New fibrillin gene mutation – possible cause of ascending aortic dilation in patients with aortic valve disease: Preliminary results

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BACKGROUND: Approximately 10% of patients who undergo surgery for aortic valve disease (stenosis or regurgitation) suffer from ascending aortic dilation (AAD). A possible genetic etiology of AAD associated with aortic valve disease has been repeatedly mentioned in the literature, but a specific responsible gene mutation has not been described.

METHODS: In the present study, two groups of patients were compared, all of whom underwent surgery for aortic valve disease. Group A was a cohort of 27 patients who suffered from aortic valve disease associated with AAD. Group B was a cohort of 29 patients with structural aortic valve disease, but without concomitant AAD (control group). Genomic DNA was extracted from the white blood cells of peripheral blood samples and was amplified using primers specific for

chosen exons of the fibrillin-1 gene, including their intron/exon boundaries. Exons 26 and 27 were selected for analysis.

RESULTS: Analysis of the intronic part situated close to exon 27 showed insertion of cytosine between nucleotide 37 682 and 37 683 of query sequence. This insertion was classified as IVS 37 682 and 37 683insC. This mutation was found in all 27 patients from group A (patients with structural aortic valve disease accompanied by significant AAD). The abovementioned mutation was not found in any of the 29 patients from group B.

CONCLUSIONS: This finding has potential implications for risk stratification and therapeutic targeting not only for patients with existing disease, but also for the general population. Future studies are needed to determine the clinical utility of the finding; however, the present hypothesis needs to be verified by further molecular studies.

Key Words: Aortic valve disease; Ascending aortic dilation; Fibrillin gene mutation

The prevalence of structural aortic valve disease (stenosis or regurgitation) in the general population is estimated to be 0.2%. According to our own observations and other sources (1), approximately 10% of patients who undergo surgery for aortic valve disease (either aortic stenosis or regurgitation) also suffer from ascending aortic dilation (AAD). Development of AAD in patients with aortic valve disease is probably initiated by pathological changes of the aortic wall. A genetically determined disorder of fibrillin-1 (FBN-1) likely plays an important role in the occurrence of pathological changes of the aortic wall and pathogenesis of AAD.

A possible genetic etiology of AAD (sometimes termed as poststenotic dilation of the ascending aorta) associated with structural aortic valve disease has been mentioned repeatedly in the literature, but a specific responsible gene mutation has not been described (2,3).

METHODS

In the present retrospective study, the clinical charts of patients who underwent surgery for aortic valve disease were analyzed to identify the subgroup of patients with aortic valve disease and concomitant AAD. From January 1996 to December 2004, 695 patients with aortic valve disease underwent surgery. Dominant aortic stenosis was present in 407 patients, and AAD was present in 38 of these patients. Dominant aortic

regurgitation was present in 288 patients and AAD was present in 28 of these patients.

To perform further analyses in the patients with aortic valve disease and concomitant AAD, attempts were made to contact all 66 of the AAD patients. Twenty-seven patients agreed to undergo further examination.

In the present study, two groups of patients were compared, all of whom underwent surgery for aortic valve disease.

Group A was a cohort of 27 patients (22 men and five women; mean [± SD] age 60±13 years, range 35 to 76 years) who suffered from aortic valve disease associated with AAD.

Group B was a cohort of 29 selected patients (18 men and 11 women; mean age 67±7 years, range 52 to 79 years) who underwent surgery for structural aortic valve disease, but did not have concomitant AAD (control group).

All patients underwent transthoracic echocardiography preoperatively.

In all group A patients, the maximal diameter of the ascending aorta was greater than 5 cm. Each ascending aorta was clearly dilated into a spindle shape. The dilations spread from the sinotubular junction to the truncus brachiocephalicus. The Valsalva sinuses were not dilated. Dominant aortic stenosis was found in 18 patients and aortic regurgitation in nine patients. A congenital bicuspid aortic valve was present in seven of 27 patients. Aortic stenosis was present in six

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Figure 1) Schema of linear reduction aortoplasty

patients and aortic regurgitation in one patient. All patients underwent aortic valve replacement and linear reduction aortoplasty (Figure 1). The evaluation of the effectiveness and durability of linear aortoplasty in these patients is the one of the main targets of long-term follow-up in this group.

In all group B patients, the maximal diameter of the ascending aorta was less than 4 cm. None of the patients had a dilated ascending aorta. Dominant aortic stenosis was found in 17 patients and aortic regurgitation was found in 12 patients. A congenital bicuspid aortic valve was present in three of 29 patients. Aortic stenosis was found in one patient and aortic regurgitation in two patients. All group B patients underwent aortic valve replacement only.

All patients from both groups underwent a clinical genetic examination and, in each of them, Marfan syndrome (MFS) according the Ghent criteria was excluded.

Molecular genetic analysis

Genomic DNA was collected from both patient groups. Blood samples were collected and used to create a DNA bank at the Institute of Computer Science, Academy of Sciences Czech Republic (Prague, Czech Republic). Genomic DNA was extracted from the white blood cells of peripheral blood samples using Miller's technique.

Exons 26 and 27 were selected for analysis because FBN-1 gene mutations are mostly localized in these areas. Exons 26 and 27 of the FBN-1 gene were amplified individually from genomic DNA. A total of 25 μ L of mixture contained genomic DNA, 2.0 mM deoxyribonucleotide triphosphate, a 0.1 mM solution of each primer, 10 $\times$ polymerase chain reaction buffer (100 mM Tris-HCl pH 8.3, 500 mM KCl), 2.5 mM MgCl₂ and 5 U/ μ L Taq DNA polymerase. All amplifications contained an initial 5 min denaturation step at 94°C, followed by 34 cycles with denaturation for 30 s at 95°C, 30 s annealing at 51°C to 56°C (depending on primer characteristics), 1 min extension at 72°C with a final 10 min extension at 72°C using a DNA Engine Dyad Dual-Bay Thermal Cycler (Bio-Rad Laboratories Inc, USA). Individual FBN-1 exons were amplified with intron-based exon-specific primers.

Primers used for genomic amplification and their size

Exon 26 (227 bp):

F: 5'-AAT TAA GGC TGT CCT GAG AC-3'

R: 5'-CAT GGA ATC CTT CTC TTT CTG-3'

Figure 2) Schema of fibrillin-1 (FBN-1) gene structure. mRNA Messenger RNA

Exon 27 (181 bp):

F: 5'-GGC CCC CAC CTT TAA CAT G-3'

R: 5'-GAA AGT CTT TGC TCC TTA C-3'

The amplified DNA fragments migrated using horizontal gel electrophoresis in 2% DNA-ase-free agarose and were visualized using an ultraviolet lamp. Exons 26 and 27 were sequenced with fluorescent terminators on an ABI Prism 3100-Avant Genetic Analyzer sequencer (Applied Biosystems Inc, USA). All fragments were sequenced in both directions. The DNA sequence of the amplified regions (exons 26 and 27) was compared with reference sequences of human genome using an online comparison tool (Genomic BLAST – Human, <http://blast.ncbi.nlm.nih.gov/Blast.cgi>) and the Chromas program (Technelysium Pty Ltd, Australia) was used for further analysis.

RESULTS

Genomic DNA from 27 patients in group A and 29 patients in group B was amplified using primers specific for chosen exons of the FBN-1 gene, including their intron/exon boundary. Amplification and sequence analysis of exons 26 and 27 were performed without changes to the DNA. Analysis of the intronic part situated close to exon 27 showed insertion of cytosine between nucleotides 37 682 and 37 683 of the query sequence. This was classified as IVS 37 682 and 37 683insC. These mutations were found in all 27 group A patients (patients with structural aortic valve disease accompanied by significant AAD). The FBN-1 gene structure and cytosine insertion is shown in Figure 2. The abovementioned mutations were not found in any of the 29 patients in group B (patient with structural aortic valve disease, but without AAD).

DISCUSSION

Patients with AAD are at risk of fatal complications such as aortic dissection and rupture. The risk correlates with the diameter of the dilated ascending aorta. If the dissection is not resolved, 90% of the patients die within one year. Replacement of the impaired aortic valve and re-establishment of the normal diameter of the ascending aorta using linear aortoplasty can resolve impaired hemodynamics caused by aortic valve disease, thus eliminating the risk of redilation and aortic dissection.

The medial layer of the aorta consists predominantly of elastic fibres organized in concentric lamellae that are responsible for the elasticity of the vessels. The extracellular matrix of the adventitia is mostly made of collagen fibrils, which are tightly associated in longitudinal bundles whose function is to limit dilation of the aorta. Microfibrils are integral components

of the elastic lamellae and the branching network that extends through the aortic wall (4).

FBN-1 is a large (approximately 320 kDa), multidomain glycoprotein comprised mainly of the three classes of cysteine-rich repeat motifs, the most common of which is a module with homology to the epidermal growth factor (EGF) precursor (EGF-like domain). FBN-1 is the major structural component of a class of 10 nm to 12 nm extracellular microfibrils with a wide tissue distribution and occurs in association with elastic fibres in tissue such as the aorta (5). Fibrillin-associating microfibrils appear to fulfill several physiological roles, including acting as scaffolding for tropoelastin deposition and elastic fibre formation during elastogenesis, contributing to the elastic properties of the elastic fibres, and maintaining tissue homeostasis (6).

Extensible fibres are important structures of the aortic wall. The present study suggests that genetic variation in the genes encoding specific proteins constituting the aortic wall and regulating the extracellular matrix turnover are likely to influence properties of these elastic fibres. FBN-1, a major constituent of extensible microfibrils, is a strong candidate for these degenerative changes. The gene for FBN-1 is located on chromosome 15q21.1, contains 65 exons and spans approximately 235 kbp of genomic DNA. The majority of mutations concerning the FBN-1 gene are linked with MFS. FBN-1 mutations have been found in individuals with isolated aortic aneurysm and dissection (7). The link between impaired aortic wall and FBN-1 mutation is quite obvious. Mutations in the FBN-1 gene on chromosome 15q21.1 have been found to cause MFS, a dominantly inherited disorder that presents with clinically variable skeletal, ocular and cardiovascular abnormalities. All FBN-1 gene mutations identified in MFS patients predict a dominant negative pathogenesis through the production of a mutant FBN-1 monomer from the nonmutant allele (8). All mutations leading to delayed secretion of FBN-1 have disrupted or inserted a cysteine in one of the EGF-like domains, leading to the conclusion that correct disulfide bonding within EGF-like domains is necessary for proper secondary structure of the protein (9).

In our study, we screened exons 26 and 27 of the FBN-1 gene. Genomic DNA from 27 patients was amplified using primers specific for the chosen exons of the FBN-1 gene, including their intron/exon boundaries. All patients were screened under identical conditions for each exon. Amplification and sequence analysis of exons 26 and 27 were performed without changes to the DNA. Analysis of the intronic part situated close to exon 27 showed a mutation (insertion of cytosine) between nucleotides 37 682 and 37 683 of the query sequence. We classified this as *IVS 37 682 and 36 683insC*. The location of the mutation (cytosine insertion) is shown by an arrow in Figure 3.

Affliction of the aortic wall, including pathological AAD, is associated with fragmented elastic fibres and accumulation of amorphous matrix elements in the medial layer. This pathology is probably caused by mutations in the FBN-1 gene.

The current study suggests that sequence variation in the genes encoding proteins that constitute the aortic wall and regulate the turnover of the extracellular matrix are likely to influence properties of the elastic fibres.

We have identified a uniform mutation in intron 27 of the FBN-1 gene in all 27 patients with aortic valve disease

Figure 3 Intron 27 sequence diagram of the samples of patients with ascending aortic dilation (group A). The arrow indicates the cytosine insertion

associated with AAD (group A). This mutation maintains the reading frame of the FBN-1 gene. On the other hand, in the randomly selected group of 29 patients with structural aortic valve disease without AAD (group B), the abovementioned FBN-1 gene mutation was not found.

The present study is an initial step and, although a causative link has not been shown, these data are very important for further research of the role of FBN-1 in relation to the cardiovascular risk associated with aortic dilation.

In light of the abovementioned facts, we can speculate about the influence of the newly revealed mutation in FBN-1 gene expression. Also remaining is the question of whether the described cytosine insertion on the intronic part of the FBN-1 gene between nucleotides 37 682 and 37 683 is a population polymorphism or a mutation.

A genetically determined disorder of FBN-1 in the aortic wall probably plays an important role in pathogenesis of AAD.

The present initial pilot study and these data are very important for further research on the role of FBN-1 in relation to cardiovascular risk associated with AAD. FBN-1 and microfibrils participate in the maintenance of elastic tissues and some evidence has suggested that a progressive loss of microfibrils may be the initiating factor in the development of aortic dilation and dissection (10). Detection of the abovementioned FBN-1 gene mutation could help to select the most appropriate surgical treatment for dilated ascending aorta in these patients and establish new indication criteria and appropriate timing of surgery in patients with structural aortic valve disease and concomitant AAD.

CONCLUSION

We believe that the newly revealed mutation of the FBN-1 gene may play an important role in development of AAD in patients with concomitant aortic valve disease.

Targets of further study include the possible mechanism of the revealed mutation of FBN-1 gene expression and the role of the revealed mutation in the etiopathology of other aneurysmatic (dilating) aortic diseases.

Our findings may have implications for risk stratification and therapeutic targeting not only for patients with existing disease, but also for the general population. Future studies will determine the clinical utility of this finding; however, the present hypothesis needs to be verified by further molecular studies.

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