

Mutation Screening of FBN1 Gene in Patients with Ascending Aortic Dilatation

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INTRODUCTION

Mutations in the FBN1 gene are known causes of Marfan Syndrome (MFS) and related disorders. In our study we suggest that genetic variation in the genes encoding specific proteins constituting aortic wall and regulating the turnover of the extracellular matrix (e.g. FBN1, TGFB1 and TGFB2) are likely to influence properties of elastic fibres. In the Czech Republic there are about 2500 of operations for structural aortic valve disease (stenosis or regurgitations) per year. These diseases are associated with valvular hemodynamic abnormality, which could be the contributory factor for developing **ascending aortic dilatation**.

CLINICAL OBSERVATION

About 10 % of patients operated for aortic valve disease suffer simultaneously from **ascending aortic dilatation (AAD)**. Cause and progression of the aortic valve disorder is considered to be the contributory factor for dilatation of primarily changed aortic wall of ascending aorta.

Patients are at risk of fatal complications like dissection or rupture of aortic wall. The risk correlates with diameter of dilatation of ascending aorta. If dissection is not cured it would end in 90 % in death in one year.

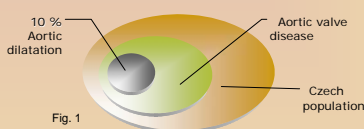


Fig. 1

Men are affected 2-5 times more than women (Meszaros et al., 2000). Substitution of impaired aortic valve regulate post-stenotic flow effects (hemodynamics) and eliminate the risk of dilatation and dissection.

The medial layer of the aorta consists predominantly of elastic fibres organized in concentric lamellae that are responsible for the elasticity of the vessel. The extracellular matrix of the adventitia is mostly made of collagen fibrils, which are tightly associated in longitudinal bundles whose function is to limit the dilatation of the aorta. Microfibrils are integral components of the elastic lamellae and the branching network that extends throughout the aortic wall.

MATERIAL

The patients were diagnosed with aortic valve disorder associated with ascending aortic dilatation and were conscientiously selected. Collection contains 22 men and 6 women, in age from 35 to 76. All patients underwent genetic examination including clinical and genealogical examination and their personal history was taken. Diagnosis of Marfan syndrome or its frust forms or any other particular genetic syndrome were excluded.

	Aortic valve disease (preoperative)	Ascending aortic diameter (mm) (preoperative)	Number of patients	Male/Female	Years of age
Patients	Aortic stenosis	55,78 (48-80)	19	22/6	55-20 (35-75)
	Aortic regurgitation	51,11 (48-60)	9		
Controls	Aortic stenosis/regurgitation	33,1 (28-42)	28	18/10	68 (49-79)

HISTOPATHOLOGY

In ascending aorta we found fusiform enlargement and the diameter was over 45 mm before the operation. Aortic dilatation was obvious from sinotubular junction to truncus brachiocephalicus involving sinuses of Valsava. We classified **Erdheim like changes** of aortic wall.

The majority of patients have a 3rd grade – severe – about 17 percent. That means that the amount of collagen was increased, atherosclerotic changes were obvious, there were fibrosis and cystic degeneration, elastic fragmentation and finally there were changes in orientation of smooth muscle fibers.

Grade of histological changes	Number of patients (%)
I. grade - low	0 (0%)
II. grade - intermediate	5 (23%)
III. grade - severe	17 (77%)

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The Gene for Fibrillin-1: FBN1 Gene

Fibrillin-1 is a large (~320 kD), multidomain glycoprotein comprised mainly of three classes of cysteine-rich repeat motifs, the most common of which is module with homology to the epidermal growth factor precursor - **EGF-like domain**. Fibrillin-1 is the major structural component of a class of 10-12 nm extracellular microfibrils with a wide tissues distribution and occur either in association with elastic fibres in tissues such as the aorta (Sakai et al., 1991). Fibrillin associated microfibrils appear to fulfil several physiological roles, including acting as a scaffolding for tropoelastin deposition and elastic fibres formation during elastogenesis, contributing to the elastic properties of the elastic fibres, and maintaining tissue homeostasis (Robinson and Godfrey, 2000).

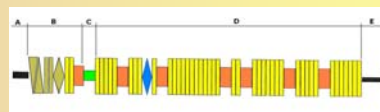


Fig.2 Based on Pereira et al., 1993

- 47 tandem repeat domains EGF (6 cysteine residues)
- 43 calcium binding - cbEGF
- 7 domains TGF-β1-bp (8 cysteine residues)
- Fib motif (2 cysteine residues)
- Proline-rich region (C)
- C- and N- terminals (A, E)

The gene for fibrillin-1 (FBN1) is located on **chromosome 15q21.1**, contains at least 65 exons, and spans about 235 kb of genomic DNA. Mutations in the FBN1 gene on chromosome 15q21.1 have been found to cause **Marfan syndrome (MFS)**, a dominantly inherited disorder characterized by clinically variable skeletal, ocular and cardiovascular abnormalities. Dilatation of the aortic root is the hallmark feature in the cardiovascular system. All FBN1 mutations identified in MFS patients predict a dominant negative pathogenesis through the production of mutant fibrillin-1 monomer on the background of normal fibrillin-1 monomer from the non-mutant allele (Dietz, 1993).

Fibrillin-1	FBN1	15q21.1	65 exons
Transforming growth factor, beta receptor I	TGFB1	9q22	9 exons
Transforming growth factor, beta receptor II	TGFB2	3p22	7 exons

GENETIC SCREENING

Genomic DNA was collected from 28 probands diagnosed as aortic valve disorder associated with ascending aortic dilatation. We performed mutation screening of the gene for fibrillin-1 FBN1 gene. Genomic DNA was extracted from white blood cells from peripheral blood samples. Screening involved specifically chosen exons of the FBN1 gene: **exon 4** and **exons 24-30** with their relevant intron/exon boundaries. Our gene scanning method was based on post-pcr analysis of high resolution melting curves using a Lightcycler® 480 instrument. Amplicons displaying sequence variations were subsequently subjected to DNA sequencing in both directions.

We performed amplification and sequence analysis of exon 26 and exon 27 and we detected **sequence variation in intronic part situated close to exon 27**, this variation was identified as insertion of guanine between nucleotide 37 682 and 37 683 of query sequence. We classified this mutation as **IVS27 37682_37683insC**.

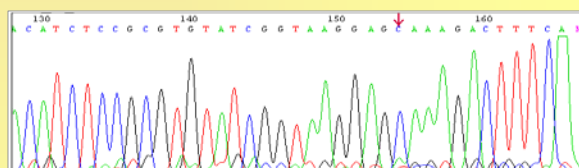


Fig. 3: The location of the insertion is shown by the red arrow

SUMMARY

The current study suggests that sequence variation in the genes encoding proteins constituting the aortic wall and regulating the turnover of the extra cellular matrix are likely to influence properties of elastic fibres.

This is an initial study and although a causative link has not been shown, these data are very important for further research of the role of Fibrillin-1 in relation to cardiovascular risk associated with aortic dilatation. These findings would have potential implication for risk stratification and therapeutic targeting not only for patients with existing disease but also for general population.

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