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Clinical research

Phenotypic variability of cardiovascular manifestations in Marfan Syndrome

Possible role of hyperhomocysteinemia and C677T MTHFR gene polymorphism

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Aims The aim of this study was to evaluate (1) homocysteinemia and the prevalence of the C677T MTHFR polymorphism in Marfan patients and (2) whether the severity of cardiovascular manifestations is associated with homocysteinemia and/or C677T polymorphism.
Methods and results We studied 107 patients subdivided into three subgroups based on the severity of cardiovascular manifestations: (A) no involvement (*n*=4); (B) mild involvement (*n*=45); (C) aortic dilatation or aortic dissection (*n*=58), and 189 controls. Total homocysteine (tHcy) was significantly higher in subgroup C than in subgroup B. In subgroup C patients with dissection tHcy was higher than in those without dissection. In subgroup C the prevalence of 677T homozygotes was higher, but not significantly, than in the subgroup B. In patients with dissection the prevalence of 677T homozygotes was significantly higher than in those without dissection and than in subgroup B. In the logistic regression analysis, severe cardiovascular manifestations and aortic dissection in Marfan patients were associated with tHcy plasma levels.
Conclusions Our data indicate an association between the severity of the cardiovascular manifestations, in particular aortic dissection, and elevated tHcy levels. This suggests an important role for tHcy in determining phenotypic variability in Marfan patients and provides further evidence for the association of homocysteinemia with the damage of the vascular system.

Introduction

Marfan Syndrome (MFS), an inherited autosomal dominant disorder of connective tissue, is characterized by variable phenotypic manifestations involving primarily the cardiovascular, ocular, musculoskeletal and central nervous systems. Cardiovascular manifestations include progressive dilatation of the aortic root, ascending aortic aneurysms, aortic dissection and rupture, and aortic and mitral valvular insufficiency.[1,2] Aortic root dilatation and related complications represent the main cause of death.[3,4] In 1995 Silverman and co-workers[5] reported an

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increase in life expectancy by more than 25% in comparison to that reported by Murdoch in 1972.[6] However, the disease continues to produce significant morbidity and mortality.[7] The histological abnormality underlying ascending aortic dilatation, aneurysm, and dissection is Erdheim's cystic medial necrosis (CMN),[8] which is characterized by loss of non-inflammatory smooth muscle cells, fragmentation of elastic fibres, and accumulation of basophilic ground substance (proteoglycans). Marfan Syndrome is due to mutations in the gene, located on chromosome 15q21.1, encoding fibrillin-1, a complex glycoprotein component of the microfibrils. These microfibrils form a scaffold for the deposition and aggregation of elastin, and the defect in their synthesis leads to structural weakness of the vascular wall.[9–11] Fibrillin-1 also has an independent structural role.[12] The relationship between the altered synthesis of microfibrils and the destruction of connective tissue components that occurs in MFS is not completely understood. In particular, it is difficult to explain the wide phenotypic variability not only inter-familial but also intra-familial.

A recent study has demonstrated an association between the presence of abdominal aortic aneurysm (AAA) in patients selected for surgical treatment of AAA and elevated homocysteine (tHcy) plasma levels and suggests that tHcy may induce endothelial perturbation and stimulation in these patients. Interestingly, the association was also observed in a subgroup of patients with AAA who did not have clinical or instrumental manifestations of atherosclerosis.[13] Moreover, a significant association between high plasma levels of tHcy and spontaneous cervical artery dissection was also found.[14] Moderate hyperhomocysteinemia,[15] a common condition that occurs in approximately 5–7% of the general population,[16] is a major independent risk factor for a number of diseases including atherosclerosis and thrombosis,[17] cognitive decline including Alzheimer's disease,[18] senile osteoporosis[19] and presbyopia.[20] The multisystem toxicity of tHcy is attributed to its spontaneous chemical reaction with many biologically important molecules, primarily proteins. tHcy plasma levels are influenced by several factors, including vitamins, age, gender, hormones; moreover, mutations in genes encoding enzymes involved in methionine metabolism, such as the methylenetetrahydrofolate reductase (MTHFR), cystathionine-β-synthase and methionine synthase,[21,22] may play an important role. Aim of this study was to evaluate tHcy plasma levels and investigate the prevalence of the C677T MTHFR polymorphism in patients with Marfan syndrome and in particular to determine whether increased tHcy plasma levels and/or C677T polymorphism are associated with the severity of the cardiovascular manifestations (aortic dilatation and dissection).

Methods

Subjects

One hundred and seven consecutive Marfan patients were selected among 150 Marfan patients referred to the Center for Marfan Syndrome and Related Disorders of the Department of Critical Area of the University of Florence from 1 July 2000 to 30 December 2001. All strictly fulfilled the diagnostic criteria for Marfan Syndrome according to De Paepe et al.[23] Selection criteria were age (>14 years), no previous aortic intervention, hypertension, diabetes mellitus, dyslipidemia, renal insufficiency and other major disease states. Hypertension was defined as systolic blood pressure higher than 140 mmHg or diastolic pressure higher than 90 mmHg. Hypercholesterolemia was defined as cholesterol levels higher than 5.18 mmol/l, hypertriglyceridemia as triglyceride levels higher than 2.26 mmol/L and hyperglycaemia as levels higher than 6.1 mmol/l. Creatinine levels were considered altered when higher than 106 μmol/l. None of the patients were receiving long-term treatment with β-blockers or other cardiovascular drug therapy before the study. During the same period, 189 apparently healthy unrelated volunteers were recruited from the staff of Hospital and University of Florence or their relatives. Subjects were selected on the basis of the history, clinical examination and comprehensive laboratory blood analysis. The selection criteria of controls were the absence of a family history of Marfan Syndrome, aortic aneurysm, aortic dissection and sudden death and those previously indicated for Marfan patients. All adult participants (Marfan patients and controls) gave informed written consent. In the case of minors, written consent was obtained from one parent.

Diagnostic criteria for cardiovascular system in Marfan patients

According to DePaepe et al.[23] a differentiation between major criterion being present in a system and the system 'being involved' (minor criteria) was applied. For the cardiovascular system (1) major criteria are: dilatation of the ascending aorta with or without aortic regurgitation and involvement of at least the sinuses of Valsalva, or dissection of the ascending aorta; (2) minor criteria are: mitral valve prolapse with or without mitral valve regurgitation, and/or dilatation of the main pulmonary artery, in the absence of valvular or peripheral pulmonic stenosis or any other obvious cause, below the age of 40 years and/or calcification of the mitral annulus below the age of 40 years, and/or dilatation or dissection of the descending thoracic or abdominal aorta below the age of 50 years.

Echocardiographic evaluation

Echocardiographic study was performed using an ultrasound system (Sequoia 812 Acuson Corporation, Mountain View, California, USA), equipped with a multiherz transducer (2.5–3.5 MHz). Echocardiographic recordings were obtained from the parasternal and apical windows with the patient in left lateral position. Complete M-mode, two-dimensional, pulse, continuous wave and colour-Doppler recordings were performed. M-mode echocardiographic measurements were made according to the guidelines of the American Society of Echocardiography.[24] Aortic dimensions were measured using M-mode recording from the left parasternal window. The two-dimensional image was used to ensure that the M-mode cursor was perpendicular to the long axis of the aorta. The aortic diameter was measured between the leading edges of the anterior and posterior aortic wall echoes, 2 to 3 cm above the aortic valve, at end-diastole (peak of R wave on electrocardiogram). The means of five diameter eight measurements in sequential cardiac cycles were used for data analysis. Aortic diameter/body surface area ratio was calculated. Aortic dilatation was established for values higher than 2.1 cm/m^2.[25]

Classification of Marfan patients based on severity of aortic involvement

On the basis of the cardiovascular manifestations patients were subdivided into three groups:

- subgroup A:patients without cardiovascular manifestations;
- subgroup B:patients with mild cardiovascular system involvement (involvement of mitral valve or pulmonary artery or descending thoracic aorta or abdominal aorta, and/or mild aortic dilatation <2.2 cm/m^2 body surface area);
- subgroup C:patients with major criteria in the cardiovascular system (moderate to severe aortic dilatation >2.2 cm/m^2 body surface area or with aortic dissection). Patients with major criteria in the cardiovascular system (subgroup C) were also subdivided between those with (subgroup C1) or those without (subgroup C2) aortic dissection.

Blood sampling

Blood withdrawal was performed between 7:30 and 9 am, after an overnight fasting. After discarding the first ml, blood was collected in tubes containing 4.4 mmol/l EDTA 2K$^+$ for tHcy and cysteine (Cy) determination and immediately placed on ice. Plasma was separated within 1 h and stored at −80 °C until tHcy and Cy determination. After the separation of plasma the samples were reconstituted with NaCl 0.9% solution and stored at −80 °C until DNA extraction.

tHcy, Cy, Folate and B12 measurement

tHcy (free and protein bound) plasma and Cy levels were determined by high-performance liquid chromatography (LKB 2248 pump, Pharmacia, Uppsala, Sweden) and fluorescence detection (fluorescence detector model 474, Waters, Milford, MA, U.S.A) according to a modification[13] of the Araki and Sako method. The intra-day and inter-day coefficients of variation were 1.6% and 4.0%, respectively. Hyperhomocysteinemia was defined as tHcy plasma level above the 95th percentile of the control subjects (10 μmol/l for females and 15 μmol/l for males). The reference values of our Hospital for tHcy concentrations are 3.5–12.5 μmol/l in females and 4.9–15.5 μmol/l in males. As regards Cy they are 125–311 μmol/l. Vitamin B12 and folate levels were determined by radioassays (ICN Pharmaceuticals, NY): reference values of our Hospital are 180–970 pmol/l and 3–17 nmol/l, respectively.

DNA extraction

Genomic DNA was extracted from peripheral blood cells according to Sambrook et al.[26]

C677T MTHFR gene polymorphism detection

The C677T MTHFR polymorphism was detected by PCR amplification of the DNA fragment containing the C677T MTHFR gene polymorphism.[27] Then 10 μl of the PCR product were digested with Hinf I according to the manufacturer's protocol (Amersham, Arlington Heights, IL, USA) in a final volume of 20 μl. After digestion, samples were loaded on an 8% polyacrylamide gel, and DNA fragments were visualized with ethidium bromide.

Statistical analysis

Statistical analysis was performed with the Stata 6.0 software for Windows (Stata Corporation, College Station, Texas). This study (107 Marfan patients and 189 control subjects) had 97.5% power at α=0.05 (one-sided) to detect a difference of 15% in the prevalence of hyperhomocysteinemia between Marfan patients and control subjects. In our previous studies on the association between hyperhomocysteinemia and vascular diseases[13,28] a significant difference in the prevalence of hyperhomocysteinemia of about 30% was observed. Departures from the Hardy–Weinberg equilibrium were assessed by the χ^2 test. The results are given as median and range. Fisher's exact test was used to compare proportions of homozygotes between groups. The rate ratio (RR) was used to compare the prevalence of homozygotes in patients and controls. The non parametric Mann–Whitney test for unpaired data was used for comparisons between single groups and the Kruskal–Wallis test among the groups. Multiple regression analysis was used to evaluate in Marfan patients the relationships among tHcy plasma levels and aortic dimensions (aortic diameter/body surface area ratio), sex, age, Cy, folate and vitamin B12 plasma levels. In order to evaluate the risk of severe cardiovascular manifestations (subgroup C) or dissection (subgroup C1) in Marfan patients we performed logistic regression analysis with severe cardiovascular manifestations or aortic dissection as dependent variable and C677T MTHFR genotypes, age, sex, tHcy, Cy, folate and vitamin B12 as independent variables. Odds ratios for severe cardiovascular manifestations and for aortic dissection in Marfan patients according to tHcy levels as continuous variable or categorical variable (to be hyperhomocysteinemic or normohomocysteinemic subjects) were evaluated. All odds ratios are given with the 95% confidence interval (CI). All *P*-values reported were two-tailed, with values of less than 0.05 considered statistically significant.

Table 1 Characteristics of subjects investigated

	Marfan patients	Control subjects	*P* value
No. of patients	107	189	
No. of males (%)	52 (49)	99 (52)	
Median age (y)	29	30	0.07
Age range (years)	(14–62)	(16–63)	
No. of smokers (%)	29 (27.3)	58 (30.1)	0.59

Results

The characteristics of the Marfan patients and control subjects are reported in Table 1. In Table 2 the characteristics of Marfan patients in the subgroups identified on the basis of the cardiovascular involvement are reported.

tHcy plasma levels

Table 3 shows tHcy plasma levels in the different groups.

The box-plots of tHcy plasma levels in our patients and control subjects are reported in Fig. 1.

Hyperhomocysteinemia, defined as tHcy plasma level above the 95th percentile of the control subjects (10 μmol/l for females and 15 μmol/l for males) was detected in 34 patients out of 107 (31.8%) and in nine control subjects out of 189 (5%). In the subgroup B and C hyperhomocysteinemia was detected in 13 out of 45 (28.9%) and in 21 out of 58 (36.2%), respectively. In the subgroup C1 hyperhomocysteinemia was detected in 15

Table 2 Grouping based on severity of cardiovascular manifestations of patients with Marfan Syndrome

Subgroups	*n*	Age (years)[a]	Involvement of cardiovascular system	Aortic measurements (cm/m^2 BSA)	Aortic diameters (mm)
A	4	24.5(19–36)	No	1.92 (1.87–2.01)	34.0 (24.0–37.0)
B	45	27.0(14–62)	Mild involvement	2.00 (1.75–2.19)	37.5 (28.0–47.0)
C	58	30.5(15–49)	Major criteria	2.60 (2.21–3.52)	50.0 (28.8–100.0)
C1	26	30.5(15–49)	Aortic dissection	2.50 (2.21–2.93)	49.8 (28.8–60.0)
C2	32	31.0(18–45)	No dissection	2.69 (2.23–3.52)	50.0 (39.0–100.0

[a]Data are given as median (range).
No=no cardiovascular manifestation; mild involvement=mild aortic dilatation <2.2 cm/m^2 BSA; major criteria=moderate to severe aortic dilatation >2.2 cm/m^2 BSA or aortic dissection.

Table 3 Total homocysteine, cysteine, folate and vitamin B12 levels in the different groups

	tHcy (μmol/l)	Cy (μmol/l)	Folate (μmol/l)	Vitamin B12 (pmol/l)
Controls	8.7 (1.6–24.0)	204 (99–331)	7.6 (3.0–25.0)	375 (200–1252)
Marfan patients				
Total	9.9 (4.0–23.1)[a]	205 (107–289)	7.5 (4.5–22.3)	375 (250–1251)
Subgroup A	7.5 (7.0–9.0)	204 (123–282)	7.7 (7.3–13.0)	377 (340–380)
Subgroup B	8.8 (4.0–20.3)	205 (148–272)	7.7 (5.0–13.0)	375 (250–625)
Subgroup C	10.7 (5.0–23.1)[b,c]	207 (107–289)	7.2 (4.5–22.3)	375 (250–1257)
C1 (with dissection)	13.5 (9.0–23.1)[d,e,f]	208 (155–289)	7.3 (4.6–12.0)	378 (305–1250)
C2 (without dissection	8.9 (5.0–19.4)	203 (107–246)	7.5 (4.5–22.3)	370 (250–800)

[a]P<0.0001 total vs controls.
[b]P<0.0001 subgroup C vs controls.
[c]P=0.017 subgroup C vs subgroup B.
[d]P<0.0001 subgroup C1 (with aortic dissection) vs controls.
[e]P<0.0001 subgroup C1 (with aortic dissection) vs subgroup B.
[f]P<0.0001 subgroup C1 (with aortic dissection) vs subgroup C2 (without aortic dissection).All data are given as median (range).

out of 26 (57.7%) and in the subgroup C2 in 6 out of 32 (18.7%).

In the multiple regression analysis tHcy levels, but not sex, age, Cy, folate and vitamin B12, significantly correlated (r=0.295, P=0.008) with aortic dimensions (aortic diameter/body surface area ratio) in Marfan patients.

Cy, folate and vitamin B12 levels

Cy, folate and vitamin B12 levels in controls, Marfan group, Marfan subgroup B and Marfan subgroups C, C1 and C2 were similar and did not differ statistically (Table 3).

MTHFR C677T polymorphism

The C677T MTHFR genotype distribution was in Hardy–Weinberg equilibrium in all groups (control subjects, Marfan patients, and subgroups B, C, C1 and C2) (Table 4). The prevalence of homozygotes for the C677T MTHFR polymorphism was higher in Marfan patients (22.4%) than in control subjects (14.3%), but the difference was not statistically significant (RR=1.39, 95% CI 0.99–1.95, P=0.075). In the subgroup C the prevalence of homozygotes for the polymorphism was significantly (P=0.04) higher (25.9%) in comparison to control subjects. This prevalence was also higher in comparison to subgroup B patients (17.8%), but there was no significant difference (P=0.35). When we consider patients with aortic dissection (subgroup C1), the prevalence of homozygotes for the polymorphism (42.3%) was significantly higher than in patients without aortic dissection (subgroup C2; 12.5%; P=0.01), in subgroup B (P=0.03), and in control subjects (P=0.0005).

tHcy plasma levels and C677T MTHFR genotype

In the whole Marfan population a significant genotype-phenotype association (P<0.00001) between tHcy plasma levels and C677T MTHFR polymorphism was observed (Table 4). A statistically significant genotype-phenotype association in the subgroup B and subgroup C (P=0.03 and P<0.00001, respectively), as well as in the control subjects (P=0.039) was also observed (Table 4). The statistical significance of the differences among tHcy levels according to genotype is indicated in Table 4. The presence of one T allele determined a median increase of 0.3 and 2 μmol/l of the tHcy levels in control subjects and Marfan patients respectively; in the presence of two T alleles the median increase was 1.2 and 6.3 μmol/l, respectively.

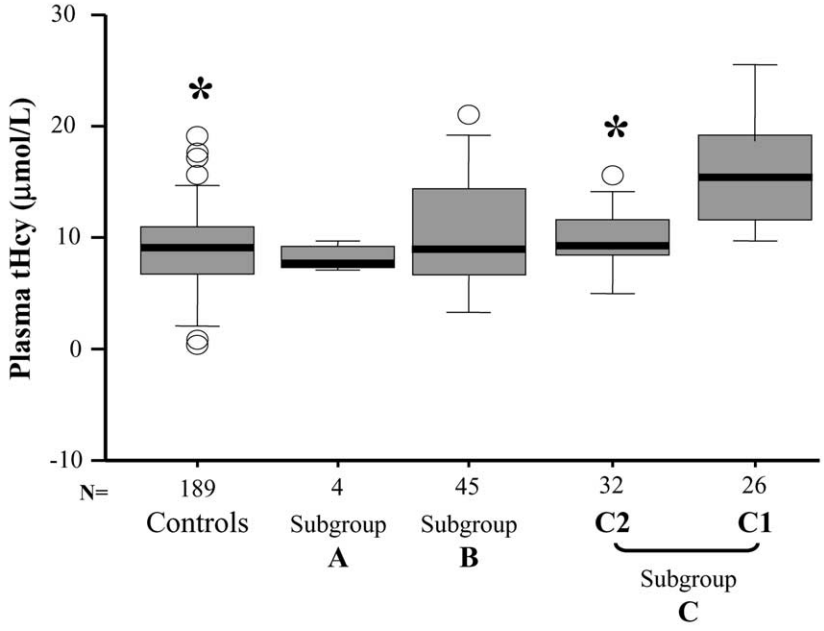

Fig. 1 Box-plots of the tHcy plasma levels in our patients and control subjects. A=no involvement; B=mild involvement; C=major criteria; C1=aortic dissection; C2=no dissection.

Table 4 Total homocysteine plasma levels in the different groups related to C677T MTHFR genotype

Groups	Genotype	*n* (%)	tHcy (µmol/l) median (range)	*P* values vs CT	*P* values vs CC
Controls	TT	27 (14.3)	9.9 (7.0–24.0)	0.14	0.009
	CT	105 (55.5)	9.0 (1.6–16.8)		0.12
	CC	57 (30.2)	8.7 (4.0–13.0)		
Marfan patients					
Total	TT	24 (22.4)	14.3 (6.0–23.1)	0.002	0.000002
	CT	49 (45.8)	10.0 (4.0–20.3)		0.001
	CC	34 (31.8)	8.0 (5.0–13.0)		
Subgroup B	TT	8 (17.8)	12.0 (6.0–15.3)	0.1	0.01
	CT	22 (48.9)	10.0 (4.0–2-0.3)		0.08
	CC	15 (33.3)	8.0 (5.0–13.0)		
Subgroup C	TT	15 (25.9)	16.0 (8.9–23.1)	0.03	0.000014
	CT	24 (41.4)	10.4 (7.8–19.4)		0.001
	CC	19 (32.7)	8.1 (5.0–13.0)		
C1 (with dissection)	TT	11 (42.3)	16.2 (11.7–23.1)	0.002	0.011
	CT	11 (42.3)	12.1 (9.0–18.0)	0.56	
	CC	4 (15.4)	11.0 (9.1–13.0)		
C2 (without dissection)	TT	4 (12.5)	10.0 (8.9–14.0)	0.82	0.014
	CT	13 (40.6)	9.8 (7.8–19.4)	0.003	
	CC	15 (46.9)	8.0 (5.0–12.1)		

Univariate and multivariate logistic regression analysis

In the univariate logistic regression analysis severe cardiovascular manifestations (subgroup C) or aortic dissection (subgroup C1) in Marfan patients were not associated with Cy, folate and vitamin B12 plasma levels, age and sex. The risk of severe cardiovascular manifestations was 1.25, 95% CI 0.78–1.98 (P=0.35) according to 677TT MTHFR genotype and 1.16, 95% CI 1.03–1.29, P=0.012 according to the increased of tHcy plasma levels. Aortic dissection was significantly associated with 677TT MTHFR genotype (OR 1.96, 95% CI 1.20–3.19, P=0.007) and with increased tHcy plasma levels (OR 1.42, 95% CI 1.21–1.65, P<0.0001).

In the multivariate logistic regression analysis, taking severe cardiovascular manifestations or aortic dissection as dependent variable and C677T MTHFR genotypes, age, sex, tHcy, Cy, folate and vitamin B12 plasma levels as independent variables, only the increase of the

continuous variable homocysteinemia was associated with the risk of severe cardiovascular manifestations (OR 1.23, 95% CI 1.06–1.44, *P*=0.007) or aortic dissection (OR 1.48, 95% CI 1.21–1.81, *P*<0.0001).

When homocysteinemia was used as categorical variable in relation to the definition of hyperhomocysteinemia, multivariate logistic regression analysis indicated that the OR for aortic dissection in hyperhomocysteinemic Marfan patients was 4.46, 95% CI 1.30–15.27, *P*=0.017.

Discussion

The present study shows, for the first time, that tHcy plasma levels are more elevated in Marfan patients with severe cardiovascular manifestations in comparison to those with mild cardiovascular manifestations and are related to aortic dilatation. Interestingly, in patients with aortic dissection higher plasma levels of tHcy and prevalence of homozygous 677TT MTHFR genotype were found in comparison to patients with severe cardiovascular manifestation but without aortic dissection and patients with mild cardiovascular manifestations. A genotype-phenotype relationship between tHcy plasma levels and C677T MTHFR polymorphism was observed. After the multivariate logistic regression analysis, tHcy but not C677T MTHFR polymorphism remained associated with severe cardiovascular manifestations and aortic dissection in our Marfan patients. On the other hand, a recent large study of Husemoen and coworker shows that the C677T MTHFR polymorphism accounts for only 6% of the variation in tHcy levels in the general population.[29]

The higher levels of tHcy in Marfan patients are not to be ascribed to reduced folate or vitamin B12 concentrations, as they were comparable in controls and in the patient groups, but we cannot rule out a difference in vitamin B6 levels. Our results add further weight to the findings obtained in patients with spontaneous cervical artery dissection[14] and suggest that high plasma levels of tHcy and the homozygous 677TT MTHFR genotype are important contributors to the weakening of the extracellular matrix of the vascular wall.

Hyperhomocysteinemia may be implicated in the vascular damage of the aorta in Marfan patients by several mechanisms. Thoracic aortic aneurysms (TAAs) in Marfan patients develop as a consequences of disruption of medial and adventitial elastin and collagen in association with foci of necrosis of the medial smooth muscle cells and accumulation of proteoglycans (CMN).[8] In CMN areas of TAAs of Marfan patients an increased immunoreactivity for metalloproteinases (MMP)-2 and MMP-9 produced by smooth muscle cells,[30] which are known to have elastolytic activity,[31] was reported. These observations support the concept, similar to that previously formulated with respect to atherosclerotic aneurysms,[32–34] that increased activity of MMPs plays an important role in the pathogenesis of the aortic aneurysms in Marfan syndrome. Segura and co-workers[30] hypothesized that the defect in fibrillin 1 in Marfan syndrome leads to formation of elastin that is abnormally aggregated and more easily degraded by MMPs than is normal elastin. It also leads to up regulation of the synthesis of MMPs, progressive destruction of connective tissue by these enzymes, and development of TAAs. Several studies demonstrated that hyperhomocysteinemia induces a marked remodelling of the extracellular matrix of the arterial wall by induction of elastolysis through the activation of metalloproteinases.[35,36] However, we have to keep in mind that in the experimental studies concentrations of homocysteine higher than those reached in circulating blood have been tested, so these possible mechanisms are not applicable *sic et simpliciter* to in vivo clinical conditions. Nevertheless, the in vivo interaction of homocysteine with the vascular wall is a long lasting challenge for an abnormal vascular structure.

Most importantly, tHcy may directly affect fibrillin-1 by interfering with intra- and/or inter-molecular disulfide bonds through disulfide exchange, or binding to free sulfhydryl groups. The multisystem toxicity of tHcy is attributed to its spontaneous chemical non-enzymatic reaction (homocysteinylation) with many biologically important molecules, primarily proteins. Irreversible homocysteinylation of long-lived proteins should lead to cumulative damage and progressive clinical manifestations. Fibrillin-1 is seen as the paradigm of extracellular connective tissue proteins that are especially susceptible to tHcy (and presumably Hcy thiolactone) attack.[20] The prominent presence of epidermal growth factor (EGF)-like domains in fibrillin-1 and in many other extracellular proteins of the coagulation, anticoagulation, and lipoprotein transport pathways, all of which malfunction in hyperhomocysteinemia, suggests that EGF-like domains may be preferential sites of homocysteinylation.[20] Fibrillin-1, a large glycoprotein with a high cysteine content (~14%), much of which (~33%) appears in the free reactive sulfhydryl form, is also found in the medial layer of all elastic arteries, in the heart, bone, periostium, cartilage, skin and lung, all structures that are compromised in both Marfan syndrome and homocysteinuria. Homocysteinuria is a recessive disease due to inborn errors of homocysteine metabolism in which polymorphisms in cystathionine-β-synthase gene are the most common causes. It is characterized by major clinical manifestations involving the eyes and the central nervous, skeletal, and vascular systems. Many of the clinical manifestations of these two disorders overlap, thus supporting the assumption that fibrillin 1 is an important target of homocysteinylation. Fibrillin-1 consists largely of 56 cysteine-rich imperfect repeat domains, 47 of which show significant homology to a motif originally found in EGF-like repeats.[37] The biological significance of these cysteinyl residues is demonstrated by the fact that many of the mutations causing Marfan syndrome substitute one of the highly conserved cysteinyl residues, add a new one, or alter their relative spacing. Free homocysteine may react with some of the numerous free cysteinyl sulfhydryl (SH) groups in fibrillin-1 or may disrupt critical Cys-Cys disulfide bridges in EGF-like domains with the formation of protein mixed disulfides via thiol-disulfide exchange reactions. Fibrillin-1 may also be irreversibly homocysteinylated at lysyl residues, many of which are concentrated in a long

region (17 587 Da) at the carboxyl end of the protein, which is conspicuously lacking in cysteinyl residues. This region may be involved in intermolecular fibrillin aggregation; its homocysteinylation may therefore impede the formation of microfibrils.[20]

Majors and Pyeritz[38] showed that in vitro deficiency of cysteine impairs the accumulation of fibrillin-1 in the extracellular matrix and hypothesized that in vivo it could contribute to the pathological changes of connective tissue found in cystathionine-synthase (CBS) deficiency. In our Marfan patients whether total or subgroup B or C, Cy plasma levels did not differ from control group nor from each other so excluding this mechanism.

A limitation of this study is the scarcity of Marfan patients in the subgroup (A) without cardiovascular involvement. This is consistent with the very low prevalence of Marfan subjects without cardiovascular manifestations. Finally, some clinical and experimental studies showed that homocysteine may be released from damaged tissues as tissue damage accelerates specific methylation reactions, generating S-adenosyl homocysteine and releasing homocysteine.[39] We measured tHcy when vascular involvement was present, therefore we cannot rule out that elevated tHcy levels are, at least in part, the expression of vascular damage.

In conclusion, our data indicate an association between the severity of cardiovascular manifestations in Marfan patients and tHcy levels. The findings that in patients with more severe cardiovascular manifestations of Marfan syndrome, and in particular with aortic dissection, tHcy levels and prevalence of homozygous 677TT MTHFR genotype are higher in comparison to patients with less severe vascular involvement suggest a role for hyperhomocysteinemia and MTHFR genotype in the phenotypic variability of cardiovascular manifestations of Marfan syndrome, and could indicate the value of vitamin supplementation (folic acid, vitamin B6 and B12) for these patients.

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