

Diseases of the ascending aorta in Marfan syndrome -
molecular biological background and surgical
interventions

PhD Doctoral Thesis

Kálmán Benke MD

Semmelweis University

Doctoral School of Basic Medicine



Tutors: Zoltán Szabolcs, M.D., Ph.D.,
Tamás Radovits, M.D., Ph.D.,

Opponents: Attila Szijártó, M.D., D.Sc.,
Balázs Gasz, M.D., Ph.D.,

Head of Examination Commission: Katalin Darvas, M.D., Ph.D.,

Members of Examination Commission: Anikó Gál, M.D., Ph.D.,

László Székely, M.D., Ph.D.,

Budapest

2017

Introduction

Marfan syndrome (MFS) is an autosomal dominant systemic disorder of connective tissue. The estimated prevalence of MFS is 1:3000-1:5000. As a connective tissue disorder, the phenotypic appearance of MFS affects several parts of the body. Besides the most visually apparent symptoms of the skeletal system such as a tall, slender figure with long limbs, fingers and toes, hypermobile joints and characteristic changes in the physiology of the chest, Marfan syndrome also results in ocular and central nervous system malfunction. However, the most severe and life-threatening manifestations are related to the cardiovascular system. Presentations of malformations in the cardiovascular system include aortic root dilation, mitral valve prolapse, aortic valve insufficiency, ascending aortic aneurysms, aortic dissection and rupture. The principal site of the lesions is in the ascending aorta. Dilation, aneurysm and dissection of the aortic root are the most prominent cardiovascular problems in Marfan patients.

FBNI has been found to be the affected gene by one of the 2900-plus described different genetic mutations in the development of Marfan syndrome. The gene encodes fibrillin-1, an extracellular matrix protein, which is a major component of microfibrils that form the extracellular matrix of both elastic and non-elastic connective tissues. Mutation in *FBNI* leads to abnormalities in the fibrillin-1 protein, and subsequently in the microfibrils as well, which ultimately results in connective tissue weakness. Folic acid metabolism enzyme polymorphisms are believed to be responsible for the elevation of homocysteine (HCY) concentration in the blood plasma, which correlates to the formation of certain cardiovascular diseases. It has been demonstrated that high (HCY) levels in homocystinuric patients can modify fibrillin-1 by abolishing calcium binding and by rendering protein domains significantly more susceptible to proteolytic degradation due to affected disulfide bonds.

Methylenetetrahydrofolate reductase (MTHFR) catalyses the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate (5-MTHF). 5-MTHF is required for methionine synthase (MTR) to remethylate HCY to methionine. MTR is activated by the methionine synthase reductase (MTRR) enzyme. A cytosine-thymine point mutation c.665C>T (p.Ala222Val) is the most frequent single nucleotide polymorphism (SNP) of the *MTHFR* gene (rs1801133, also known as C677T) and results in a 50-60% less active MTHFR enzyme. A more moderate lowering of the enzyme activity comes from the *MTHFR* polymorphism c.1286A>C (p.Glu429Ala; rs1801131, also known as A1298C). Guanine instead of adenine commonly occurs in *MTR* and *MTRR* at positions 2756 (c.2756A>G,

p.D919G; rs1805087, also known as A2756G) and 66 (c.66A>G, p.Ile22Met; rs1801394, also known as A66G), respectively.

Among individuals who do not have Marfan syndrome but develop aortic aneurysms the prevalence of *MTHFR* and *MTR* polymorphisms is higher. *MTHFR* polymorphisms also correlate with cervical dissections in non-Marfan patients. So far, only one study reported that the *MTHFR* C677T SNP associated with aortic aneurysms and dissections in Marfan patients. No studies have been published analysing association between *MTR* and *MTRR* polymorphisms and dissections in Marfan syndrome. Although elevated plasma HCY level and polymorphisms of enzymes in the folate metabolism have been evaluated to some extent, data concerning their interactions in Marfan syndrome are very few and not comprehensive. A more detailed investigation of *MTR* and *MTRR* SNPs and their roles in Marfan syndrome and other cardiovascular diseases is also required. Therefore, we sought to explore the association between homocysteinaemia, the severity of cardiovascular diseases, *MTR* A2756G and *MTRR* A66G polymorphisms in patients with Marfan syndrome.

Aims

Although Marfan syndrome is an inhomogenic disease, the life threatening manifestations are related to the ascending aorta such as aortic aneurysm, aortic dissection or rupture. These manifestations could form in the twenties, much more earlier than in the normal patient population.

Therefore our aims were to,

1. identify gene polymorphisms of the folate metabolism's enzymes, which could strongly correlate with the malignant cardiovascular manifestations in Marfan syndrome.
2. help in the surgical decision making for the optimal timing of the intervention (operation).
3. find biomarkers, which could be significant predictors of the severe cardiovascular manifestations and could predict the „aggressivity” of Marfan syndrome.

Methods

Subjects

A cohort of 71 consecutive Marfan patients (56 unrelated and 15 first degree relatives) enrolled in the Marfan biobank and 117 control subjects were involved in this study. The diagnosis of Marfan syndrome was established according to the revised (2010) Ghent criteria which ensure an excellent specificity and sensitivity to identify Marfan syndrome by clinical features. In the absence of family history, Marfan syndrome was confirmed as involvement of the ascending aorta and systemic score ≥ 7 pts(points). In the presence of family history, MFS was diagnosed if a patient had aortic root enlargement or aortic dissection, or had a systemic score of 7 pts or more according to the internationally excepted revised Ghent nosology. The characteristics of Marfan patients are shown in Table 1. Between October 2011 and April 2012 clinical data and biological samples (serum, blood, genomic DNA, cDNA from (peripheral blood mononuclear cell) PBMC) from 34 women (47.8%) and 37 men (52.1%) with Marfan syndrome were collected in the biobank. Average age was 36 years [27-43] at the time of the investigation. During the same period, we studied 117 apparently healthy individuals (90 males and 27 females) with a median age of 39 [34-49] years. The selection criteria of controls were absence of aortic aneurysm/dissection and family history of Marfan syndrome in the anamnesis. Total DNA samples were obtained from control groups too. All individuals participating in the study provided written informed consent in accordance with regulations of the Institutional Review Board of the Ethics Committee (ETT 13699/2011).

Table.1

Groups	A (n=27)	B (n=17)	C (n=27)	C1 (n=14)	C2 (n=13)
Age (years)*	29 ± 12	29 ± 10	35 ± 13	34 ± 14	37 ± 12
Male	16	5	12	4	8
Involvement of cardiovascular system	No intervention required	Mild involvement required intervention	Severe cardiovascular involvement	Annuloaortic ectasia	Aortic dissection
Anthropometric					

(measured)					
Height	178 [170-180]	187 [182-200]	182 [173-190]	185 [181-196]	175 [172.7-187]
Lower segment (cm)	95 [89-98]	99 [94.7-106]	95 [90-104]	99 [90-113]	91 [87.5-99]
Arm span (cm)	182 [177-184]	194 [183-207]	189 [180-200]	189 [184-202]	188.5 [179-198.5]
Foot size	41 [40-43]	44 [42-48]	43 [41-46]	44 [42-46]	42 [41-44]
Weight (kg)	62 ± 22.5	74.5 ± 26.4	72.1 ± 21.0	69.6 ± 25.4	69.2 ± 25.1
Aortic diameters (mm)	32 [27 - 37]	49 [46.8 - 50]	62.5 [54.8 - 70]	59.5 [50 - 67.8]	67.5 [60 - 75]
Anthropometric (calculated)					
Body Mass Index (BMI; kg/m ²)	19.9 ± 6.3	20.7 ± 6.7	21.6 ± 5.7	20.0 ± 6.7	21.6 ± 7.4
Body surface area (m ²) /Mosteller/	1.75 [1.61-1.87]	1.99 [1.81-2.26]	1.94 [1.85-2.08]	1.94 [1.89-2.09]	1.95 [1.80-2.01]
Ghent nosology (%)					
Positive family history	100%	76%	59%	64%	54%
Systemic score (SSc)	8 [7-9.5]	9 [8-9]	7.5 [7-8]	8 [7-8]	7 [7-8]
SSc < 7 pts	0%	0%	15%	14%	15%
SSc 7-10 pts	93%	100%	79%	79%	77%
SSc > 10 pts	7%	0%	7%	7%	8%
Ectopia lentis	36%	29%	22%	21%	23%

Classification based on the severity of cardiovascular manifestations of patients with MFS

*Patients age at the time of the operation.

Classification of Marfan patients based on the severity of aortic involvement

On the basis of the cardiovascular manifestations, Marfan patients were divided into three groups:

- Group A (n=27): Patients who at the time of investigation do not have cardiovascular manifestations which require elective surgery;
 - no aortic dissection, no aortic valve insufficiency
 - ascending aorta diameter < 45 mm
- Group B (n=17): Patients with mild cardiovascular involvement, which required an elective surgery of the ascending aorta;

Prophylactic surgery was indicated according to ESC/EACTS guidelines, which denote:

- ascending aorta diameter 45-50 mm or sinus of Valsalva 45-48 mm with grade I-II aortic valve insufficiency
- And one of the below mentioned risks:
 - size increase of 2 mm/year in repeated examinations
 - family history of aortic root dissection

— Group C (n=27): Patients with major and severe cardiovascular involvement, which were subdivided between those who were operated for annuloaortic ectasia (subgroup C1, n=14) or those with repair of type A aortic dissection (subgroup C2, n=13). Annuloaortic ectasia (subgroup C1, n=14) surgery was indicated according to ESC/EACTS guidelines, which denote:

- ascending aorta diameter > 50mm or sinus of Valsalva > 48mm with III-IV degree of aortic valve insufficiency

Aortic dissection (subgroup C2, n=13) surgery was indicated according to ESC/EACTS guidelines, which denote:

- type A aortic dissection confirmed by CT (Group C2)

Histopathology of the aortic wall and valve

During aortic root surgery aortic wall and valve samples were sent for pathological examination to assess the etiology of aortic root disease.

DNA extraction

Total genomic DNA (gDNA) was extracted from EDTA-stabilised blood samples using the ZR Genomic Kit (Zymo Research, Irvine, USA) and DNA concentration was measured by a NanoDrop 1000 spectrophotometer (NanoDrop, Wilmington, USA). The gDNA quality was monitored with Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, USA) after the measurement of DNA concentration. Total gDNA was used for polymerase chain reaction (PCR) amplification, hybridization and quantitative real-time – PCR.

HCY, Folate and vitamin B12 measurements

78 serum samples from MFS patients and 117 serum samples from healthy controls were collected and stored at -70°C . Total (free plus disulfide-bound) HCY plasma concentrations were determined by a fully automated fluorescence polarization immunoassay (Abbott IMx analyzer, Ontario, Canada). Hyperhomocysteinemia was defined as HCY plasma levels $>14\ \mu\text{mol/L}$. Vitamin B12 and folate plasma concentrations were determined by the fully automated Beckman Coulter UniCel DxI 800 instrument using Access B12 and Access Folate Determination reagents (Beckman Coulter, Brea, USA).

Gene polymorphism detection

Two polymorphisms of the *MTHFR* gene (NM_005957.4:c.665C>T and c.1286A>C) were detected by a GenoType MTHFR Test System (Hain Bioscience, Nehren, Germany) using PCR hybridization and alkaline phosphatase reaction on a membrane strip. Using PCR TaqMan probes, the *MTR* gene polymorphism c.2756A>G (NM_000254.2) was detected by a Duplica RealTime MTR A2756G Kit (Euro Clone, Milano, Italy), while the *MTRR* gene polymorphism c.66A>G (NM_002454.2) was detected by a Duplica RealTime MTRR A66G Kit (Euro Clone, Milano, Italy).

SNP score

We evaluated all four gene polymorphisms in all groups and set up a single nucleotide polymorphism (SNP) score system, with no points for absence of gene polymorphism, 1 point for each heterozygous polymorphism, and 2 points for each homozygous mutation. The maximal score was 8, which means 4 homozygous polymorphisms in all measured genes in the same subject.

Statistical analysis

All continuous variables were expressed as mean \pm SD or median with interquartile ranges, whereas categorical variables were expressed as percentage. The Shapiro-Wilk test was used to check for normality of the data before further analysis. Mann-Whitney U-test was used to

compare continuous parameters with non-normally distributed variables. Spearman rank correlation was assessed to calculate the correlations between HCY, folate and vitamin B12. Departures from the Hardy–Weinberg equilibrium were assessed by the χ^2 - test. Investigation for differences among three or more groups was performed using Kruskal-Wallis analysis of variance (ANOVA) test when normality assumption was violated. Post hoc comparisons were done by using Mann-Whitney test with Bonferroni correction. We used univariate and multivariate logistic regression analysis to evaluate the risk factors of aortic dissection and major cardiovascular involvement. A two-tailed p-value of <0.05 was considered significant. All analyses were performed using SPSS 20.0 software (Chicago, IL, USA).

Results

Table 1 shows the clinical data of Marfan patients in each groups identified on the basis of the cardiovascular involvement.

Hcy, Folate and B12 plasma levels and SNP score

Table 2 shows the plasma levels of HCY, folate and B12 vitamin in the five groups. The box-plots of HCY plasma levels in Marfan patients and control subjects are shown in Figure 1. HCY differed significantly among the 5 study groups ($p < 0.0001$). Multiple comparisons showed a significantly higher level of HCY in Group C2 compared to Groups A, B, C1 and control group ($p < 0.0001$, $p < 0.0001$, $p = 0.001$ and $p = 0.003$, respectively) (Table 2). Similarly, folate differed significantly between the groups ($p < 0.0001$), being lower in Group C2 than in Groups A, B, C1 and control subjects ($p < 0.0001$, $p = 0.02$, $p < 0.0001$ and $p < 0.0001$, respectively) (Table 2). We also found significant difference in vitamin B12 plasma levels among groups according to Kruskal-Wallis test ($p = 0.015$) (Table 2). Multiple comparisons showed significantly decreased levels of vitamin B12 in Group C2 compared to Groups A and B ($p = 0.001$ and $p = 0.012$, respectively). SNP score was higher in Group C2 compared to all other groups with a p value of less than 0.0001 (Fig. 2). The correlation curves of HCY, folate and vitamin B12 in Marfan patients are depicted in Figure 3.

Table.2

Plasma levels of HCY, folate and vitamin B12, and the median SNP score of the investigated groups.

HCY ($\mu\text{mol/l}$)	Folate (nmol/l)	Vitamin B12 (pmol/l)	SNP score
---------------------------	-----------------	----------------------	-----------

Controls	11.7 [9.6-14.2] †	27.1 [23.2-33.6] †	256.4 [174.7-364.4]	0 [0-1] †
Marfan patients				
Group A	9.2 [8.8-10.5] †	29.8 [22.6-34.5] †	356. [285.2-419,8] †	1 [0-1] †
Group B	10.7 [9.9-11.5] †	19.9 [19.3-27.8] †	322.9 [247.7-379.1] ‡	1 [0-1] †
Group C*	13.4 [10.9-15.1]	20.7 [15.0-26.1]	246.7 [206.3-299.5]	2 [1-4]
C1 Annuloaortic ectasia	11.5 [10.2-12.6] †	24.8 [21.0-28.5] †	262.3 [243.4-321.4]	1 [1-2] †
C2 Aortic dissection	14.9 [14.1-16.6]	15.5 [10.5-17.6]	223.2 [197.8-261.6]	4 [3-5]

* Group C was excluded from analysis

† P<0.01 vs Group C2 Aortic dissection

‡ P<0.05 vs Group C2 Aortic dissection

The main pathological finding were cystic medial degeneration (CMD) in all samples which is consistent with the diagnosis of Marfan syndrome.

Gene polymorphisms of folic acid metabolism enzymes

The genotype distributions of the investigated four gene polymorphisms are depicted in Table 3. All gene polymorphism were in Hardy-Weinberg equilibrium in the Marfan population and in control population, except the C677T *MTHFR* genotype distribution of the control group ($p=0.003$). The prevalence of heterozygotes and homozygotes for the *MTHFR* polymorphism c.665C>T was higher in Marfan patients (19.3% and 7.7%) than in control subjects (10.3% and 2.7%). The heterozygous and homozygous polymorphisms in the two other examined genes were also more frequent in the Marfan groups compared to control subjects (Table 3). Group C2 had the highest prevalence of homozygotes for all four gene polymorphisms. In the Marfan cohort a significant genotype-phenotype association between HCY plasma levels and the polymorphisms of the enzymes of folic acid metabolism was observed as shown in Table 3.

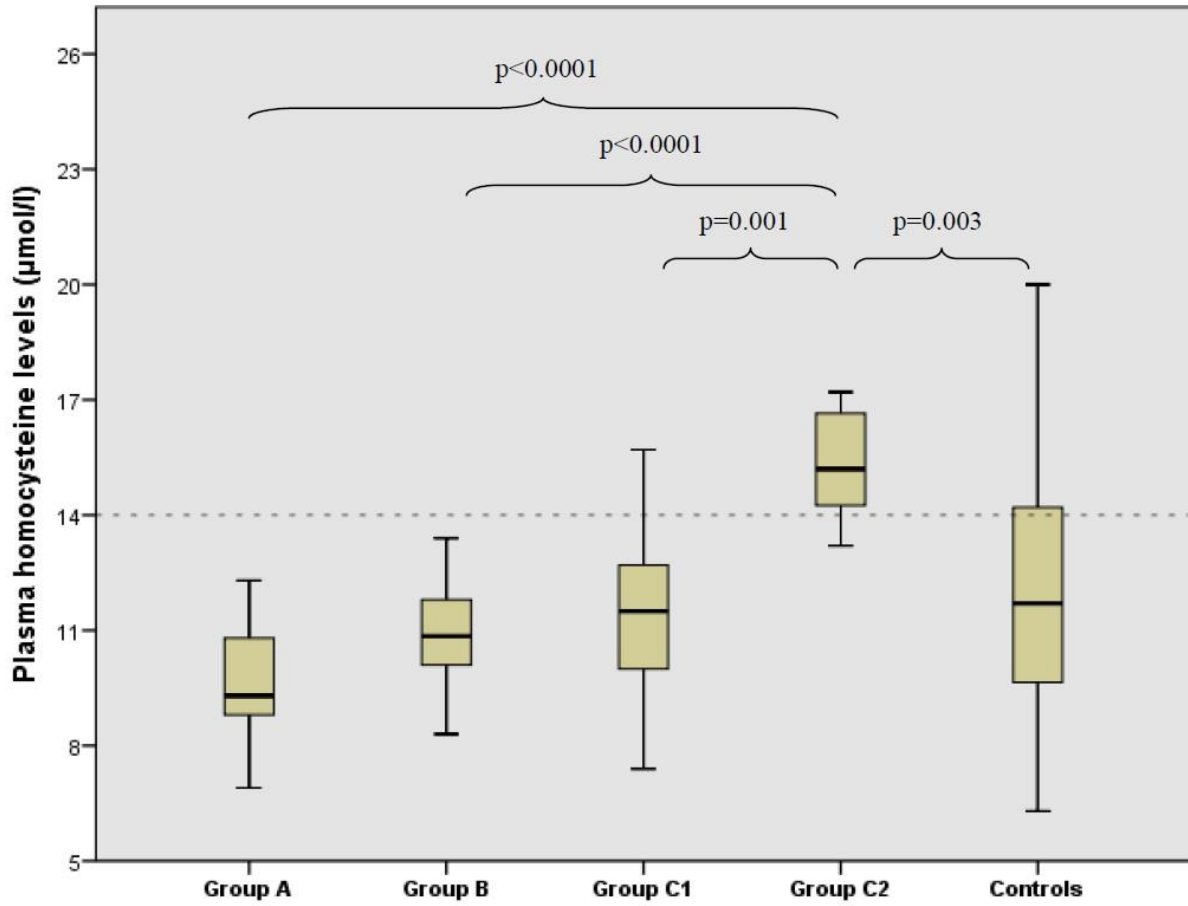


Figure 1: HCY plasma levels in Marfan patients and control subjects. A=no intervention required, B=mild involvement, C1=annuloaortic ectasia, C2=aortic dissection. Multiple comparisons showed significant higher levels of HCY in Group C2 compared to Groups A, B, C1 and control group ($p<0.0001$, $p<0.0001$, $p=0.001$ and $p=0.003$, respectively)

Univariate and multivariate analysis

In the univariate logistic regression analysis major cardiovascular involvement (Group C) and aortic dissection (Group C2) in Marfan patients were associated with HCY, folate, and vitamin B12 plasma levels. However, multivariate logistic regression analysis revealed that only HCY plasma level was an independent risk factor for the severe cardiovascular involvement (Group C; OR 1.85, 95% CI 1.28-2.67, $P=0.001$) as well as for aortic dissection (Group C2; OR 2.49, 95% CI 1.30-4.78, $p=0.006$).

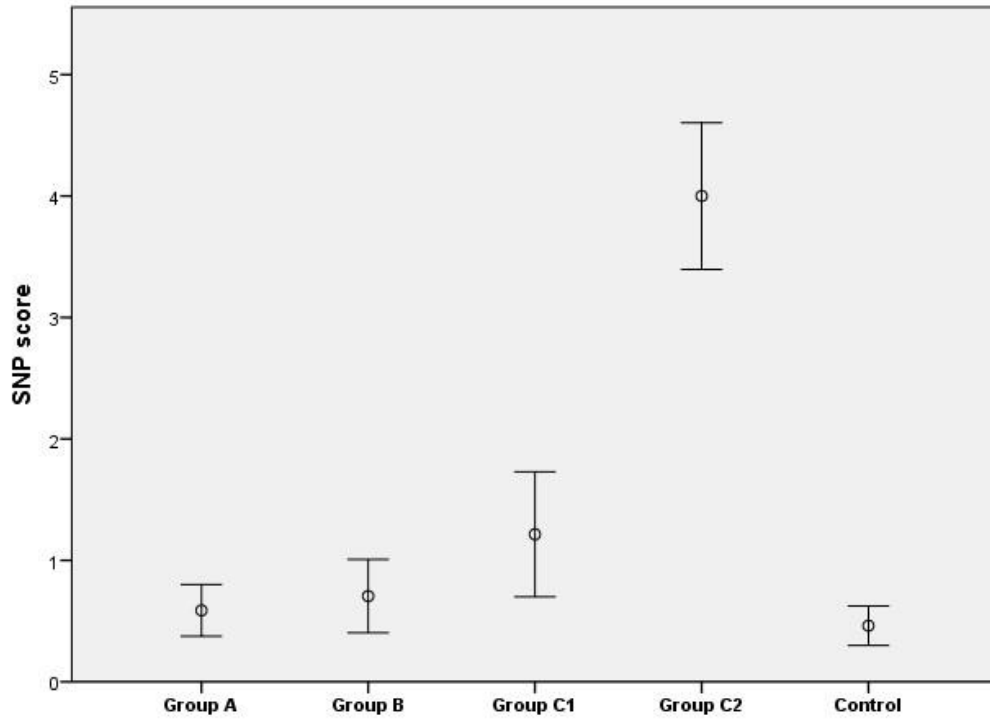


Figure 2: SNP scores of Marfan patients and control subjects. A=no intervention required, B=mild involvement, C1=annuloaortic ectasia, C2=aortic dissection. SNP score was higher in Group C2 compared to all other groups ($p < 0.0001$).

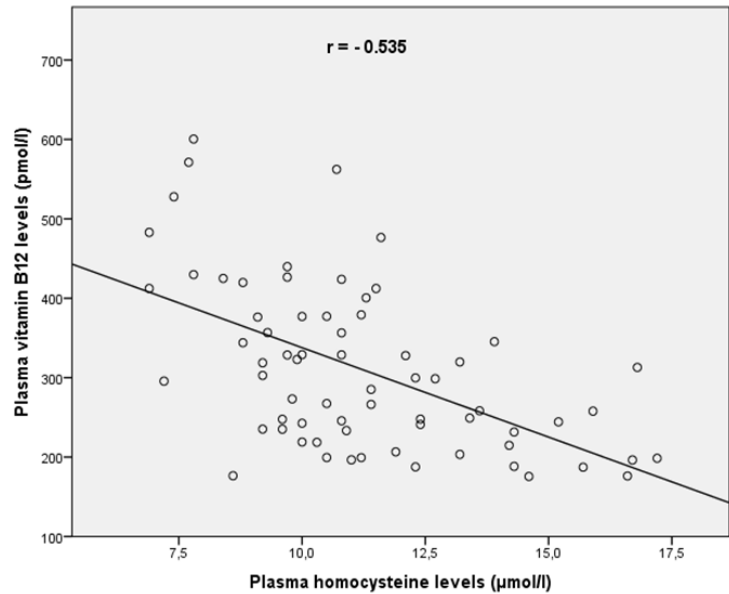
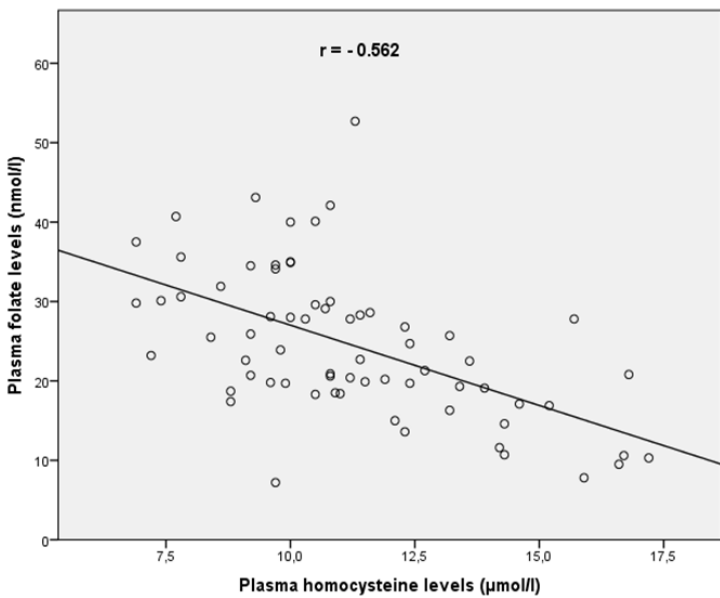


Figure 3 : Correlation curves of HCY, folate and vitamin B12 in Marfan patients. (all $p < 0.001$)

Discussion

To the best of our knowledge this is the first study to investigate four gene polymorphisms of the three most investigated folic acid metabolism enzymes in Marfan syndrome. We found that Marfan patients with type A aortic dissection present with significantly higher plasma level of HCY and lower levels of folate and vitamin B12 compared to Marfan patients with mild or no cardiovascular manifestations. Interestingly, a higher SNP score was observed in Marfan patients with severe involvement of the cardiovascular system as well as in aortic dissection group when compared to other Marfan groups and to controls. A higher prevalence of heterozygous and homozygous genotypes of the four examined gene polymorphisms was found in patients with aortic dissection. A genotype-phenotype correlation between HCY plasma levels and *MTHFR*, *MTR* and *MTRR* gene polymorphisms was observed. Indeed, genotypes thought to lower the activity of folic acid metabolism enzymes were associated with a relatively high plasma level of HCY. The multivariate logistic regression analysis revealed that higher HCY plasma level is an independent predictor of severe cardiovascular manifestation and aortic dissection.

Our results support the findings obtained in Marfan patients with hyperhomocysteinaemia, suggesting that the decreased activity in the above enzymes' leads to a high plasma level of HCY, and that plays an important role in weakening the extracellular matrix of the vascular wall. Doronzo et al. described that HCY increases MMP-2 synthesis and secretion in cultured arterial human vascular smooth muscle cells derived from aorta and this effect is concentration and time-dependent. The effective HCY concentrations were in the range of mild to severe hyperhomocysteinemia. The increased levels of matrix metalloproteinases (MMP-2, -3, and -9) are responsible for loss of of the aortic wall integrity leading to aneurysm formation and dissection in Marfan syndrome. According to Raaf et al., hyperhomocysteinemia was associated with increased expression and signalling of transforming growth factor- β (TGF- β), a molecule with a key role in the pathomechanism of Marfan syndrome, and one that upregulates the expression of many matrix metalloproteases. While increased level of MMPs enhances elastin and microfibril degradation of the extracellular matrix, MMPs seem to be responsible for disintegration of elastic fibres. Therefore, their overproduction of these proteases reduces connective tissue elasticity, and leads to weakness of the aortic wall (Figure 4). Fibrillin-1 is a target for HCY, reducing significantly N- to C-terminal self-interaction properties, which represent a critical step in the biogenesis of microfibrils. Irreversible homocysteinylation of these long-lived proteins could occur a cumulative progression in the cardiovascular manifestations of Marfan syndrome.

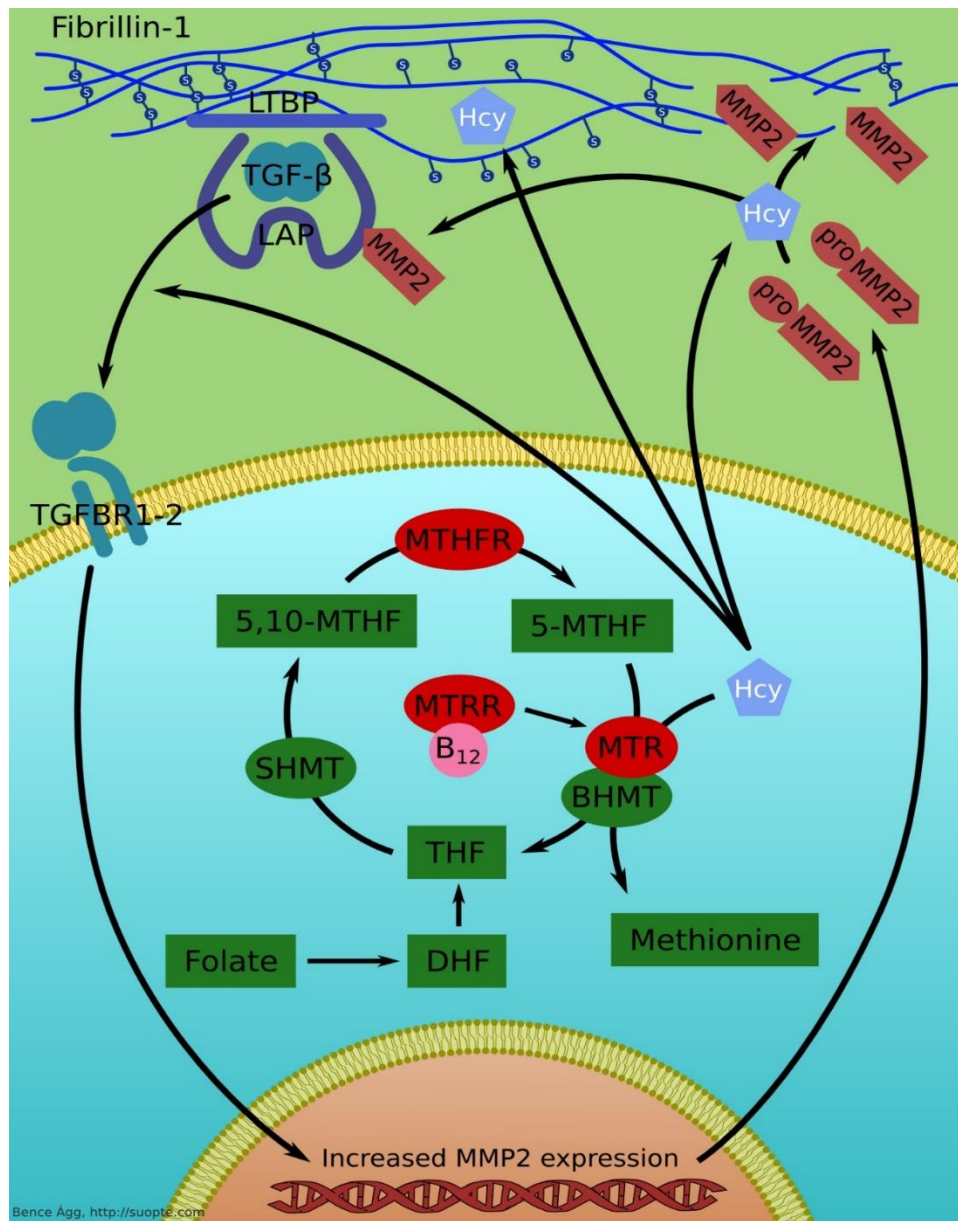


Figure 4: The role of folic acid metabolism enzymes in the pathomechanism of the homocysteine-fibrillin-1 interactions. Fibrillin-1 is a target for HCY, reducing significantly N- to C-terminal self-interaction properties, which represent a critical step in the biogenesis of microfibrils. The higher level of HCY could react with some of the numerous free cysteinyl sulfhydryl (SH) groups in fibrillin-1 or could disrupt critical Cys-Cys disulfide bridges. Irreversible homocysteinylolation of these long-lived proteins could occur a cumulative progression in the cardiovascular manifestations of Marfan syndrome. HCY increases matrix metalloproteinase-2 (MMP-2) synthesis and secretion, and this increased levels of MMP-2 are responsible for the destruction of the aortic wall leading to formation of aneurysm and dissection in Marfan syndrome. Hyperhomocysteinemia were associated with increased expression of TGF- β , which molecule upregulates the expression of many matrix

metalloproteinases and elastases. While increased level of elastases and MMPs enhances elastin and microfibril degradation of the extracellular matrix, MMPs seem to be responsible for disintegration of elastic fibres. *LTBP*: Latent TGF- β Binding Protein; *LAP*: Latency Associated Protein; *TGF- β* : Transforming Growth Factor- β ; *HCY*: Homocysteine; *TGFBRI-2*: TGF- β binding receptors 1 and 2; *MMP-2*: Matrix metalloproteinase-2; *MTHFR*: Methylene tetrahydrofolate reductase; *MTR*: Methionine synthase; *MTRR*: Methionine synthase reductase; *SHMT*: Serine-hydroxymethyltransferase; *DHF*: Dihydrofolate; *THF*: Tetrahydrofolate; *BHMT*: Betain-homocysteine methyltransferase; *5-MTHF*: 5-methyltetrahydrofolate; *5,10-MTHF*: 5,10-Methenyltetrahydrofolate; *B12*: Vitamin B12.

Sbarouni et al. found that non-Marfan patients with type A aortic dissection had significantly higher levels of HCY and B12 and lower levels of folate compared to patients with chronic aneurysms and normal controls. Although many studies examined the folic acid metabolism enzymes separately, none investigated all four polymorphisms in the same time like we did in our current work. Our SNP scoring provides a potential risk stratification system which together with the HCY, folate and B12 plasma levels, could help improve the prediction of cardiovascular events in Marfan syndrome. Combining blood based biomarkers and genetic profiling with clinical monitoring and echocardiographic measurements could further help guide treatment, whether prophylactic surgery or pharmacotherapy.

One of the limitations of this study might be the control group. Although all of the controls were apparently healthy (without history of cardiovascular diseases), we found that the HCY plasma levels were higher than the previously published data by Giusti et al. However, these results do not influence the fact that within Marfan patients a higher HCY plasma level– and a more adverse genotype with genotype are strongly associated with pathological lesions of the aortic wall such as aortic dissection and annuloaortic ectasia. A well-defined prospective investigation is warranted to confirm the results of our retrospective study. The classification of patients according severity of cardiovascular involvement is based on the the internationally accepted guidelines and principally reflects a clinical/surgical decision making perspective. Therefore this classification could have ignored some rational theoretical aspects (such as age, diameter-dilatation rate, Z-score and involvement of the mitral valve), that can be a main limitation of the study.

Conclusion

In conclusion, Marfan patients who develop type A aortic dissection have significantly higher plasma level of HCY and lower levels of folate and vitamin B12 compared to Marfan patients with mild or no cardiovascular manifestations. Marfan patients with severe cardiovascular involvement and in particular with aortic dissection had a higher SNP score, HCY plasma levels and prevalence of homozygous genotypes of folic acid metabolism enzymes when compared to the patients with mild or no cardiovascular manifestations. This suggests an important role of folic acid metabolism in the development and remodelling of the extracellular matrix of the aorta. These results could indicate a preventive supplementation of folic acid and vitamin B12 for Marfan patients with high SNP score.

List of publications in the topic

Ágota Annamária, Ágg Bence, **Benke Kálmán**, Joó József, Langmár Zoltán, Marosi Krisztina, Lelovics Zsuzsanna, Deé Kitti, Nagy Péter, Köles Bernadett, Horváth Endre, Crespo Zsuzsanna, Szabolcs Zoltán, Nagy Zsolt. (2012) A Marfan szindróma biobankjának létrehozása. **Orvosi Hetilap** 153: (8) 296-302

Benke Kálmán, Ágg Bence, Szilveszter Bálint, Odler Balázs, Nagy Zsolt, Pólos Miklós, Merkely Béla, Szabolcs Zoltán. (2014) A Marfan-szindróma molekuláris pathomechanizmusa. **Cardiologia Hungarica** 44: (2) 115-121.

Benke Kálmán*, Sayour Alex Ali*, Ágg Bence, Radovits Tamás, Szilveszter Bálint, Odler Balázs, Németh Balázs Tamás, Oláh Attila, Pólos Miklós, Mátyás Csaba, Ruppert Mihály, Hartványosky István, Maurovich-Horvát Pál, Merkely Béla, Szabolcs Zoltán. (2016) Génpolimorfizmusok, mint rizikófaktorok a Marfan-szindróma kardiovaszkuláris manifesztációinak előrejelzésében. **Cardiologia Hungarica** 46: (2) 76-81. *megosztott elsőszersőség

Benke Kálmán , Ágg Bence, Szilveszter B , Tarr F, Nagy ZsB , Pólos M, Daróczi L, Merkely B, Szabolcs Zoltán. (2013) The role of TGF- β in Marfan syndrome.

Cardiology Journal 20: (3) 227-234. **IF: 1,209**

Benke Kálmán, Ágg Bence, Mátyás Gábor, Szokolai Viola, Harsányi Gergely, Szilveszter Bálint, Odler Balázs, Pólos Miklós, Maurovich-Horvat Pál, Radovits Tamás, Merkely Béla, Nagy B. Zsolt, Szabolcs Zoltán. (2015) Gene polymorphisms as risk factors for predicting the cardiovascular manifestations in Marfan syndrome. **Thrombosis and Haemostasis.** 114: (4) 748-756. **IF= 5,25**

Benke Kálmán, Ágg Bence, Szabó Lilla, Szilveszter Bálint, Odler Balázs, Pólos Miklós, Cao Chun, Maurovich-Horvat Pál, Radovits Tamás, Merkely Béla, Szabolcs Zoltán. (2016) Bentall procedure: quarter century of clinical experiences of a single surgeon. **Journal Cardiothoracic Surgery.** 11: (1) 19.9 p. **IF=1,03**

List of other publications

Szabolcs Zoltán, Ágg Bence, **Benke Kálmán**, Pólos Miklós, Hüttl Kálmán, Bartha Elektra. (2013) Marfan-szindrómás beteg aortadisszekciója.

Orvostovábbképző Szemle. 20: (5) 63-67.

Kárpáti Soma Zsolt, Szokolai Viola, Harsányi Gergely, Ágg Bence, **Benke Kálmán**, Langmár Zoltán, Elbert Gábor, Merkely Béla, Szabolcs Zoltán, Nagy Zsolt. (2015) A prenatális genetikai diagnosztika alkalmazásának lehetősége Marfan-szindrómában. **Egészség Akadémia** 6: (1) 5-11.

Szentmihályi Iлона, Barabás János Imre, Bali Ágnes, Kapus Gábor, Tamás Csilla, Sax Balázs, Németh Endre, Pólos Miklós, Daróczi László, Kőszegi Andrea, Cao Chun, **Benke Kálmán**, Kovács Péter Barnabás, Fazekas Levente, Szabolcs Zoltán, Merkely Béla, Hartyánszky István. (2016) Heart transplantation and long-term lvad support cost-effectiveness model. **Magyar Sebészet.** 69: (4) 186-193.

Ágg Bence, **Benke Kálmán**, Szilveszter Bálint, Pólos Mikós, Daróczi László, Odler Balázs, Nagy Zsolt, Tarr Ferenc, Merkely Béla, Szabolcs Zoltán. (2014) Possible extracardiac predictors of aortic dissection in Marfan syndrome. **BMC Cardiovascular Disorders** 14: 47.11 p. **IF: 1,88**

Édes István Ferenc, Ruzsa Zoltán, Szabó György, Nardai Sándor, Becker Dávid, **Benke Kálmán**, Szilveszter Bálint, Merkely Béla. (2015) Clinical predictors of mortality following rotational atherectomy and stent implantation in high risk patients – a single center experience. **Catheterization and Cardiovascular Intervention.** 86: (4) 634-641. **IF=2,181**

Szilveszter Bálint, Elzomor Hesham, Károlyi Mihály, Kolossvary Márton, Raaijmakers Rolf, **Benke Kálmán**, Celeng Csilla, Bartykowszki Andrea, Bagyura Zsolt, Lux Árpád, Merkely Béla, Maurovich-Horvat Pál. (2016) The effect of iterative model reconstruction on coronary artery calcium quantification. **International Journal of Cardiovascular Imaging.** 32: (1) 153-160 **IF=1,88**

Odler Balázs, Ivancsó István, Somogyi Vivien, **Benke Kálmán**, Tamási Lilla, Gálffy Gabriella, Szalay Balázs, Müller Veronika. (2015) Vitamin D deficiency is associated with impaired disease control in Asthma COPD Overlap Syndrome patients. **International Journal of Chronic Obstructive Pulmonary Disease.** 10: (1) 2017-2025. **IF=3,046**

Odler Balázs, Cseh Áron, Constantin Tamás, Fekete György, Losonczy György, Tamási Lilla, **Benke Kálmán**, Szilveszter Bálint, Müller Veronika. (2017) Long time enzyme replacement therapy stabilizes obstructive lung disease and alters peripheral immune cell subsets in Fabry patients. **Clinical Respiratory Journal.** DOI: 10.1111/crj.12446 **IF=2,147**

Oláh Attila, Kellermayer Dalma, Mátyás Csaba, Németh Balázs Tamás, Lux Árpád, Szabó Lilla, Török Mária, Ruppert Mihály, Meltzer Anna, Sayour Alex Ali, **Benke Kálmán**, Hartyánszky István, Merkely Béla, Radovits Tamás. (2017) Complete Reversion of Cardiac Functional Adaptation Induced by Exercise Training. **Medicine and Science in Sports and Exercise.** 49: (3) 420-429. **IF=4,041**

Benke Kálmán*, Sayour Alex Ali*, Mátyás Csaba, Ágg Bence, Németh Balázs Tamás, Oláh Attila, Ruppert Mihály, Hartyánszky István, Szabolcs Zoltán, Radovits Tamás, Merkely Béla,

Szabó Gábor. (2017) Heterotopic Abdominal Rat Heart Transplantation as a Model to Investigate Volume Dependency of Myocardial Remodeling. **Transplantation.** 101: (3) 498-505. **IF=3,69** *megosztott elsőszerzőség

Korkmaz-Icöz Sevil, Radovits Tamás, Loganathan Sivakannan, Li Shiliang, Ruppert Mihály, **Benke Kálmán**, Brlecic Paige, Szabó Csaba, Karck Matthias, Szabó Gábor. (2017) Prolonging hypothermic ischemic cardiac and vascular storage by inhibiting the activation of the nuclear enzyme poly (adenosine diphosphate ribose) polymerase. **European Journal of Cardiothoracic Surgery.** 50: (5). 829-835. **IF= 2,803**