Marfan syndrome and related connective tissue disorders

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CHAPTER 6

Relation between genotype and left-ventricular dilatation in patients with Marfan syndrome

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ABSTRACT

Cardiovascular manifestations in patients with Marfan syndrome (MFS) are related to aortic and valvular abnormalities. However, dilatation of the left ventricle (LV) can occur, even in the absence of aortic surgery or valvular abnormalities. We evaluated genetic characteristics of patients with MFS with LV dilatation. 182 patients fulfilling the MFS criteria, without valvular abnormalities or previous aortic surgery, with a complete *FBN1* analysis, were studied. *FBN1* mutations were identified in over 81% of patients. Twenty-nine patients (16%) demonstrated LV dilatation (LV end diastolic diameter corrected for age and body surface area >112%). *FBN1*-positive patients carrying a non-missense mutation more often had LV dilatation than missense mutation carriers (14/74 versus 5/75; $p<0.05$). Finally, *FBN1*-negative MFS patients significantly more often demonstrated LV dilatation than *FBN1*-positive patients (10/33 versus 19/149; $p<0.05$). It is concluded that LV dilatation in MFS patients is more often seen in patients with a non-missense mutation and in those patients without an *FBN1* mutation. Therefore physicians should be aware of the possibility of LV dilatation in these patients even in the absence of valvular pathology.

INTRODUCTION

Marfan syndrome (MFS; MIM#154700) is an autosomal dominant disorder of the connective tissue, caused in a majority of cases by mutations in the gene encoding fibrillin-1 (*FBN1*; MIM#134797). More than 600 *FBN1* mutations have been reported so far and about 25-30% of cases represent *de novo* mutations (for the *FBN1* mutation database see: www.umd.be). The mutation detection rate reaches over 80% in patients fulfilling the diagnostic revised Ghent criteria for MFS.[1-3] Recently, mutations in the transforming growth factor-β receptor 1 and 2 genes (*TGFBR1* and *TGFBR2*) have also been identified in a subset of MFS patients who did not carry an *FBN1* mutation.[4-7]

MFS affects the cardiovascular system amongst others, with the main cardiovascular manifestations being aortic dilatation/dissection and valvular abnormalities. In addition, the presence of mild left ventricular (LV) dilatation (i.e. increased LV end diastolic dimension (LVEDD)) and/or mild impairment of both LV systolic and diastolic function) has been reported, even in patients without significant valvular abnormalities or previous aortic surgery.[8-14] Elaborating on our previous study, in the present study we focussed on LV dilatation and we investigated whether a relationship exists between LV dilatation in patients with MFS and genotype.[10]

PATIENT DATA/MATERIAL/METHODS

Patient selection

Patient selection has been reported previously.[10] Briefly, 529 consecutive adult (over 17 years of age) patients fulfilled the diagnostic revised Ghent criteria for MFS and did not show features suggestive for Loeys-Dietz syndrome.[6] In 295 patients previous aortic surgery or significant valvular abnormalities noted on echocardiogram (moderate or severe aortic and/or mitral regurgitation) precluded participation in this study.[15] Of the remaining 234 MFS patients, DNA data were available for 184 patients. Two more patients were excluded because their DNA results did not allow for a single interpretation. We thus had 182 patients in our study group.

Echocardiography

Echocardiograms were performed in accordance with standard techniques and as reported previously.[10,15] Briefly, LVEDD was derived from two-dimensional echocardiograms. For each patient the predicted normal value for LVEDD was calculated according to their age and body surface area using the regression equations proposed by Henry.[17] The LVEDD was expressed as a percentage of the predicted value: observed dimension/predicted normal value x100. In accordance with the guidelines, 112% (mean + 2SD) was used as the cut-off value for increased LV dimension.[18]

DNA analysis

Genomic DNA was extracted from peripheral blood leukocytes or skin fibroblasts. Mutational analysis of the *FBN1* gene was performed by Denaturing High Performance Liquid Chromatography (DHPLC) with subsequent sequencing of fragments with aberrant patterns.[19] The effect of potential

splice-site mutations was confirmed using reverse-transcription PCR. If DHPLC analysis was negative, additional investigations were performed to elucidate a potential pathogenic role of the *FBN1* gene, namely: (a) Multiplex Ligation-dependent Probe Amplification (MLPA) analyses to assess large genomic duplications or deletions, and (b) determination of potential mono-allelic expression on messenger RNA of heterozygous polymorphisms representative for a null allele. If this screening was negative, *TGFBR1* and *TGFBR2* were screened like we described earlier.[20]

If an *FBN1* mutation was present, we defined its characteristics and the type of mutation, as reported previously.[3] Mutations were classified into missense and non-missense mutations. The missense mutations were categorized into mutations affecting cysteine residues (cysteine mutations) or mutations affecting other amino-acids (non-cysteine mutations). Non-missense mutations were subcategorized further based upon the results of in-silico prediction-programmes and RNA studies when available. The following categories were defined: nonsense mutations, mutations leading to in-frame events, mutations leading to out-of-frame events and null alleles. When mono-allelic expression of heterozygous polymorphisms on messenger RNA was found, in the absence of a mutation in genomic DNA, a null allele was considered causative. Silent mutations were defined as mutations which do not lead to an amino-acid change.

Statistics

Data are presented as a mean with SD or proportions/percentages. Differences between groups were evaluated using the Student's t-test for continuous variables, the Mann-Whitney U test was used for non-parametric variables and the Chi-square test for proportions/ percentages. A p-value <0.05 was considered to indicate statistical significance. Analyses were performed using IBM SPSS Statistics 20.

RESULTS

Patients

Data on DNA analyses were available for 184 patients. Two of these patients carried two *FBN1* mutations that may both influence the phenotype. They were therefore excluded from further analysis, leaving 182 patients from 120 different families. Ninety-five mutation positive index-patients, carried 84 different mutations (supplementary Table 1). The mean age of the total group was 33.5 ± 11.8 years and 92 (50.5%) were male (Table 1). The mean body surface area was 2.0 ± 0.2 m^2 and the mean aortic root diameter was 41 ± 5.8 (25-57) mm. Two-thirds of patients (122/182) were on beta-blocker therapy (Table 1).

Echocardiography

The mean LVEDD in the group of 182 patients was 50.7 ± 5.7mm, which corresponded with 103.3 ± 9.8% of the predicted value. In 29 patients, from 26 different families, the LVEDD exceeded the upper limit of normal (112%)(Table 1). The records of these 29 patients with LV dilatation were reviewed for

Table 1. Characteristics of the total study group and the subgroups +/- *FBN1* mutation, ≤ />LVEDD112% and combinations thereof.

	Subgroup	Male Sex (%)		Age in years (SD)		On Beta-blockade
Total group (n=182)		92 (51%)		33.5 (11.8)		122 (67%)
Total group (n=182)	FBN1+ (n=149)	68 (46%)	p<0.05	33.6 (11.8)		99 (66%)
	FBN1- (n=33)	24 (73%)		33.1 (11.7)		23 (70%)
Total group (n=182)	LVEDD≤112 (n=153)	70 (46%)	p<0.01	34.2 (12.1)		101 (66%)
	LVEDD>112 (n=29)	22 (76%)		29.5 (8.8)		21 (72%)
FBN1 + group (n=149)	FBN1+/LVEDD≤112 (n=130)	55 (42%)		33.9 (12.2)		84 (65%)
	FBN1+/LVEDD>112 (n=19)	13 (68%)		31.7 (9.1)		15 (79%)
FBN1 – group (n=33)	FBN1-/LVEDD≤112 (n=23)	15 (65%)		36.4 (11.9)	p<0.05	17 (74%)
	FBN1-/LVEDD>112 (n=10)	9 (90%)		25.5 (7.0)		6 (60%)

FBN1+=with *FBN1* mutation; FBN-=without *FBN1* mutation; LVEDD= left ventricular end diastolic dimensions, SD= standard deviation,
All differences are non-significant, unless indicated

factors other than MFS that might explain the dilatation (e.g. hypertension, coronary disease), but none of these factors could be identified. No patients suffered from heart failure. Twenty-two of the 29 patients with LVEDD >112% were male (76%), which is significantly more than in the group of MFS patients with LVEDD≤112% (p<0.01)(Table 1).

DNA analysis

In 149/182 (81.9%) patients a putative pathogenic mutation in the *FBN1* gene or a null allele was identified, whereas no mutation (n=30) or a silent mutation (n=3 patients carrying 4 silent mutations) in *FBN1* was found in 33 (18.1%) patients. The groups of patients with or without an *FBN1* mutation did not differ in terms of diagnostic criteria, mean body surface area, mean aortic root diameter or beta-blocker use, but more males (24/33) without an *FBN1* mutation were identified (Table 1). One *TGFBR2* variant (c.1274T>A; p.Met425Lys) was identified in a patient who also demonstrated LVEDD >112%. Although a mutation affecting this identical amino-acid residue has been described before, its pathogenic character remains to be established as this mutation could not be found in the patient's brother who showed a similar clinical picture.[21] In total, 84 different *FBN1* mutations

were identified, 57 of which were unique and 27 of which were found more often.

Genotype - phenotype relationship

Table 2 shows the relationship between LV dilatation (LVEDD >112%) and the categories: missense mutations, non-missense mutations and absence of an *FBN1* mutation. Patients carrying missense mutations less frequently (5/75; 6.7%) showed LV dilatation compared to patients having a non-missense mutation (14/74; 18.9%) ($p<0.05$) (Table 2). LV dilatation was present in 10/33 patients without an *FBN1* mutation or silent mutation carriers (30.3%) versus 19/149 patients carrying an *FBN1* mutation (12.8%)($p<0.05$) (Table 2). Patients in the *FBN1*-negative group with LVEDD >112% were younger than those with normal LVEDDs (25.5 vs. 36.4 years, $p<0.05$) (Table 1).

Table 2: Distribution of number of patients according to type of *FBN1* mutation in the two LV dilatation categories

	Mutation according to groups	Subtype of mutation	LVEDD≤112%		LVEDD>112%		
FBN1+ (n=149)	Missense (n=75)	non-cysteine missense	37	70	3 (7.5%)	5 (6.7%)*	19 (12.8%)†
		cysteine-missense	33		2 (5.7%)		
	Non-missense (n=74)	in frame	13	60	5 (27.7%)	14	
		nonsense	24		2 (7.7%)		
		out of frame	23		(19.2%)*		
		null allele	0		5 (17.8%)		
					2		
FBN1- (n=33)			23		10	10 (30.3%)	10 (30.3%)†
Total			153		29		

*=$p<0.05$, †=$p<0.05$

FBN1+ = with *FBN1* mutation; FBN1- =without *FBN1* mutation; LVEDD= left ventricular end diastolic dimension

DISCUSSION

Dilated cardiomyopathy, characterised by both marked LV dilatation and systolic dysfunction, in the absence of significant valvular pathology is very rare in MFS.[18] However, isolated mild LV dilatation is relatively common, occurring in about one in six patients with MFS.[9,10] This is not an entirely unexpected finding given the fact that fibrillin-1 is a component of the myocardium. In the present study, we investigated the genotype-phenotype relationship regarding LV dilatation in a large group of patients with MFS. Our data show that patients carrying non-missense mutations, more often had LV dilatation than patients carrying missense *FBN1* mutations. We speculate that this might be due to the fact that in the non-missense mutation group haploinsufficiency is believed to be the main mechanism leading to disease and this may underlie LV dilatation as well. However, this needs confirmation in other series because we were not able to investigate haploinsufficiency due to

nonsense mediated mRNA decay in all non-missense mutations (supplementary Table 1). Although a dominant negative effect of premature stop-codon-introducing mutations, leading to production of shortened proteins, can be expected, this is probably largely eliminated due to preferential degradation of transcripts (nonsense mediated mRNA decay), which is believed to be common in *FBN1* premature termination mutations.[22,23] For in-frame deletions, it has been established that the mutant protein product interferes with microfibril assembly by a dominant negative mechanism, which might also underlie the more severe ventricular phenotype.[24] However, the explanations for these differences are highly speculative, in particular given the complex interactions of fibrillin with other extracellular matrix proteins and growth factors.[25-27] Our data also show that it is important whether an *FBN1* mutation is present or not, because in those patients without such a mutation the prevalence of LV dilatation was significantly higher than in those in whom an *FBN1* mutation was identified. In fact, in nearly one-third of all patients without an *FBN1* mutation, the phenotype was characterized by LV dilatation. However, there appeared to be a possible sex effect, since males more often than females did not harbour an *FBN1* mutation and they also showed LV-dilatation more often (Table 1). A possible explanation for this phenomenon might be that aortic root size in males is on average 2.4 mm larger than females, even after correction for larger body size and thus may lead to more MFS diagnoses in males, as enlarged aortic root size is a major criterion in the diagnostics of MFS.[28,29] As this can be considered a phenocopy, this subsequently may lead to a lower yield in *FBN1* analysis. Another explanation might be the presence of a yet unrecognised phenocopy with MFS (-like) features and enlarged LV diameters, which is more predominant in males. Although we can not provide a conclusive explanation for the observed male predominance, this may have led to a potential confounder effect in the analyses. Recently, mutations in *TGFBR2*, encoding transforming growth factor β (TGFβ) receptor type 2, were identified in MFS patients.[4,30] TGFβ regulates proliferation and differentiation of cells.[31] Several lines of evidence have implicated TGFβ signalling in the pathogenesis of connective tissue disorders, including MFS. Based on the above considerations, it can be speculated that the present study should have revealed *TGFBR1* or *-2* mutations in the subset of patients without a mutation in *FBN1* and that these mutations could play a causative role in the process of LV dilatation. However, we excluded overrepresentation of *TGFBR1* or *-2* in LV dilatation in MFS patients who do not carry an *FBN1* mutation because we only identified one single variant of unknown significance, while in the literature *TGFBR* mutations are also being identified in a few percent of cases.[4-7]

Strengths and weaknesses of the study

This is the first study on the relationship between genotype and LV dilatation in MFS patients and is unique given the size of the population studied. Nonetheless, the sizes of different subgroups that were compared were sometimes still relatively small. As a result, certain trends may not have reached significance, and reproduction of our findings in an independent and larger population would be desirable. In addition, not all of the initial 234 MFS patients had undergone *FBN1* mutation

analysis. This may have given rise to a certain bias. Furthermore, since the definition of LV dilatation (in the context of familial dilated cardiomyopathy) is based on M-mode echocardiography, we used M-mode but the validity of this technique can be questioned.[17,18] Finally, given our previous study in which we demonstrated the presence of LV dilatation but not impairment of LV function in MFS patients, the present study was also only about LV dilatation, not about LV function.[10] A definite strength of this study lies in the extensive and complete genetic analyses we performed, including MLPA to exclude large rearrangements, the demonstration of bi-allelic *FBN1* expression, and complete *TGFBR1* and *-2* analyses in patients in whom no *FBN1* mutation was identified.

CONCLUSION

Dilatation of the LV is not uncommon in patients with MFS. We have shown for the first time that this LV dilatation may occur particularly in MFS patients carrying non-missense mutations and in those in whom no *FBN1* mutation is demonstrable. Therefore, clinicians should be aware of the possibility of LV dilatation developing in these patients even in the absence of predisposing factors like valvular pathology.

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