Marfan syndrome and related connective tissue disorders in the current era in Switzerland in 103 patients: medical and surgical management and impact of genetic testing

Bombardieri, Elisa ; Rohrbach, Marianne ; Greutmann, Matthias ; Matyas, Gabor ; Weber, Roland ; Radulovic, Jovana ; Fasnacht Boillat, Margrit ; Linka, André ; De Pasquale, Gabriela ; Bonassin, Francesca ; Attenhofer Jost, Christine H

Abstract: INTRODUCTION Marfan syndrome (MFS) and related connective tissue disorders (CTDs) are increasingly recognised. Genetic testing has greatly improved the diagnostic outcome/power over the last two decades. In this study we describe a multicentre cohort of adults with MFS and related CTDs, with a particular focus on results from genetic testing. METHODS All patients with MFS and related CTDs were identified from the databases of five centres in the canton of Zurich. Echocardiographic and clinical findings including systemic Marfan score, use of medication and genetic results were retrospectively analysed. MFS was diagnosed using the revised Ghent criteria (including FBN1 genetic testing if available); other CTDs (Loeys-Dietz syndrome) were diagnosed by genetic testing only. RESULTS A cohort of 103 patients were identified (62 index patients, 41 relatives of family members): 96 patients with MFS and 7 patients with other CTD, 54 males (52%), median age 23 years (range 1ndash;75). The median systemic Marfan score was 5 (range 0ndash;18). Only 40 patients (40/103, 39%) fulfilled criteria for systemic involvement (ge;7 points). A history of aortic dissection was present in 14 out of 103 patients (14%). Echocardiographic data were available for all: aortic root enlargement (Z-score ge;2 in adults, Z-score ge;3 in children) was found in 49 patients (48%) and mitral valve prolapse in 64 (62%). Genetic testing had been performed in 80 patients (78%); FBN1 mutations were present in 69 patients (86%); other pathogenic mutations could be identified in seven patients (9%); no disease-causing mutation was found in four patients, three of them fulfilling the Ghent criteria of MFS. Of the mutation-positive patients, 33 had a systemic score of ge;7 and 43 had a systemic score of ge;5. Revised Ghent criteria were fulfilled in 70 patients: in 69 patients with FBN1 mutations and 1 patient with another CTD. Recommended treatment (beta-blocker, angiotensin receptor blocker) was taken by 63% of patients. CONCLUSIONS In this cohort a high percentage of patients fulfilling the revised Ghent criteria for MFS underwent genetic testing, often leading to or confirming the diagnosis of MFS. Other CTDs could be discriminated best by genetic testing. With respect to the diagnosis of MFS and related CTDs, the usefulness of the systemic score is limited, showing the importance of genetic testing, which enabled definitive diagnosis in 95% of tested patients. Patient education on medical treatment still has to be improved. (Trial registration no: KEK-ZH-Nr. 2013-0241).

DOI: https://doi.org/10.4414/smw.2020.20189

Originally published at:
Bombardieri, Elisa; Rohrbach, Marianne; Greutmann, Matthias; Matyas, Gabor; Weber, Roland; Radulovic, Jovana; Fasnacht Boillat, Margrit; Linka, André; De Pasquale, Gabriela; Bonassin, Francesca; Attenhofer Jost, Christine H (2020). Marfan syndrome and related connective tissue disorders in the current era in Switzerland in 103 patients: medical and surgical management and impact of genetic testing. Swiss Medical Weekly, 150:w20189.
DOI: https://doi.org/10.4414/smw.2020.20189

Established in 1871

Swiss Medical Weekly

Formerly: Schweizerische Medizinische Wochenschrift

An open access, online journal • www.smw.ch

Original article | Published 03 April 2020 | doi:10.4414/smw.2020.20189
Cite this as: Swiss Med Wkly. 2020;150:w20189

Marfan syndrome and related connective tissue disorders in the current era in Switzerland in 103 patients: medical and surgical management and impact of genetic testing

Bombardieri Elisa[a], Rohrbach Marianne[b], Greutmann Matthias[a], Matyas Gabor[c], Weber Roland[d], Radulovic Jovana[e], Boillat Margrit Fasnacht[f], Linka André[g], De Pasquale Gabriella[h], Bonassin Francesca[a], Attenhofer Jost Christine H.[e]

[a] Cardiology, University Hospital, Zurich, Switzerland
[b] Division of Metabolism, University Children's Hospital, Zurich, Switzerland
[c] Centre for Cardiovascular Genetics and Gene Diagnostics, Schlieren, Switzerland
[d] Cardiology, Division of Cardiology, University Children's Hospital, Zurich; and Children's Research Centre, University Children's Hospital, Zurich, Switzerland
[e] Cardiovascular Centre, Klinik Im Park, Zurich, Switzerland
[f] Department of Paediatric Cardiology, Paediatrics, Kantonsspital Winterthur, Switzerland
[g] Department of Cardiology, Kantonsspital Winterthur, Switzerland
[h] Department of Cardiology, Stadtspital Triemli, Zurich, Switzerland

Summary

INTRODUCTION: Marfan syndrome (MFS) and related connective tissue disorders (CTDs) are increasingly recognised. Genetic testing has greatly improved the diagnostic outcome/power over the last two decades. In this study we describe a multicentre cohort of adults with MFS and related CTDs, with a particular focus on results from genetic testing.

METHODS: All patients with MFS and related CTDs were identified from the databases of five centres in the canton of Zurich. Echocardiographic and clinical findings including systemic Marfan score, use of medication and genetic results were retrospectively analysed. MFS was diagnosed using the revised Ghent criteria (including *FBN1* genetic testing if available); other CTDs (Loeys-Dietz syndrome) were diagnosed by genetic testing only.

RESULTS: A cohort of 103 patients were identified (62 index patients, 41 relatives of family members): 96 patients with MFS and 7 patients with other CTD, 54 males (52%), median age 23 years (range 1–75). The median systemic Marfan score was 5 (range 0–18). Only 40 patients (40/103, 39%) fulfilled criteria for systemic involvement (≥7 points). A history of aortic dissection was present in 14 out of 103 patients (14%). Echocardiographic data were available for all: aortic root enlargement (Z-score ≥2 in adults, Z-score ≥3 in children) was found in 49 patients (48%) and mitral valve prolapse in 64 (62%). Genetic testing had been performed in 80 patients (78%); *FBN1* mutations were present in 69 patients (86%); other pathogenic mutations could be identified in seven patients (9%); no disease-causing mutation was found in four patients, three of them fulfilling the Ghent criteria of MFS. Of the mutation-positive patients, 33 had a systemic score of ≥7 and 43 had a systemic score of ≥5. Revised Ghent criteria were fulfilled in 70 patients: in 69 patients with *FBN1* mutations and 1 patient with another CTD. Recommended treatment (beta-blocker, angiotensin receptor blocker) was taken by 63% of patients.

CONCLUSIONS: In this cohort a high percentage of patients fulfilling the revised Ghent criteria for MFS underwent genetic testing, often leading to or confirming the diagnosis of MFS. Other CTDs could be discriminated best by genetic testing. With respect to the diagnosis of MFS and related CTDs, the usefulness of the systemic score is limited, showing the importance of genetic testing, which enabled definitive diagnosis in 95% of tested patients. Patient education on medical treatment still has to be improved. (Trial registration no: KEK-ZH-Nr. 2013-0241)

Keywords: *Marfan syndrome, connective tissue disorders, Loeys-Dietz syndrome, genetic testing in Marfan syndrome and related conditions*

Correspondence:
Dr Christine H. Attenhofer Jost, MD, Cardiovascular Centre Klinik Im Park, Seestrasse 220, CH-8027 Zürich, christine.attenhoferjost[at]hirslanden.ch

Introduction

Marfan syndrome (MFS) is an autosomal-dominant connective tissue disorder caused by mutations in the fibrillin-1 (*FBN1*) gene [1]. Over 1000 different mutations causing MFS and related disorders have been described so far [2, 3]. About 25% of the mutations are *de-novo*.

The mutations lead to a qualitative or quantitative lack of FBN1 protein, a 350 kDa glycoprotein that is a major component of elastin associated microfibrils in the extracellular matrix [4]. The structural change of FBN1 protein in MFS explains the resulting changes in elastin-con-

taining tissue such as abnormalities of the eye (ectopia lentis), aorta, heart valves and, although partially, skeleton (dolichostenomelia, arachnodactyly, pectus deformity and joint laxity) [5].

There is a wide spectrum of clinical expression, ranging from severe neonatal MFS or fatal aortic dissection to milder forms without cardiovascular involvement. Cardiac problems include aortic dilatation, aortic dissection, aortic regurgitation, mitral valve prolapse and sudden cardiac death. Nowadays, MFS is diagnosed using the revised Ghent criteria of 2010 [6]. The most important differential diagnoses of MFS are shown in table 1. Frequently, other CTDs, including more aggressive Loeys-Dietz syndrome (LDS) and ACTA2 mutations can be identified by genetic testing only.

Before genetic testing became widely available, MFS was diagnosed solely on the basis of clinical findings (Ghent criteria). However, these clinical diagnostic criteria are imperfect. Genetic testing has much improved the diagnostic process over the last two decades. In this retrospective cohort study, we summarise clinical characteristics and findings from genetic testing in a multicentre cohort.

Materials and methods

Setting

The study was based on a retrospective chart review and data analysis between 2001 and 2013 at five large hospitals in the Canton of Zurich: University Hospital of Zurich (Department of Cardiology), University Children Hospital of Zurich (Divisions of Metabolism and Cardiology), Stadtspital Triemli (Department of Cardiology), Klinik Im Park (Cardiovascular Centre) and Kantonsspital Winterthur (Department of Cardiology).

The study was approved by the ethics review committee of the Canton of Zurich (KEK-ZH-Nr. 2013-0241).

Patients

Within the participating institutions all patients followed up in their dedicated programmes for Marfan syndrome, suspected Marfan syndrome or Marfan-like conditions were identified from their clinical databases. For the purpose of this study, the charts of all patients with a diagnosis of MFS were carefully reviewed. All patients had at least one echocardiographic examination or other imaging of the aorta, and at least one consultation at one of the study centres between 2001 and 2013.

Definitions

The aortic root was measured at the sinuses of Valsalva. In children and adolescents (2–18 years old), the root was measured in mm from inner edge to inner edge during diastole, thus excluding the thickness of aortic wall. In adults, the aortic root was measured in mm from leading edge to leading edge at end-diastole.

Aortic root ectasia is defined as a Z-score ≥2 in adults or ≥3 in children. The Z-scores were calculated using the nomograms for aortic root diameters by Gautier for the group aged <15 years and by Devereux for adults (age >15 years) [8, 9]. For the calculation of the Z-scores for the ascending aorta the formula according to Campens was used [8, 9].

In patients who underwent aortic root surgery, the preoperative diameters of the aorta were used as baseline, if available.

Diagnosis of Marfan syndrome

The diagnosis of MFS was defined as fulfilment the 2010 Ghent criteria [6]. These criteria include aortic dilatation, ocular involvement, systemic findings and results from genetic testing. Molecular genetic testing included analysis of *FBN1* and MFS-related genes (see table 1) [6]. In the absence of a positive family history of MFS, a diagnosis of MFS can be confirmed in the following situations: (a) presence of aortic root aneurysm (aortic Z-score ≥2 above

Table 1: Differential diagnosis of Marfan syndrome.

	Inheritance	Prevalence	Aortic aneurysm	Early aortic dissection	Other cardiovascular findings	Gene
MFS	AD	1:5000	++	+	IA, MVP	*FBN1*
LDS1, LDS2	AD	Unknown	++	+++	BAV, IA, MVP	*TGFBR1, TGFBR2*
LDS3	AD	Unknown	++	++/+++	BAV, IA, MVP	*SMAD3*
LDS4, LDS5	AD	Unknown	++/+++	+	BAV, MVP	*TGFB2, TGFB3*
LDS6	AD	Unknown	++	+	MVP	*SMAD2*
TAAD	AD	Unknown	++/+++	++/+++	BAV, MVP	For example, *ACTA2, BGN, FOXE3, HCN4, LOX, MAT2A, MFAP5, MYH11, MYLK, PRKG1*
Shprintzen-Goldberg syndrome	AD	Unknown	++	-	MVP	*SKI*
Ehlers-Danlos syndrome, vascular type (EDS IV)	AD	1:50 000	+	++	IA, MVP	*COL3A1*
Aortic valve disease	AD	>1:100	+	+	BAV	*NOTCH1*
ELN-related cutis laxa	AD	<1:4 Mio.	+	+	-	*ELN*

ACTA2 = actin-alpha; AD = autosomal dominant; BAV = bicuspid aortic valve; BGN = biglycan gene; COL3A1 = type 3 collagen pro-α1; ELN = elastin; FBN1 = fibrillin 1; FOXE3 = forkhead transcription factor 3; HCN4 = hyperpolarisation-activated cyclic nucleotide-gated channels gene 4; IA = interatrial septum aneurysm; LDS = Loeys-Dietz syndrome; LOX = lysyl oxidase; MAT2A = methionine adenosyltransferase 2A; MFAP = microfibrillar-associated protein; MFS = Marfan syndrome; MVP = mitral valve prolapse; MYH11 = myosin heavy chain 11; MYLK = myosin light-chain kinase; NOTCH1= neurogenic locus notch homologue protein 1; PRKG1 = cGMP-dependent protein kinase 1; SKI = Sloan Kettering Institute proto-oncoprotein; SMAD = SMAD proteins homologues of both the drosophila protein, mothers against decapentaplegic (MAD) and the *Caenorhabditis elegans* protein SMA; TAAD = thoracic aortic aneurysm and dissection; TGFBR1 and 2 = transforming growth factor β 1 and 2 Table modified from Attenhofer Jost CH, Greutmann M, et al. [7]

20 years old or a Z-score ≥3 below 20 years) and ectopia lentis; (b) aortic root Z-score ≥2 above 20 years old or a Z-score ≥3 below 20 years and *FBN1* mutation; (c) aortic root Z-score ≥2 above 20 years old or a Z-score ≥3 below 20 years and systemic involvement (systemic score ≥7); or (d) ectopia lentis and the presence of an *FBN1* mutation known to cause an aortic phenotype [6].

In the presence of a positive family history for MFS (diagnosed in the index patients with the criteria mentioned above), the presence of only one of the following features confirms MFS: ectopia lentis OR aortic root aneurysm (aortic Z-score ≥2 above 20 years old or a Z-score ≥3 below 20 years) or aortic dissection OR a systemic involvement (systemic score ≥7) [6].

Genetic testing

DNA extraction from fibroblasts or ethylenediamine tetraacetic acid (EDTA)-anticoagulated whole blood samples, as well as exon-by-exon polymerase chain-reaction testing, denaturing high performance liquid chromatography (DHPLC) screening, and/or direct Sanger sequencing and multiplex ligation-dependent probe amplification (MLPA) analysis were performed as previously described [10–12].

Statistical analysis

Continuous variables are presented as mean and standard deviation or median with interquartilce range (IQR) as appropriate. Categorical variables are presented as percentages. For the analysis of Z-scores, average absolute deviation was used. Statistical comparisons of distribution between groups were made using the student t-tests. The analysis was performed using the SPSS standard statistical software.

Results

Patients

We identified a total of 103 patients from 62 distinct families. Of these, 96 (93%) had MFS, 7 (7%) LDS or another MFS-related CTD. Sixty-two patients (60%) of the cohort were newly diagnosed index patients, whereas 41 (40%) had a family member with an established diagnosis. Baseline patient characteristics are summarised in table 2.

Results of genetic testing

Genetic testing was performed in a total of 80/103 (78%) patients. In most of these patients, disease causing mutations in the *FBN1* gene were found (69/80, 86%). One patient had a *TGFBR1* (LDS1), three a *TGFBR*2 (LDS2), two a *SMAD3* (LDS3) and one a *TGFB2* mutation (LDS4) mutation. In four patients, three of whom fulfilled the Ghent criteria for MFS, no disease-causing mutations could be identified.

Clinical findings

The clinical findings are shown in tables 2 and 3. Median age was 23 years (range 1–75) and 43/103 patients (42%) were under the age of 18 years.

In 40 patients (40/103, 39%) a systemic score of ≥7 was documented. Of the 96 patients with a definitive diagnosis of MFS, 38 (40%) had a systemic score of ≥7, 44 (46%) a systemic score of ≥6, 50 (52%) a systemic score of ≥5 and 59 (61%) a systemic score of ≥4.

Other features commonly seen included hypermobility of the joints in 28 (27%), leg length discrepancy in 10 (10%), arthralgia in 12 (12%) and varicose veins in 8 (8%) patients. Hypertelorism and a cleft palate were present in only one patient with LDS; a bifid uvula was present in four (4%) patients, three of them with LDS (LDS1 and LDS3) and one with MFS and a disease-causing mutation in the *FBN1* gene. Twelve (12%) patients had a history of scoliosis surgery and prior foot surgery was reported by 7 (7%) patient; 12 (12%) patients had previous lensectomy.

Cardiovascular findings

The echocardiographic findings are summarised in table 4. Aortic root enlargement was found in 49 patients (49/103, 48%). Mitral valve prolapse was common (64/103 patients, 62%); however, severe mitral regurgitation was rare. Left ventricular ejection fraction was <50% in nine patients (9/103, 9%). There was no difference in echocardiographic findings between *FBN1*-positive patients and those with other CTDs (for each p-value see table 4).

Cardiac surgery

Cardiac surgery was performed in 34 patients (33%): 28 patients (27%) underwent aortic root surgery, 2 (2%) mitral valve surgery, (3%) combined aortic root and mitral valve surgery, and 1 (1%) combined aortic root and tricuspid valve surgery. Among patients undergoing aortic root

Table 2: Background of all patients and separate analysis of participants who underwent genetic analysis.

Variable	All patients (n = 103)	FBN1-positive patients (n = 69)	Other CTD (n = 7)
Mean age (years), range 1–75 years	23	17	15
Men	54 (52%)	31 (45%)	3 (43%)
Ghent positive	95 (92%)	64 (93%)	7 (100%)
Ectopia lentis	29 (28%)	20 (29%)	0
Aortic dissection type A	10 (10%)	4 (6%)	0
Aortic dissection type B	4 (4%)	0	1 (14%)
Spontaneous pneumothorax	3 (3%)	3 (4%)	0
Stretch marks	22 (21%)	20 (29%)	1 (14%)
Dural ectasia	13 (13%)	5 (7%)	0
Family history of Marfan syndrome	41 (40%)	30 (43%)	1 (14%)

CTD = connective tissue disorder; FBN1 = fibrillin 1

surgery, 30 (30/31, 97%) had MFS and only 1 patient LDS. Fifty-eight percent (18/31) of the aortic root aneurysms were repaired using the valve-sparing Tirone David technique (in one patient combined with tricuspid valve repair), whereas 35% (11/31) received a composite graft (in two patients combined with mitral valve replacement and one another patient combined with mitral valve repair). One patient received an isolated replacement of the ascending aorta, one patient underwent isolated mitral valve replacement and another one mitral valve repair. In two patients, details about the exact surgical technique for aortic root treatment were not available.

Four out of five patients (80%) who underwent mitral valve surgery had genetically proven MFS. In 60% (3/5) of these patients, replacement of the valve was needed; 40% (2/5) were repaired.

One patient of the cohort needed an implantable cardioverter defibrillator (ICD) because of ventricular tachycardia and a positive family history for sudden cardiac death. Two more patients needed implantation of pacemaker owing to postoperative complete atrioventricular block.

All cardiac interventions are summarised in table 5.

Cardiovascular complications and death

Fourteen percent (14/103) of the cohort experienced an aortic dissection; in 10/14 (71%) patients it was a type A dissection, the others had type B aortic dissection. Overall, 93% of these patients (13/14) had MFS, only one patient had LDS.

Three patients died, all of whom had MFS. A 15-year-old girl died from a hypoxic brain injury after out-of-hospital resuscitation because of ventricular fibrillation and an epileptic storm. A 16-year-old boy died from high-grade osteosarcoma in the lateral left femoral condyle. A 45-year-old woman died from infectious complications (mediastinitis) after repeated complex surgeries for type A aortic dissection.

Table 3: Summary of Ghent criteria and other skeletal findings.

Criterion	All patients (n = 103)	FBN1-positive patients (n = 69)	Other CTD (n = 7)
Ectopia lentis	29 (28%)	20 (29%)	0
Family history of Marfan syndrome	41 (40%)	30 (43%)	1 (14%)
Pectus carinatum	20 (19%)	16 (23%)	3 (43%)
Pectus excavatum	33 (32%)	22 (32%)	3 (43%)
Arm span/height ratio >1.05	2 (2%)	2 (3%)	0
Wrist or thumb sign	17 (17%)	13 (19%)	2 (29%)
Wrist and thumb sign	26 (25%)	21 (30%)	2 (29%)
Scoliosis	53 (51%)	37 (54%)	4 (57%)
Back surgery	12 (12%)	8 (12%)	1 (14%)
Reduced extension of the elbows (<170°)	8 (8%)	7 (10%)	0
Pes planus or valgus	54 (52%)	43 (62%)	5 (71%)
Protrusio acetabuli	6 (6%)	4 (6%)	0
Joint hypermobility	28 (27%)	21 (30%)	5 (71%)
Arachnodactyly	41 (40%)	30 (43%)	5 (71%)
Facial appearance and craniosynostosis	8 (8%)	6 (9%)	2 (29%)

CTD = connective tissue disorder; FBN1 = fibrillin 1

Table 4: Echocardiographic findings.

Parameter	All patients (n = 103)	FBN1-positive patients (n = 69)	Other CTD (n = 7)	p-value FBN1 vs CTD	Children / young adults <20 years old (n = 46)	Adults ≥20 years old (n = 57)	p-value children vs adults
Aortic root (cm), mean ± SD	3.4 ± 0.8	3.2 ± 0.7	3.4 ± 0.8	0.4781	2.9 ± 0.7	3.7 ± 0.7	0.0001
– Z-score (average absolute deviation)	1.77	1.72	1.37	0.0711	1.55	1.95	0.5925
Ascending aorta (cm), mean ± SD	2.7 ± 0.8	2.6 ± 0.7	2.2 ± 0.4	0.2789	2.1 ± 0.4	3.3 ± 0.6	0.0001
Aortic annulus (cm), mean ± SD	2.3 ± 0.5	2.2 ± 0.4	2.4 ± 0.3	0.3252	2.1 ± 0.5	2.5 ± 0.3	0.0001
Mitral valve prolapse, n (%)	64 (62%)	39 (57%)	4 (57%)	0.9752	29 (63%)	35 (61%)	0.8662
Degree mitral regurgitation, n (%)							
– none/trivial	74 (72%)	50 (72%)	5 (71%)	0.9542	31 (67%)	43 (75%)	0.3716
– mild	22 (21%)	14 (20%)	2 (29%)	0.6142	12 (26%)	10 (18%)	0.2976
– moderate	6 (6%)	4 (6%)	0	0.5192	3 (7%)	3 (5%)	0.7888
– severe	1 (1%)	1 (1%)	0	0.7524	0	1 (2%)	0.3716
Tricuspid valve prolapse, n (%)	16 (16%)	9 (13%)	2 (29%)	0.2719	6 (13%)	10 (18%)	0.5354
LVEDD (cm), mean ± SD	4.8 ± 0.8	4.7 ± 0.8	4.5 ± 0.8	0.6645	4.3 ± 0.8	5.2 ± 0.7	0.0001
Shortening fraction (%), mean ± SD	36.1 ± 7.6	36.6 ± 7.6	35 ± 5.9	0.6295	35.2 ± 6.5	36.9 ± 8.3	0.2924
LVEF (%), mean ± SD	57.6 ± 7.8	59.4 ± 6.7	55.7 ± 5.8	0.1944	57.9 ± 6.7	57.5 ± 8.4	0.8414
LVEF <50%, n (%)	9 (9%)	2 (3%)	1 (14%)	0.2616	1 (2%)	8 (14%)	0.0343

CTD = connective tissue disorder; FBN1 = fibrillin 1; LVEDD = left ventricular end-diastolic diameter; LVEF = left ventricular ejection fraction; SD = standard deviation

Medication

A total of 64% (66/103) of patients were under therapy with a beta-blocker, an angiotensin II receptor blocker (ARB) and/or an angiotensin converting-enzyme (ACE) inhibitor (more than one medication possible). Forty patients (39%) were on beta-blockers, 44 (43%) were on an ARB and 21 (20%) were on a combination of beta-blocker and ARB. Other medication included ACE inhibitors in 13 (13%) of patients. Nine percent of patients (9/103) were under treatment with a statin. Among the 37 patients not on any medication, we identified 5 adults with a Z-score >3.0.

Family history

Eleven patients (11%) had a family member who suffered or even died from aortic dissection, 26 (25%) patients had a relative with an aortic aneurysm, and 14 (14%) had a positive family history for sudden cardiac death.

Role of genetic testing for confirmation of diagnosis

A systemic score ≥7 (required to document systemic involvement according to the Ghent criteria) was found in only 40 patients (39%). Thus in 41 patients (41/103, 40%) a definitive diagnosis was achieved only with additional information from genetic testing.

Discussion

In our group of 103 patients with clinical suspicion of MFS, 69 were confirmed as having MFS by genetic testing and the 2010 Ghent criteria. In seven patients MFS-related LDS due to mutation in the genes *TGFBR1* (LDS1), *TGFBR2* (LDS2), *SMAD3* (LDS3) or *TGFB2* (LDS4) was identified by genetic testing. In 27 patients (26%), MFS was diagnosed on the basis of the Ghent criteria without genetic testing. In our cohort, genetic testing was quite frequent. In three patients fulfilling the revised Ghent criteria no mutation in any of the tested genes has yet been found. In a large number of affected patients, the diagnosis of MFS or a related disorder could be confirmed only by genetic testing in addition to clinical and echocardiographic examination. These results demonstrate the steadily growing impact of genetic testing in patients with suspected CTDs. Genetic testing often not only confirms the specific diagnosis, but may in many cases also has an impact on medical treatment and timing of life-saving aortic surgery.

The frequency and type of cardiovascular surgery in our cohort was comparable to other centres, with valve-sparing surgery used as often as possible [13, 14].

Medical therapy was used according to guidelines in 63% of patients [15, 16].

To date, over 200 heritable CTDs have been described, affecting various organ systems, including heart, blood vessels, bone, eyes, skin, joints and lungs [17]. Over the past few decades, many of the underlying molecular defects have been identified, including genes encoding for structural proteins (e.g., *FBN1*, *COL1A1*, *COL3A1*, *COL5A1*, *BGN*), modifying enzymes (e.g., *ADAMTS2*, *PLOD1*), or components of the transforming growth factor-beta (TGFβ)-signalling pathways (e.g., *SKI, SMAD2/3, TGFBR1/2* and *TGFB2/3*). However, as of now these genes do not exclusively explain all causes of CTDs and thus many genes still await discovery [17]. In the majority of patients with clinical suspicion of MFS, mutations (disease-causing sequence variants) in *FBN1* have been identified; however, mutations in the genes causing LDS (*TGFBR1, TGFBR2, SMAD3, TGFB2, TGFB3*), one of the most important differential diagnosis of MFS, or in other aortopathy-related genes such as *ACTA2, MYH11, MYLK, MAT2A, MFAP5* and *PRKG1* have also been detected. In particular, patients with mutations in genes causing LDS can present with distinct and characteristic clinical phenotypes including hypertelorism and bifid uvula, both features not found in MFS. Patients with *ACTA2* mutations often have livedo reticularis and iris flocculi. Such signs can help in the clinical differentiation. However, bifid uvula is not specific for LDS and has been found in other diseases such as cleft palate (e.g., *SKI*-related Shprintzen-Goldberg Syndrome) or phosphoglucomutase 1 (*PGM1*) deficiency, a disease not related to a CTD.

Some CTDs present with distinct clinical phenotypes, but many of them lead to overlapping clinical features despite the fact that they are have different genetic aetiologies. This is one explanation of why the clinical Ghent score is insufficient for a final diagnosis and illustrates the importance of molecular testing in the diagnosis of MFS and other CTDs. The frequency of genetic testing in this study was relatively high and comparable to the data published by Schoenhoff et al. from Berne [18], the largest MFS centre in Switzerland (76% in 2014), but considerably higher than those from the Mayo Clinic involving 59 patients

Table 5: Summary of cardiac interventions.

Intervention	All patients (n = 103)	FBN1-positive patients (n = 69)	Other CTD (n = 7)
Cardiac surgery	34 (33%)	18 (26%)	1 (14%)
Aortic root surgery	31 (30%)	15 (22%)	1 (14%)
– Tirone David	18 (17%)	11 (16%)	1 (14%)
– composite graft	11 (11%)	4 (6%)	0
– exact surgical technique unknown	2 (2%)	0	0
Isolated replacement of aorta ascendens	1 (1%)	1 (1%)	0
Mitral valve surgery	5 (5%)	4 (6%)	0
– repair	2 (2%)	2 (3%)	0
– replacement	3 (3%)	2 (3%)	0
Tricuspid valve repair	1 (1%)	1 (1%)	0
ICD implantation	1 (1%)	1 (1%)	0
PM implantation	2 (2%)	0	0

CTD = connective tissue disorder; FBN1 = fibrillin 1; ICD = implantable cardioverter defibrillator; PM = pacemaker

with suspected MFS undergoing aortic root surgery from a similar time period, where genetic testing was performed in only 25% [13].

The criteria of the revised Ghent Marfan systemic score were published in 2010 [6]. A total score of at least 7 is considered essential in the diagnosis of MFS. However, as shown in our cohort, the majority of patients (61%) did not score 7 points. This might be because two of the clinical features, protrusio acetabuli and dural ectasia, can be found only radiologically and are thus not routinely assessed, although it is known that dural ectasia, for example, is present in 95% of children with MFS [19]. In addition, the specificity of dural ectasia has never been examined to the best of our knowledge. Therefore, in current practice the use of the 2010 Ghent criteria is only somewhat helpful as they include two criteria that cannot be commonly assessed.

Ectopia lentis, which is not present in LDS, is found in 62% of cases of MFS [20], but can also be an isolated finding not associated with MFS. In our cohort, ectopia lentis was present in only 28%, which is considerably less than reports in the current literature. To exclude glaucoma and cataracts in the MFS eye, patients should have an annual ophthalmological examination. In order to offer tailored treatment and clinical follow up, molecular genetic testing should be performed in all patients with suspected MFS or CTD.

Cardiac interventions

Cardiac surgery was needed in 33%, including aortic root surgery (30%) and mitral valve surgery (5%). Additionally, one patient needed an ICD. As expected, most of the cardiac procedures were on the aortic root. The most frequent surgical technique was the valve-sparing Tirone David procedure, which was comparable to other centres [13, 21, 22]. The thresholds used for intervention in the aortic root were 4.5–5.0 cm in adults with MFS (depending on family history); the thresholds may be lower if fast growth of the aortic root is observed or before planned pregnancy. In LDS, the threshold is 4.2 cm in adults and in children if the 99th percentile of the aortic root is exceeded and the aortic annulus surpasses 1.8–2.0cm [15, 16]. Lower thresholds are used depending on growth of the aorta, planned pregnancy or small size of the patient.

Benefits of the valve-sparing operation are that the patients keep their own aortic valve and that there is no need of lifelong anticoagulation, which is desirable especially in young women who wish to have a child. For 1-year survival there is no difference between the two techniques, but severe mitral regurgitation was observed more often after the valve-sparing surgery [21]. Currently, other surgical techniques such as PEARS (personalised external aortic root support) and the Florida sleeve operation play a negligible role in MFS patients [23, 24]. Another option would be to use an aortic bioprothesis with subsequent percutaneous aortic valve replacement if needed. There are limited data on percutaneous mitral valve reconstruction with MitraClip in MFS [25]. However, there are no data on the performance of percutaneous cardiac interventions on subsequent risk of aortic dissection in MFS. Long-term outcome data in MFS show that aortic surgery in other segments is quite frequent; even after elective aortic root replacement the risk of reintervention is more than 10% with about 15% of patients suffering from type B dissection over time. After type A dissection the risk of reintervention is 50% or higher [26–28].

Medical treatment

Overall, 64% of the patients in our cohort were on some form of cardiac medical treatment: 39% were treated with a beta-blocker, 43% with an ARB and 13% with an ACE inhibitor. These results are similar to recently published studies [13]. Of the 37 patients not on medication, 23 (62%) were <17 years old and the mean diameters were 3.1 ± 0.9 cm for the aortic root and 2.4 ± 0.7 cm for the ascending aorta. Of those >17 years of age, after excluding the patients who undergone Tirone David or composite graft procedures or having Z-score <2.0, we identified 5 patients with Z-score >3.0 not on medication,

Since 1994, when Shores and colleagues showed that treatment with beta-blockers in MFS patients slows aortic growth rate, beta-blockers belong to the standard of care in MFS patients with either aortic root dilatation and/or positive family history for aortic root dilatation and/or a mutation know to be associated with an aortic phenotype [29–32]. Therefore we anticipated a much higher percentage of patients under beta-blocker therapy. Our data show that patient education is important and should be a main focus in treatment of these patients.

Since ARB therapy has been shown in a MFS mouse model to have a protective effect on the aorta for aneurysms and dissection, its use has been widespread [33]. In 2014, Mueller et al. showed in a cohort of paediatric MFS patients that ARB and beta-blocker therapy both slow aortic root dilatation [34]. Losartan and beta-blockers such as atenolol seem to be equivalent [35]. The Taiwan Marfan trial and the compare trial both demonstrated that combined therapy with a beta-blocker and ARB was more effective than beta-blockers alone [36, 37]. Larger trials such as the Pediatric Heart Network randomised trial of atenolol versus losartan in children and young adults showed similar efficacy [38]. Therefore it is up to the treating clinician to choose a beta-blocker and/or ARB in patients with MFS; however, at least one of these medications should be used.

There are no data showing that ACE inhibitors are non-inferior to ARB or beta-blocker therapy; some studies have suggested less efficacy [39]. Calcium channel blockers are not routinely recommended in the treatment of MFS patients [40].

Other organ involvement

Regular orthopaedic assessment is strongly recommended as noncardiovascular procedures are often needed in MFS patients, including scoliosis surgery, foot surgery, herniotomy, lensectomy and pleurodesis after spontaneous pneumothorax. In our cohort, 12% of patients had back surgery with an incidence of scoliosis of 51%.

Study limitations

Assessment of dural ectasia with magnetic resonance imaging and hip radiography to assess protrusio acetabuli were not routinely performed without clinical indication. Therefore, the Ghent score was most likely underestimat-

ed. However, in clinical practice, dural ectasia and hip are not routinely assessed radiographically in most centres.

Clinical genetic testing has become more important to make or confirm the diagnosis, allowing the identification and counselling of at-risk relatives as well. However, the current ability to sequence is greater than the ability to interpret the detected sequence variants with diagnostic and lifetime value [41].

We retrospectively analysed a limited group of patients from cardiovascular centres without taking into account patients from orthopaedic and ophthalmology clinics. We do not know the impact of this limitation on our data. Besides, we cannot exclude an ascertainment bias and some patients supposed to have MFS were retrospectively found to have LDS. However, genetic testing was offered to all patients in this study group, so there was no bias in that regard. The selection criteria were such that we included only patients from the echocardiography database with an aortic root aneurysm or type A aortic dissection where analysis of the clinical reported showed at least one feature suggestive of a CTD. Therefore, the prevalence is falsely low.

It is a retrospective study from a relatively small cohort, thus the results have to be confirmed in larger studies. We are not aware of any patients in the five centres fulfilling the inclusion criteria who were not included and therefore this study fulfils the criteria in the STROBE statement [42].

Conclusion

This study shows that in our centres in Switzerland a high percentage of patients fulfilling the revised Ghent criteria for MFS undergo genetic testing, often leading to or confirming the diagnosis of MFS. Clinical evaluation in combination with genetic testing is by far superior to clinical evaluation alone.

Cardiac surgery was performed according to guidelines and most often for the dilated aortic root [15, 16]. This study did not analyse long-term outcome and cannot report the frequency of reintervention, which is considerable in this cohort.

Only 63% of the patients were under therapy with beta-blockers, ARBs and/or ACE inhibitors. Thus conservative management in MFS has to be improved, necessitating more time for physician/patient interaction.

Disclosure statement

The study was supported by the HerzGefäss Stiftung Zürich der Klinik Im Park. Otherwise there was no any financial interest/arrangement or affiliation with one or more organisations that could be perceived as a real or apparent conflict of interest in the context of the subject of this paper.

References

1 Kainulainen K, Pulkkinen L, Savolainen A, Kaitila I, Peltonen L. Location on chromosome 15 of the gene defect causing Marfan syndrome. N Engl J Med. 1990;323(14):935–9. doi: http://dx.doi.org/10.1056/NEJM199010043231402. PubMed.

2 Vollbrandt T, Tiedemann K, El-Hallous E, Lin G, Brinckmann J, John H, et al. Consequences of cysteine mutations in calcium-binding epidermal growth factor modules of fibrillin-1. J Biol Chem. 2004;279(31):32924–31. doi: http://dx.doi.org/10.1074/jbc.M405239200. PubMed.

3 Faivre L, Collod-Beroud G, Loeys BL, Child A, Binquet C, Gautier E, et al. Effect of mutation type and location on clinical outcome in 1,013 probands with Marfan syndrome or related phenotypes and FBN1 mutations: an international study. Am J Hum Genet. 2007;81(3):454–66. doi: http://dx.doi.org/10.1086/520125. PubMed.

4 Dietz HC, Cutting GR, Pyeritz RE, Maslen CL, Sakai LY, Corson GM, et al. Marfan syndrome caused by a recurrent de novo missense mutation in the fibrillin gene. Nature. 1991;352(6333):337–9. doi: http://dx.doi.org/10.1038/352337a0. PubMed.

5 Pyeritz RE, McKusick VA. The Marfan syndrome: diagnosis and management. N Engl J Med. 1979;300(14):772–7. doi: http://dx.doi.org/10.1056/NEJM197904053001406. PubMed.

6 Loeys BL, Dietz HC, Braverman AC, Callewaert BL, De Backer J, Devereux RB, et al. The revised Ghent nosology for the Marfan syndrome. J Med Genet. 2010;47(7):476–85. doi: http://dx.doi.org/10.1136/jmg.2009.072785. PubMed.

7 Attenhofer Jost CH, Greutmann M, Connolly HM, Weber R, Rohrbach M, Oxenius A, et al. Medical treatment of aortic aneurysms in Marfan syndrome and other heritable conditions. Curr Cardiol Rev. 2014;10(2):161–71. doi: http://dx.doi.org/10.2174/1573403X10021405061249O2. PubMed.

8 Devereux RB, de Simone G, Arnett DK, Best LG, Boerwinkle E, Howard BV, et al. Normal limits in relation to age, body size and gender of two-dimensional echocardiographic aortic root dimensions in persons ≥15 years of age. Am J Cardiol. 2012;110(8):1189–94. doi: http://dx.doi.org/10.1016/j.amjcard.2012.05.063. PubMed.

9 Campens L, Demulier L, De Groote K, Vandekerckhove K, De Wolf D, Roman MJ, et al. Reference values for echocardiographic assessment of the diameter of the aortic root and ascending aorta spanning all age categories. Am J Cardiol. 2014;114(6):914–20. doi: http://dx.doi.org/10.1016/j.amjcard.2014.06.024. PubMed.

10 Mátyás G, De Paepe A, Halliday D, Boileau C, Pals G, Steinmann B. Evaluation and application of denaturing HPLC for mutation detection in Marfan syndrome: Identification of 20 novel mutations and two novel polymorphisms in the FBN1 gene. Hum Mutat. 2002;19(4):443–56. doi: http://dx.doi.org/10.1002/humu.10054. PubMed.

11 Mátyás G, Arnold E, Carrel T, Baumgartner D, Boileau C, Berger W, et al. Identification and in silico analyses of novel TGFBR1 and TGFBR2 mutations in Marfan syndrome-related disorders. Hum Mutat. 2006;27(8):760–9. doi: http://dx.doi.org/10.1002/humu.20353. PubMed.

12 Mátyás G, Alonso S, Patrignani A, Marti M, Arnold E, Magyar I, et al. Large genomic fibrillin-1 (FBN1) gene deletions provide evidence for true haploinsufficiency in Marfan syndrome. Hum Genet. 2007;122(1):23–32. Published online May 10, 2007. doi: http://dx.doi.org/10.1007/s00439-007-0371-x. PubMed.

13 Attenhofer Jost CH, Connolly HM, Scott CG, Ammash NM, Bowen JM, Schaff HV. Aortic Root Surgery in Marfan Syndrome: Medium-Term Outcome in a Single-Center Experience. J Heart Valve Dis. 2017;26(1):45–53. PubMed.

14 Price J, Magruder JT, Young A, Grimm JC, Patel ND, Alejo D, et al. Long-term outcomes of aortic root operations for Marfan syndrome: A comparison of Bentall versus aortic valve-sparing procedures. J Thorac Cardiovasc Surg. 2016;151(2):330–8. doi: http://dx.doi.org/10.1016/j.jtcvs.2015.10.068. PubMed.

15 Erbel R, Aboyans V, Boileau C, Bossone E, Bartolomeo RD, Eggebrecht H, et al., The Task Force for the Diagnosis and Treatment of Aortic Diseases of the European Society of Cardiology (ESC). 2014 ESC Guidelines on the diagnosis and treatment of aortic diseases: Document covering acute and chronic aortic diseases of the thoracic and abdominal aorta of the adult. Eur Heart J. 2014;35(41):2873–926. doi: http://dx.doi.org/10.1093/eurheartj/ehu281. PubMed.

16 Hiratzka LF, Bakris GL, Beckman JA, Bersin RM, Carr VF, Casey DE, Jr, et al.; American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines; American Association for Thoracic Surgery; American College of Radiology; American Stroke Association; Society of Cardiovascular Anesthesiologists; Society for Cardiovascular Angiography and Interventions; Society of Interventional Radiology; Society of Thoracic Surgeons; Society for Vascular Medicine. 2010 ACCF/AHA/AATS/ACR/ASA/SCA/SCAI/SIR/STS/SVM guidelines for the diagnosis and management of patients with Thoracic Aortic Disease: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines, American Association for Thoracic Surgery, American College of Radiology, American Stroke Association, Society of Cardiovascular Anesthesiologists, Society for Cardiovascular Angiography and Interventions, Society of Interventional Radiology, Society of Thoracic Surgeons, and Society for Vascular Medicine. Circulation. 2010;121(13):e266–369. PubMed.

17 Meester JAN, Verstraeten A, Schepers D, Alaerts M, Van Laer L, Loeys BL. Differences in manifestations of Marfan syndrome, Ehlers-Danlos

syndrome, and Loeys-Dietz syndrome. Ann Cardiothorac Surg. 2017;6(6):582–94. doi: http://dx.doi.org/10.21037/acs.2017.11.03. PubMed.

18 Schoenhoff FS, Mueller C, Czerny M, Matyas G, Kadner A, Schmidli J, et al. Outcome of aortic surgery in patients with Loeys-Dietz syndrome primarily treated as having Marfan syndrome. Eur J Cardiothorac Surg. 2014;46(3):444–9, discussion 449. doi: http://dx.doi.org/10.1093/ejcts/ezu002. PubMed.

19 Veldhoen S, Stark V, Mueller GC, Derlin T, Bley TA, Weil J, et al. Pediatric patients with Marfan syndrome: frequency of dural ectasia and its correlation with common cardiovascular manifestations. RoFo Fortschr Geb Rontgenstr Nuklearmed. 2014;186(1):61–6. PubMed.

20 Maumenee IH. The eye in the Marfan syndrome. Birth Defects Orig Artic Ser. 1982;18(6):515–24. PubMed.

21 Coselli JS, Volguina IV, LeMaire SA, Sundt TM, Connolly HM, Stephens EH, et al.; Aortic Valve Operative Outcomes in Marfan Patients Study Group. Early and 1-year outcomes of aortic root surgery in patients with Marfan syndrome: a prospective, multicenter, comparative study. J Thorac Cardiovasc Surg. 2014;147(6):1758–1767.e4. doi: http://dx.doi.org/10.1016/j.jtcvs.2014.02.021. PubMed.

22 Schoenhoff FS, Langhammer B, Wustmann K, Reineke D, Kadner A, Carrel T. Decision-making in aortic root surgery in Marfan syndrome: bleeding, thromboembolism and risk of reintervention after valve-sparing or mechanical aortic root replacement. Eur J Cardiothorac Surg. 2015;48(6):931–5, discussion 935–6. doi: http://dx.doi.org/10.1093/ejcts/ezu553. PubMed.

23 Böckler D, Meisenbacher K, Peters AS, Grond-Ginsbach C, Bischoff MS. Endovascular treatment of genetically linked aortic diseases. Gefasschirurgie. 2017;22(S1, Suppl 1):1–7. doi: http://dx.doi.org/10.1007/s00772-016-0221-z. PubMed.

24 Treasure T, Takkenberg JJ, Pepper J. Republished review: Surgical management of aortic root disease in Marfan syndrome and other congenital disorders associated with aortic root aneurysms. Postgrad Med J. 2016;92(1084):112–7. doi: http://dx.doi.org/10.1136/postgradmedj-2013-305132rep. PubMed.

25 Sung S-H, Tsai YL, Lee C-W. Emergent MitraClip to Rescue Patient with Acute Chordae Rupture and RV Failure. J Am Coll Cardiol. 2017;69(16, Supplement):S360–1. doi: http://dx.doi.org/10.1016/j.jacc.2017.03.508.

26 Schoenhoff FS, Jungi S, Czerny M, Roost E, Reineke D, Matyas G, et al. Acute aortic dissection determines the fate of initially untreated aortic segments in Marfan syndrome. Circulation. 2013;127(15):1569–75. doi: http://dx.doi.org/10.1161/CIRCULATIONAHA.113.001457. PubMed.

27 Rylski B, Bavaria JE, Beyersdorf F, Branchetti E, Desai ND, Milewski RK, et al. Type A aortic dissection in Marfan syndrome: extent of initial surgery determines long-term outcome. Circulation. 2014;129(13):1381–6. doi: http://dx.doi.org/10.1161/CIRCULATIONAHA.113.005865. PubMed.

28 Rylski B, Pacini D, Beyersdorf F, Quintana E, Schachner T, Tsagakis K, et al.; EACTS Vascular Domain, EJCTS and ICVTS Editorial Committees. Standards of reporting in open and endovascular aortic surgery (STORAGE guidelines). Eur J Cardiothorac Surg. 2019;56(1):10–20. doi: http://dx.doi.org/10.1093/ejcts/ezz145. PubMed.

29 Halpern BL, Char F, Murdoch JL, Horton WB, McKusick VA. A prospectus on the prevention of aortic rupture in the Marfan syndrome with data on survivorship without treatment. Johns Hopkins Med J. 1971;129(3):123–9. PubMed.

30 Shores J, Berger KR, Murphy EA, Pyeritz RE. Progression of aortic dilatation and the benefit of long-term beta-adrenergic blockade in Marfan's syndrome. N Engl J Med. 1994;330(19):1335–41. doi: http://dx.doi.org/10.1056/NEJM199405123301902. PubMed.

31 Rossi-Foulkes R, Roman MJ, Rosen SE, Kramer-Fox R, Ehlers KH, O'Loughlin JE, et al. Phenotypic features and impact of beta blocker or calcium antagonist therapy on aortic lumen size in the Marfan syndrome. Am J Cardiol. 1999;83(9):1364–8. doi: http://dx.doi.org/10.1016/S0002-9149(99)00101-0. PubMed.

32 Salim MA, Alpert BS, Ward JC, Pyeritz RE. Effect of beta-adrenergic blockade on aortic root rate of dilation in the Marfan syndrome. Am J Cardiol. 1994;74(6):629–33. doi: http://dx.doi.org/10.1016/0002-9149(94)90762-5. PubMed.

33 Habashi JP, Judge DP, Holm TM, Cohn RD, Loeys BL, Cooper TK, et al. Losartan, an AT1 antagonist, prevents aortic aneurysm in a mouse model of Marfan syndrome. Science. 2006;312(5770):117–21. doi: http://dx.doi.org/10.1126/science.1124287. PubMed.

34 Mueller GC, Stierle L, Stark V, Steiner K, von Kodolitsch Y, Weil J, et al. Retrospective analysis of the effect of angiotensin II receptor blocker versus β-blocker on aortic root growth in paediatric patients with Marfan syndrome. Heart. 2014;100(3):214–8. doi: http://dx.doi.org/10.1136/heartjnl-2013-304946. PubMed.

35 Forteza A, Evangelista A, Sánchez V, Teixidó-Turà G, Sanz P, Gutiérrez L, et al. Efficacy of losartan vs. atenolol for the prevention of aortic dilation in Marfan syndrome: a randomized clinical trial. Eur Heart J. 2016;37(12):978–85. doi: http://dx.doi.org/10.1093/eurheartj/ehv575. PubMed.

36 den Hartog AW, Franken R, van den Berg MP, Zwinderman AH, Timmermans J, Scholte AJ, et al. The effect of losartan therapy on ventricular function in Marfan patients with haploinsufficient or dominant negative FBN1 mutations. Neth Heart J. 2016;24(11):675–81. doi: http://dx.doi.org/10.1007/s12471-016-0905-8. PubMed.

37 Chiu HH, Wu MH, Wang JK, Lu CW, Chiu SN, Chen CA, et al. Losartan added to β-blockade therapy for aortic root dilation in Marfan syndrome: a randomized, open-label pilot study. Mayo Clin Proc. 2013;88(3):271–6. doi: http://dx.doi.org/10.1016/j.mayocp.2012.11.005. PubMed.

38 Lacro RV, Dietz HC, Sleeper LA, Yetman AT, Bradley TJ, Colan SD, et al.; Pediatric Heart Network Investigators. Atenolol versus losartan in children and young adults with Marfan's syndrome. N Engl J Med. 2014;371(22):2061–71. doi: http://dx.doi.org/10.1056/NEJMoa1404731. PubMed.

39 Habashi JP, Doyle JJ, Holm TM, Aziz H, Schoenhoff F, Bedja D, et al. Angiotensin II type 2 receptor signaling attenuates aortic aneurysm in mice through ERK antagonism. Science. 2011;332(6027):361–5. doi: http://dx.doi.org/10.1126/science.1192152. PubMed.

40 Doyle JJ, Doyle AJ, Wilson NK, Habashi JP, Bedja D, Whitworth RE, et al.; GenTAC Registry Consortium; MIBAVA Leducq Consortium. A deleterious gene-by-environment interaction imposed by calcium channel blockers in Marfan syndrome. eLife. 2015;4:. doi: http://dx.doi.org/10.7554/eLife.08648. PubMed.

41 Caspar SM, Dubacher N, Kopps AM, Meienberg J, Henggeler C, Matyas G. Clinical sequencing: From raw data to diagnosis with lifetime value. Clin Genet. 2018;93(3):508–19. doi: http://dx.doi.org/10.1111/cge.13190. PubMed.

42 von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP; STROBE Initiative. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement: guidelines for reporting observational studies. Int J Surg. 2014;12(12):1495–9. doi: http://dx.doi.org/10.1016/j.ijsu.2014.07.013. PubMed.