Original Article

Serum Levels of Matrix Metalloproteinases 2 and 9 and *TGFBR2* Gene Screening in Patients with Ascending Aortic Dilatation

(aortic dilatation / MMP-2 / MMP-9 / *TGFBR2*)

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Abstract. Development of ascending aortic dilatation (AAD) in about 10 % of patients operated for aortic valve disease (AVD) is probably based on intrinsic pathology of the aortic wall. This may involve an abnormality in the process of extracellular matrix remodelling. The present study evaluated the serum levels of specific metalloproteinases (MMP-2 and MMP-9) and investigated the gene for transforming growth factor receptor 2 (TGFBR2) in 28 patients with AVD associated with AAD (mean age 60.6 years), in 29 patients (68.9 years) with AVD without AAD, and in 30 healthy controls (45.3 years). The serum levels of MMPs were determined by ELISA. Further, we focused on genetic screening of the *TGFBR2* gene. Plasma MMP-2 concentrations were significantly higher in the groups of patients compared to the controls: median 1315.0 (mean 1265.2 ± SD 391.3) in AVD with AAD, 1240.0 (1327.8 ± 352.5) in AVD without AAD versus 902.5 (872.3 ± 166.2) ng/ml in the healthy controls, in both cases P < 0.001. The serum levels of MMP-9 were significantly higher in AVD with AAD patients [107.0 (202.3 ± 313.0)] and in AVD without AAD patients [107.0 (185.8 ± 264.3)] compared to the healthy controls [14.5 (21.2 ± 24.8) ng/ml], in both cases P < 0.001. No significant correlation was observed between plasma MMP-2 and MMP-9 and ascending aorta diameter. Genetic screening did not reveal any variation in the *TGFBR2* gene in the patients. Measurement of MMP levels is a simple and relatively rapid laboratory test that could be used as a biochemical indicator when evaluated in combination with imaging techniques.

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Abbreviations: AAD – ascending aortic dilatation, AVD – aortic valve disease, BAV – bicuspid aortic valve, BMI – body mass index, ECM – extracellular matrix, *FBN1* – fibrillin 1 gene, HDL – high-density lipoprotein, LDL – low-density lipoprotein, LDS – Loyez-Dietz aortic aneurysm syndrome, MFS – Marfan syndrome, MMP – matrix metalloproteinase, TAV – tricuspid aortic valve, TG – triglyceride, TGFBR1(2) – transforming growth factor β receptor 1 (2).

Introduction

The prevalence of structural aortic valve disease (stenosis or regurgitations) in the population is estimated at 0.2 %, and in the Czech Republic there are about 2083 of operations for this disorder per year (Šetina et al., 2012). Approximately 10 % of subjects who undergo surgery for aortic valve disease (AVD) simultaneously suffer from ascending aortic dilatation (AAD), no matter whether it is stenosis or regurgitation. Aortic valve disease is associated with valvular haemodynamic abnormality, which could be the contributory factor for developing AAD. Development of dilatation in 10 % of subjects is probably based on pathologically changed

aortic wall. The cause and progression of the aortic valve disorder is considered to be the contributory factor for dilatation of primary changed aortic wall of the ascending aorta. This may involve an abnormality in the process of extracellular matrix remodelling in the aortic wall including inadequate synthesis, degradation and transport of extracellular matrix proteins. Patients are at risk of fatal complications such as dissection or rupture of aortic wall. The risk correlates with the diameter of the dilated ascending aorta. Replacement of the impaired aortic valve and re-establishment of the normal diameter of ascending aorta can resolve impaired haemodynamics caused by AVD and eliminate the risk of re-dilatation and aortic dissection.

It is important to focus attention on the processes occurring in the extracellular matrix of aortic wall. Elastic fibres are essential extracellular matrix (ECM) macromolecules. They endow connective tissue, such as blood vessels, with the critical properties of elasticity and resilience. Their physiological importance is underscored by the spectrum of clinical manifestations associated with mutations in the genes encoding microfibrils. Vascular tissues with deficient fibrillin 1 microfibrils release metalloproteinases. These enzymes weaken the vessel wall by degrading the elastic matrix components, with subsequent matrix disruption and consequent dilatation of the vessel.

Matrix metalloproteinases (MMPs) represent a large family of zinc-dependent proteases with a wide range of substrates, including extracellular matrix components, cytokines, receptors, and cell motility factors (Yong et al., 2007; Morrison et al., 2009). They are recognized as the main proteolytic enzyme group involved in remodelling the ECM and modifying cell-cell and cell-matrix interactions (Murphy and Nagase, 2008). MMPs are not generally expressed in normal, healthy, resting tissue. Up-regulation of the activity of individual MMPs is seen in a wide range of diseased or inflamed tissues. Many different human MMPs have been identified (Yong et al., 2007). On the basis of substrate specificity and homology, MMPs can be divided into six groups: collagenases (MMP-1, -8, -13 and -18), gelatinases (MMP-2 and -9), stromelysins (MMP-3, -10, -11 and -17), matrilysins (MMP-7 and -26), membrane-type MMPs (MT-MMPs: MMP-14, -15, -16, -17, -24 and -25) and other MMPs (MMP-12, -19, -20, -21, -22, -23, -28 and -29) (Zhang et al., 2009; Sekhon, 2010). Most MMPs are initially synthesized as enzymatically inactive zymogens (pro-MMPs). Some MMPs are stored within the cell after synthesis, but most of them are either secreted freely into the extracellular space or anchored to the surface of cell membranes (Page-McCaw et al., 2007; Raffetto and Khalil, 2008). Rigorous regulation of MMP expression and activity is a crucial part of ECM homeostasis. This regulation occurs primarily at the level of gene expression, pro-MMP secretion, extracellular localization, zymogen activation, and enzyme inhibition by interaction with endogenous inhibitors (Zhang et al., 2009). MMPs have been proved to be involved in the pathology of serious cardiovascular diseases, such as aortic aneurysm, dissection, and coronary artery disease (Galis et al., 1994; Dollery et al., 1995; Isselbacher, 2005). Fragmentation of the elastic fibres and aortic dilation are associated with the local release of proteolytic enzymes, including serine proteases, cysteine proteases, and MMPs. These proteolytic enzymes cause degeneration of the ECM and destruction of the vascular framework. MMP-2 and MMP-9 play an important role in vascular remodelling (Bendeck et al., 1994; Gibbons and Dzau, 1994; Godin et al., 2000). Increased MMP-2 and MMP-9 activity is associated with destruction of the elastic laminae of arteries and aneurysm formation in animals (Allaire et al., 1998) and humans (Longo et al., 2002).

Transforming growth factor β receptor 2 (TGFBR2) belongs to the transmembrane-spanning protein serine/threonine kinases and activates numerous signalling pathways by autophosphorylation, as well as downstream signalling proteins on specific Ser/Thr residues (Hanks and Hunter, 1995). The TGFBR2 protein is encoded by 567 codons distributed in seven exons and the *TGFBR2* gene is located on chromosome 3p22 (Takenoshita et al., 1996). The ligand TGF-β 1 activates TGFBR2 directly by binding to a homodimer of TGFBR2 on the cell surface. This ligand-receptor complex recruits a homodimer of transforming growth factor β receptor 1 (TGFBR1) and activates it by multiple phosphorylation of the glycine-serine-rich region of this receptor (Wrana et al., 1994). Activated TGFBR1 phosphorylates members of the Smad family of transcription factors, transducing the extracellular signal of TGF-β 1 into the cytoplasm and nucleus, and thus regulating gene transcription in interaction with DNA-binding cofactors. TGFBR1 and TGFBR2 are involved in several cellular processes, including growth inhibition, apoptosis, proliferation, and extracellular matrix production (Chang et al., 2002). TGF-β signalling can regulate production of critical vascular matrix proteins as well as matrix-degrading enzymes. Alterations in the TGF-β signalling pathway may be damaging to normal vascular function and architecture and have been implicated in the pathophysiology of aortic aneurysm syndromes (Jones et al., 2009).

The aim of our research was to gain insight into the processes occurring in the extracellular matrix of aortic wall. We evaluated the levels of serum MMP-2 and MMP-9 in patients with AVD associated with AAD, in patients with impaired aortic valve but not accompanied with dilatation compared to healthy individuals, and we assessed their clinical significance. Further, we investigated the potential link between the *TGFBR2* gene and impaired aortic wall.

Material and Methods

In the present study, the clinical charts of patients who underwent surgery for aortic valve disease were analysed to identify the subgroups of subjects with aortic valve disease and concomitant AAD. Six hundred

ninety-five patients with aortic valve disease underwent surgery at the Department of Cardiovascular Surgery, General University Hospital in Prague from January 1996 to December 2004. Dominant aortic stenosis was present in 407 subjects and dominant aortic regurgitation was present in 288 subjects. AAD was detected in 38 subjects with aortic stenosis and in 28 subjects with aortic regurgitation. All 66 subjects with AVD associated with AAD were contacted and a total of 27 subjects agreed to undergo further examination (cases A). The second group consisted of subjects that underwent surgery for structural aortic valve disease at the same department and who had no concomitant AAD. From March to May 2006 a total of 29 control subjects agreed with participation in the study (cases B). The diameter of ascending aorta was a key criterion and it was measured preoperatively by transthoracic echocardiography in both groups of patients. The third group involved 30 healthy individuals serving as the control group.

In cases A, the median maximal diameter of the ascending aorta was 51.5 mm (54.4 ± SD 6.9). Each ascending aorta was clearly dilated into a spindle shape. The dilatations spread from the sinotubular junction to the truncus brachiocephalicus. The Valsava sinuses were not dilated. Dominant aortic stenosis was found in 18 subjects and aortic regurgitation in nine subjects. A congenital bicuspid aortic valve (BAV) was present in seven of 27 subjects – aortic stenosis was present in six subjects and aortic regurgitation in one subject. All patients underwent aortic valve replacement and linear aortoplasty. The evaluation of the effectiveness and durability of linear aortoplasty in these subjects is one of the main targets of long-term follow-up in this group.

In cases B, the median maximal diameter of the ascending aorta was 36.0 mm (35.3 ± SD 4.1). None of the subjects had a dilated ascending aorta. Dominant aortic stenosis was found in 17 subjects and aortic regurgitation in 12 subjects. A congenital bicuspid aortic valve was present in three of 29 subjects – aortic stenosis was present in one subject and aortic regurgitation in two subjects. All subjects from control group B underwent aortic valve replacement only.

The control group consisted of healthy young individuals with stable and physiological biochemical laboratory results, without any manifestation of cardiovascular or any chronic disease and with no acute or inflammatory disease. Subjects were selected from available blood donors from September to December 2008 at the same hospital.

Subjects from all groups underwent clinical genetic examination and in each subject, diagnosis of Marfan syndrome (MFS) was excluded according to the Ghent criteria. Blood samples were collected and a DNA bank was created with the support of the project LN00B107 at the European Centre of Medical Informatics, Statistics and Epidemiology-Cardio. The study was approved by the ethical committee of the First Faculty of Medicine, Charles University in Prague, Czech Republic.

Biochemical analyses

The following biochemical markers were evaluated from blood according to standards: total cholesterol in mmol/l (reference ranges: 2.90–5.00 (15–40 years), 2.90–5.20 (40–110 years)), LDL in mmol/l (reference ranges: 1.2–3.0), HDL in mmol/l (reference ranges: 1.0–2.1 for males, 1.2–2.7 for females), triglyceride (TG) in mmol/l (reference ranges: 0.45–1.70), glycaemia in mmol/l (reference ranges: 3.9–5.6). Body mass index (BMI) was calculated from the subject weight and height.

Immunochemical analyses

MMP-2 and MMP-9 were measured in the blood serum of all individuals. Blood samples in groups A and B were collected before surgical intervention. All the sera were separated within an hour of blood collection and stored at –80°C until assayed. Serum levels of MMP-2 and MMP-9 were measured using enzyme-linked immunosorbent assay kits (ELISA) (Biotrak GE Healthcare, Amersham Bioscience, Little Chalfont, UK) according to the manufacturer's instructions. All samples were measured as duplicates. The serum samples were diluted 50-fold before determination for MMP-2, and 10-fold for MMP-9. The manufacturer of assay kits described the intra-assay coefficient of variation (%) for MMP-2 as 5.3–5.4 % and the intra-assay coefficient of variation for MMP-9 was 4.92–5.50 %.

Molecular genetic analysis

Genomic DNA was extracted from white blood cells from samples of peripheral blood using Miller's technique. For genetic screening we selected the gene for TGFBR2. This gene spans seven exons and we decided to use eight intron-based primer pairs for mutation analysis of the entire coding region of the gene (Table 1). All

Table 1. Primers used for genomic amplifications of each exon of the TGFBR2 gene (Takenoshita et al., 1997)

Exon	Size	Forward primer	Reverse primer
1	178 bp	- tcg gtc tat gac gag cag -	- ggg acc cca gga aga ccc -
2	365 bp	- ggg ctg gta tca agt tca tttg -	- gga gac aga gat aca ctg act gtg -
3	241 bp	- tgc aat gaa tct ctt cac tc -	- ccc aca ccc tta aga gaa ga -
4(a)	444 bp	- cca act cct tct ctc ctt gtt ttg -	- tcc aag agg cat act cct cat agg -
4(b)	540 bp	- gtc gct ttg ctg agg tct ata agg -	- cca ggc tca agg taa agg gga tct agca -
5	261 bp	- ggc agc tgg aat taa atg atg ggc -	- tgc tcg agg caa cac atg -
6	243 bp	- ttt cct ttg ggc tgc aca tg -	- cct aag agg caa ctt ggt tga atc -
7	252 bp	- cca act cat ggt gtc cct ttg -	- tct ttg gac atg ccc agc ctg -

exons were amplified individually from genomic DNA using a Thermalcycler PTC-220 Engine Dyad® Cycler (F. Hoffmann-La Roche Ltd., Basel, Switzerland). The amplified DNA migrated in horizontal gel electrophoresis using 2% DNAase-free agarose and was visualized by an UV lamp. All exons were screened for unknown sequence variations using the LightCycler 480 System (Roche Diagnostics, Mannheim, Germany). We carried out high-resolution melting analysis performed with a high-resolution dye. The system provides highly accurate results based on the analysis of melting curve profiles.

Statistical methods

The normal distribution of continuous variables was tested using the Shapiro-Wilk test, and explored using normal Q-Q plot. The Levene test was used to assess the equality of variances in the case and control groups. Due to the small size of the groups, frequent heteroskedasticity, and non-normality distribution of data, the Kruskal-Wallis test was applied for testing whether variables in the groups originate from the same distribution. The Mann-Whitney test was used to identify the group pairs for significant differences with Bonferroni corrections of the P values. The differences in categorical variables were explored by the χ^2 test and the Fisher's exact test (if any expected value was below 5). Spearman correlation was calculated to measure the association between variables. A detailed multivariate analysis was not done because of the small number of the study groups. Statistical analysis was performed by R software (R Development Core Team, Vienna, Austria, 2010).

Results

Baseline characteristics of the cases and controls are shown in Table 2. In univariate analysis, there were significant differences in all presented variables among the three groups. Triglyceride was on the border of statistical significance at the 0.05 level. Due to the fact that the controls (healthy blood donors) were non-randomly younger than the case groups, the most favourable values were observed in the control group only. There was an insignificant difference in female distribution among the groups: 21.4 % in the cases from group A, 37.9 % in the cases from group B, 20.0 % in the controls from group C (P = 0.227). Family anamnesis was positive in no subject from group B and in 20 % subjects of the group A (unknown in the controls).

Proteomic analysis

There were significant differences in the serum levels of MMP-2 and MMP-9 among the three groups, Table 3. In the pair comparison of cases A with the controls, there were significant differences in the serum levels of both MMP-2 (P < 0.001) and MMP-9 (P < 0.001). Likewise, the differences between the cases B and the controls were significant (P < 0.001 for both MMP-2 and MMP-9). The significant results were observed even if we restricted age to 35–65 years to keep the age under partial control.

The serum level of MMP-2 was significantly increasing with age (r = 0.50, P < 0.001) associated with the health status (Fig. 1). The same conclusion holds for

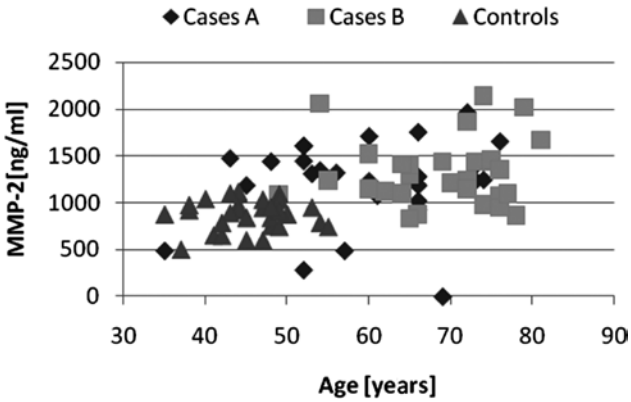

Fig. 1. Association between serum levels of MMP-2 and age

Table 2. Characteristics of the study groups

Characteristic	Cases A (AVD with AAD)			Cases B (AVD without ADD)			Controls (Healthy blood donors)			P value
	Median	Mean ± SD	(N)	Median	Mean ± SD	(N)	Median	Mean ± SD	(N)	
Age	60.5	60.6 ± 11.0	(28)	72.0	68.9 ± 8.1	(29)	45.0	45.3 ± 4.9	(30)	<0.001
BMI	26.5	26.9 ± 4.0	(26)	27.8	27.5 ± 4.6	(29)	23.2	23.3 ± 2.2	(30)	<0.001
HDL	1.3	1.2 ± 0.4	(17)	1.4	1.4 ± 0.4	(29)	1.5	1.6 ± 0.3	(30)	0.011
LDL	3.4	3.5 ± 0.7	(16)	2.8	2.9 ± 0.9	(25)	2.2	2.2 ± 0.4	(30)	<0.001
Cholesterol	5.2	5.3 ± 1.0	(26)	5.1	5.2 ± 1.3	(29)	4.1	4.0 ± 0.5	(30)	<0.001
TG	1.3	1.4 ± 0.8	(23)	1.4	1.7 ± 1.2	(29)	1.0	1.1 ± 0.3	(30)	0.055
Glycaemia	5.6	5.9 ± 1.3	(28)	5.7	6.0 ± 1.6	(29)	5.0	4.8 ± 0.6	(30)	<0.001

Table 3. Serum levels of MMP-2 [ng/ml] and MMP-9 [ng/ml]

Serum level	Cases A (AVD with AAD)			Cases B (AVD without ADD)			Controls (Healthy blood donors)			P value
	Median	Mean ± SD	(N)	Median	Mean ± SD	(N)	Median	Mean ± SD	(N)	
MMP-2	1315.0	1 265.2 ± 391.3	(27)	1240.0	1 327.8 ± 352.5	(29)	902.5	872.3 ± 166.2	(30)	<0.001
MMP-9	107.0	202.3 ± 313.0	(27)	107.0	185.8 ± 264.3	(29)	14.5	21.2 ± 24.8	(30)	<0.001

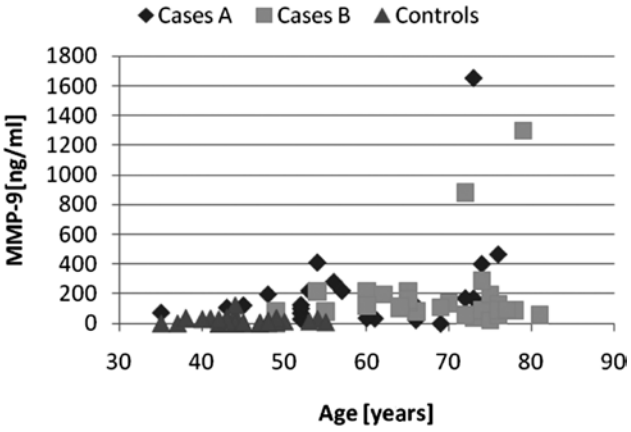

Fig. 2. Association between serum levels of MMP-9 and age

MMP-9 ($r = 0.54$, $P < 0.001$, Fig. 2). However, it is important to stress that the correlation between MMP-2/MMP-9 and age was not significant in the controls, similarly as in both case groups.

No significant differences were found in MMP-2 ($P = 0.957$) and MMP-9 ($P = 0.864$) between males and females across all the groups. Between genders, no significant differences were observed within the groups. No significant dependence of MMP-2 and MMP-9 on BMI, maximal aortic diameter, and occurrence of aortic stenosis and regurgitation in the patients was confirmed.

Genetic analysis

Genomic DNA from 27 cases and 29 controls was amplified using primers specific for the exons of the *TGFBR2* gene including their intron/exon boundary. High-resolution melting analysis did not reveal any variance in any examined sequence.

Discussion

MMPs play a significant role in the pathogenesis of aortic disease. In this study we evaluated the levels of serum MMP-2 and MMP-9 in patients with aortic valve disease associated with ascending aortic dilatation and in patients with aortic valve disease only. These were compared with values obtained from the control group of healthy individuals.

The levels of MMP-2 and MMP-9 were found to be lower in healthy individuals compared to patients that underwent surgery because of aortic disease. These results indicate that there may be some changes in extracellular matrix of the aortic wall. Due to the fact that the control group was systematically younger and any standardization of the results can be troublesome, the conclusion could be influenced by age (Figs. 1, 2). On the contrary, there was no significant correlation between age and MMP concentrations in the control group of healthy individuals at the age of 35–55 years, similarly as in the patients aged 35–81. Apart from that, the differences in MMP concentrations among the three groups kept significant even if we restricted the age to less than 65 years.

Fedak et al. (2003) suggested that the decrease in the amount of fibrillin 1 in the vasculature of patients with abnormal aortic valve may trigger MMP production, leading to matrix disruption and vascular dilatation. They carried out a study to assess the vascular matrix remodelling in patients with BAV and its implication for aortic dilatation. Samples of aorta and pulmonary artery were obtained from patients undergoing surgery with BAV and tricuspid aortic valve (TAV). These authors have shown significantly reduced fibrillin 1 content in both the aorta and pulmonary artery of BAV patients compared to that seen in TAV, suggesting a systemic deficiency of this protein. It was independent of the valve function and patient age. The amount of other examined matrix components, elastin and collagen, were unchanged. The production of MMP-2 in fibrillin 1-deficient tissues was increased and MMP-9 activity was not significantly different. The increased level of MMP-2 is in concord with our results, as we detected a significant increase in MMP-2 values in both case groups (AVD with and without AAD) compared to healthy controls. Unlike what was reported by Fedak et al. (2003), we found a significantly increased level of MMP-9 in patients with aortic valve disease compared to controls.

The pattern of MMP expression in ascending aortic tissue was also examined by Segura et al. (1998) in seven patients with MFS (three of whom had aortic dissections) using immunohistochemical analysis. MMPs or their inhibitors TIMPs showed little expression in the areas of cystic medial degeneration in the aorta of MFS patients. However, all MMPs, especially MMP-2 and MMP-9, were more extensively expressed in the smooth muscle cells at the borders of the areas of medial degeneration than in other regions. MMP-2 and MMP-9 were also expressed at the surfaces of disrupted elastic fibres, indicating the potential role of MMPs in elastin degradation. Most studies looked at the increased expression of MMPs within aneurysmal tissue. Chung et al. (2007) have shown up-regulation of MMP-2 and MMP-9 during thoracic aortic aneurysm formation in MFS. They also showed that the up-regulation of MMP generally correlated with the extent of loss of elastic fibre architecture during aneurysm formation and suggested that the pathogenesis of thoracic aortic aneurysm may be due to elastic fibre degeneration with deterioration of the aortic contraction and mechanical properties.

The degeneration of elastic fibres and resultant increased extracellular protease concentration may be caused by genetic factors as we can see in the pathogenesis of MFS. Still, the aetiology of ascending aortic dilatation remains controversial. It may as well be due to haemodynamic factors caused by impaired aortic valve, involving high velocity and turbulent flow downstream of the impaired valve. In diseased or degenerated congenitally deformed valves, increased local MMP activity could alter their elastic and collagen components and lead to structural and functional failure. Histological and immunohistochemical analysis of the aortic valve in the patients with non-rheumatic tricuspid aortic valve

and aortic stenosis revealed an inflammatory infiltrate within the valve leaflets and increased expression of MMP-1, -2, and -3 in patients with severe aortic stenosis. It was also found that MMP-9 was only present in the leaflets of patients with aortic stenosis (Edep et al., 2000; Kaden et al., 2005).

Patients with ascending aortic dilatation are at risk of dissection or rupture of aortic wall. The link between serum levels of MMPs and the dissection of ascending aorta has been investigated in several studies. Karapanagiotidis et al. (2009) evaluated the serum levels of MMP-1, -2, -3 and -9 in thoracic aortic disease and acute myocardial ischaemia. They reported the mean value of MMP-2 to be lower in patients with acute aortic dissection and the mean value of MMP-9 higher in patients with chronic aortic dissection and lower in patients with thoracic aortic aneurysm. However, the levels of MMP-2 and MMP-9 were not statistically different in patients with chronic aortic dissection, aortic aneurysm and healthy individuals. This is in contradiction with the results of the present study. Tamura et al. (2000) reported increased MMP-9 expression in macrophages at the site of the intimal tear in six patients with atherosclerosis-related dissections. Similarly, Ishii and Asuwa (2000) reported immunohistochemical evidence of MMP-2 and -9 at the initial tear in 21 postmortem acute dissection specimens. Using *in situ* hybridization for MMP-9 mRNA, Schneiderman et al. (1998) demonstrated MMP-9 involvement in four subacute dissections. A cDNA array analysis of tissue from six acute aortic dissections recently revealed up-regulation of inflammatory and proteolytic genes and down-regulation of ECM, adhesion, and cytoskeletal proteins, suggesting a degenerative process. A semi-quantitative study by Koullias et al. (2004) showed significant increases in MMP-1 and -9 in dissection tissue when compared with control tissue from cadavers.

The significant dependences of MMP-2 and MMP-9 concentrations on diameter of ascending aorta and occurrence of stenosis or regurgitation were not confirmed in the patients. Likewise, age, gender, BMI and evaluated biochemical markers did not indicate the significant dependence of MMP-2 and MMP-9 values. In cases A we detected borderline high LDL levels (3.5 ± 0.7); however, the group was older compared to the group B and the controls. An increased level of LDL cholesterol is a major risk factor for heart disease. Along with biochemical markers, overweight can put patients at greater risk of heart disease. BMI is a measure of fat based on the height and weight. The BMI value for normal weight is considered to be 18.5–24.9, and for overweight 25–29.9. Calculation of BMI in our patients showed that we could consider cases A (BMI 26.9 ± 4.0) and B (27.8 ± 4.6) to be overweight. This could be due to their style of life and limitations of the cardiovascular system as a consequence of AVD.

A further task of the present study was to investigate the potential involvement of the *TGFBR2* gene in the pathology of aortic wall in our patients. While researching the pathologies of aortic wall we cannot omit the impact of elastic fibres, the essential components of ECM. The biology of elastic fibres and microfibrils is complex because they have multiple components, tightly regulated developmental deposition, and unique biomechanical functions. Their fragmentation has been widely documented in the aorta of patients with MFS (Nataatmadja et al., 2003). Over the last decade, a considerable amount of new information has emerged describing the protein components of microfibrils. Fibrillin-associating microfibrils appear to fulfil 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 (Robinson and Godfrey, 2000). Their physiological importance is underscored by the complex spectrum of clinical manifestations associated with mutations in genes encoding microfibrils. Fibrillin 1 is the major structural component of a class of 10–12 nm extracellular microfibrils with a wide tissue distribution, and it occurs in association with elastic fibres in tissues such as the aorta (Sakai et al., 1991). Mutation in the gene for fibrillin 1 (*FBN1*) may increase the susceptibility of fibrillin 1 fragments to proteolysis. This was reported several times in connection with the pathogenesis of MFS (Robinson and Booms, 2001; Reinhardt et al., 2000), a dominantly inherited disorder characterized by clinically variable skeletal, ocular and cardiovascular abnormalities.

The most common cardiovascular complication of this condition is progressive aortic root enlargement, initially occurring at the sinus of Valsalva, and ascending aortic aneurysms. The aneurysms develop as a consequence of disruption of the medial and adventitial elastin and collagen in association with foci of cystic medial necrosis of the medial smooth muscle. We have reported the preliminary results from ongoing screening of the *FBN1* gene in our group of subjects with AAD, though the causative link has not yet been shown and further molecular studies are needed (Dudra et al., 2009). The investigation of the *FBN1* gene suggested that a mutation in the *FBN1* gene may be in part a developmental disorder, with dysregulation of transforming growth factor β activation and signalling (Neptune et al., 2003).

We suggested that genetic variation in the genes encoding specific proteins regulating the turnover of extracellular matrix may influence the degenerative changes in aortic tissue and we focused our attention on TGF-β receptors. Heterozygous mutations in the genes encoding TGFBR1 and TGFBR2 have been reported in Loyez-Dietz aortic aneurysm syndrome (LDS). Dominant *TGFBR2* mutations have been identified in MFS type 1 and type 2 and familiar thoracic aortic aneurysms and dissections (TAAD). Pannu et al. (2005) have studied four unrelated families and found mutations in the *TGFBR2* gene, but tested negative for any signs of MFS. The loss of signalling through TGF-β receptors 1 and 2

may therefore be associated with the development of familial TAAD. However, we did not reveal any variant change in the coding sequence in our study.

The pathogenesis of impaired aortic wall is likely to involve multiple factors acting on many levels. It is clear that the pathological alterations in the structure and composition of the vascular extracellular matrix are associated with reduced aortic compliance and resiliency and lead to aortic dysfunction. Still, there is a question regarding the regulation of specific upstream signalling and pathways involved in the remodelling process in the ascending aorta.

Ascending aortic dilatation is a potentially devastating disease, which often causes death by rupture in the absence of symptoms. Replacement of the ascending aorta at the time of aortic valve replacement is controversial because the risk of progressive dilatation following the aortic valve replacement is uncertain. There are currently no effective non-surgical clinical treatment protocols available which will halt or reverse the aortic remodelling process once diagnosed.

Conclusion

Measurement of serum MMP levels in patients with ascending aortic dilatation is a simple and relatively rapid laboratory test, which can be easily obtained even in the primary healthcare setting. Although the levels of MMP-2 and MMP-9 are not specific and cannot provide a definite clinical diagnosis, they could be used as a biochemical indicator of aortic disease when evaluated in combination with imaging techniques.

The current study suggests that genetic variation in the genes encoding proteins constituting the aortic wall and regulating the turnover of the extracellular matrix are likely to influence the properties of elastic fibres. Genetic analysis did not reveal any significant discrepancy in the *TGFBR2* gene in a strictly defined cohort of Czech patients with ascending aortic dilatation.

This is an initial study and although a causative link has not been shown, these data are very important for further research on the role of extracellular matrix components in relation to cardiovascular risk associated with aortic dilatation.

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