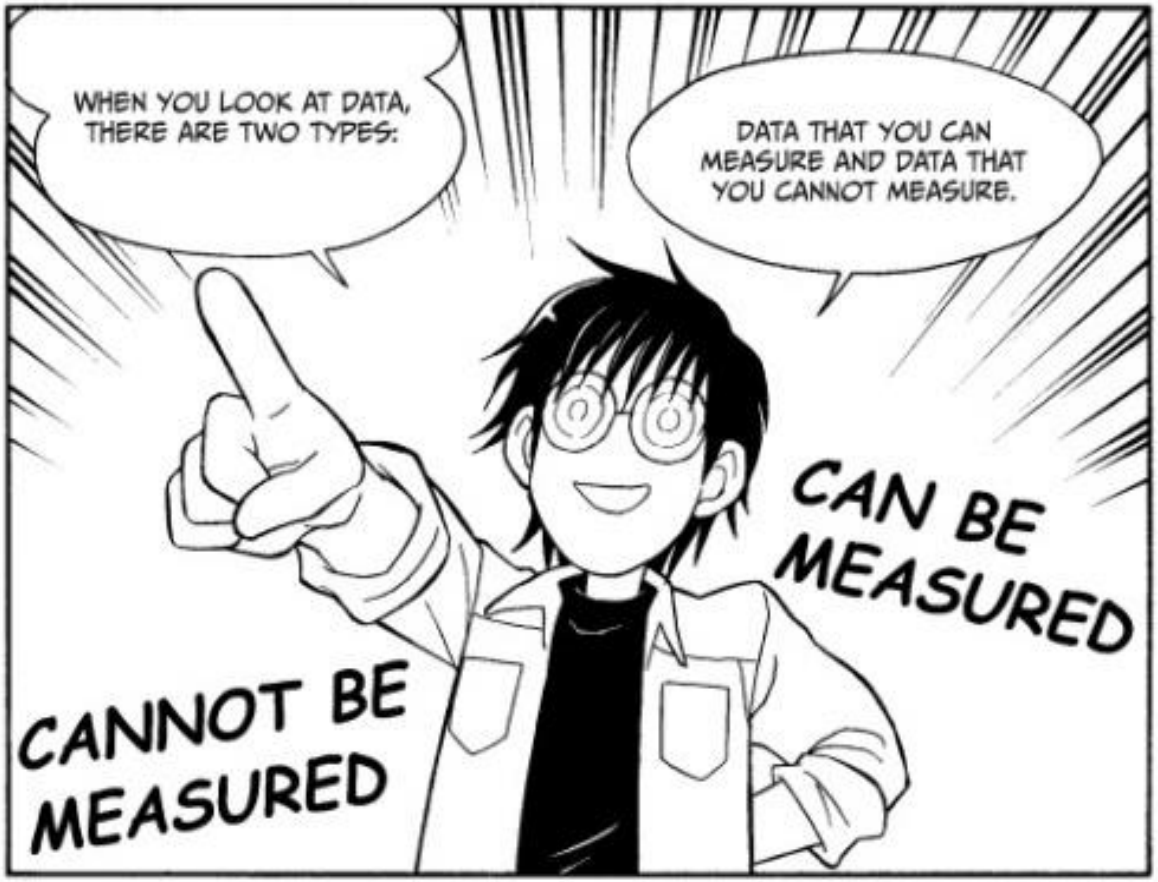
Thank you!



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

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

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