Contribution of molecular analyses in diagnosing Marfan syndrome and type I fibrillinopathies: an international study of 1009 probands.

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ABSTRACT

Background: The diagnosis of Marfan syndrome (MFS) is usually initially based on clinical criteria according to the number of major and minor systems affected following international nosology. The number of *FBN1* mutation carriers, at risk of aortic complications who would not be properly diagnosed based only on clinical grounds, is of growing importance owing to the increased availability of molecular screening. The aim of the study was to identify patients who should be considered for *FBN1* mutation screening.

Methods: Our international series included 1009 probands with a known *FBN1* mutation. Patients were classified as either fulfilling or not fulfilling "clinical" criteria. In patients with unfulfilled "clinical" criteria, we evaluated the percentage of additional patients who became positive for international criteria when the *FBN1* mutation was considered. The aortic risk was evaluated and compared in patients fulfilling or not fulfilling the "clinical" international criteria.

Results: Diagnosis of MFS was possible on clinical grounds in 79% of the adults, whereas 90% fulfilled the international criteria when including the *FBN1* mutation. Corresponding figures for children were 56% and 85%, respectively. Aortic dilatation occurred later in adults with unfulfilled "clinical criteria" when compared to the Marfan syndrome group (44% vs 73% at 40 years, $p<0.001$), but the lifelong risk for ascending aortic dissection or surgery was not significantly different in both groups.

Conclusions: Because of its implications for aortic follow-up, *FBN1* molecular analysis is recommended in newly suspected MFS when two systems are involved with at least one major system affected. This is of utmost importance in patients without aortic dilatation and in children.

Marfan syndrome (MFS; MIM 154700) is a connective tissue disorder with autosomal dominant inheritance and a prevalence of 1/5000 individuals,[1] caused mainly by *FBN1* mutations.[2] The cardinal features of MFS involve the ocular, cardiovascular and skeletal systems.[3] The skin, lungs and dura may also be involved. In order to facilitate appropriate patient management and counselling, clinical criteria for the diagnosis of MFS were established.[4 5] The initial criteria were proven to be too loose once molecular data became available.[4 6 7] For MFS to be diagnosed in an individual, revised diagnostic criteria were established, requiring at least two major systems affected (lens dislocation, ascending aortic dilatation or dissection, dural ectasia, a specified combination of skeletal features, family history or *FBN1* mutation) and involvement of at least one other organ system (table 1).[5]

In practice, only clinical data are readily available and are used for initial evaluation. Reviews of patients referred for *FBN1* analysis indicated that an *FBN1* mutation was identified in 91% of patients when diagnosis of MFS was possible on clinical grounds; therefore, clinical data are usually specific and sufficient to establish the diagnosis of MFS in this population.[8] Besides classical MFS, *FBN1* mutations are also responsible for a large phenotypic spectrum ranging from neonatal Marfan syndrome to incomplete clinical entities. The latter are referred to as "other type I fibrillinopathies" and include patients with at least one system involvement but not fulfilling the clinical criteria listed in the Ghent nosology ("clinical" Ghent criteria). Therefore the question arises of when to ask for the *FBN1* mutation screening in patients with incomplete phenotype since, despite technical advances, determining the presence of an *FBN1* mutation remains labour intensive and costly.

From an international series of 1009 probands with a known *FBN1* mutation, we assessed the contribution of molecular analyses in recognising patients at risk and suggest practical guidelines for indications for molecular analysis. We also describe the clinical characteristics of patients in whom a diagnosis can be made on clinical grounds alone, and those of patients not fulfilling "clinical" criteria, and compared the prognosis between the two groups.

PATIENTS AND METHODS

A total of 1191 probands carrying an *FBN1* mutation, registered between 1995 and 2005, were identified via the Universal Marfan database *FBN1* (UMD-*FBN1*; http:/www.umd.be)[2 9] and the network of participating centres. Inclusion criteria in the present study were (1) heterozygosity for a pathogenic *FBN1* gene mutation and (2) the availability of clinical information. Of 1191 probands with an *FBN1* mutation, 182 probands were

Table 1 Diagnostic criteria of Marfan syndrome according to Ghent nosology[5]

Criterion	Major manifestations	Minor manifestations
Skeletal system	► Pectus carinatum, or excavatum requiring surgery ► Arm span to height ratio >1·05, or reduced US/LS <0.86 ► Positive wrist and thumb sign ► Scoliosis >20° or spondylolisthesis ► Limited elbow extension <170° ► Flat feet ► Protrusio acetabulae *4 of 7 major present (system affected)*	► Facial appearance ► Joint hypermobility ► Moderate pectus excavatum ► High arched palate *2 of 7 major present, or 1 of 7 major and 2 of 4 minor present (system involvement)*
Ocular system	► Ectopia lentis *Ectopia lentis present (system affected)*	► Myopia ► Flat cornea ► Iris or ciliary muscle hypoplasia *2 of 3 minor present (system involvement)*
Cardiovascular system	► Ascending aortic dilatation with or without aortic regurgitation and involving the sinuses of Valsalva ► Ascending aortic dissection *1 of 2 major present (system affected)*	► Mitral valve prolapse ► Annulus mitralis calcification ► Dilatation or dissection of descending aorta or abdominal aorta before 50 years ► Dilatation of the main pulmonary artery before 40 years *1 of 4 minor present (system involvement)*
Pulmonary system	None indicated	► Pneumothorax ► Apical blebs *1 of 2 minor present (system involvement)*
Skin	None indicated	► Striae atrophicae ► Recurrent or incisional herniae *1 of 2 minor present (system involvement)*
Dural	► Lumbosacral dural ectasia *Dural ectasia present (system affected)*	None indicated
Family	► First degree family member independently fulfilling diagnostic criteria ► Pathogenic *FBN1* mutation *1 of 2 major present (system affected)*	None indicated

US/LS, upper segment/lower segment.
Requirements of the diagnosis of the Marfan syndrome:
For the index case: if the family/genetic history is not contributory, major criteria in at least two different organ systems and involvement of a third organ system; if a mutation known to cause Marfan syndrome in others is detected, one major criterion in an organ system and involvement of a second organ system.
For a relative of an index case: presence of a major criterion in the family history and one major criterion in an organ system and involvement of a second organ system.

excluded from the study (no clinical data available in 129, insufficient data on cardiovascular, ocular or skeletal involvement in 44, autosomal dominant Weill–Marchesani syndrome in four since they represent another well defined type I fibrillinopathy, two mutations on the same allele in four, and compound heterozygosity in one) (fig 1). The patients included in the study originated from 38 different countries located across five continents. The clinical data were collected either from a questionnaire sent to referring physicians and clinical investigators or from previous publications wherein sufficient information was available. All questionnaires were collected and referenced by one individual (LF) to avoid duplication of patients in the study. The clinical information required included a range of qualitative and quantitative clinical parameters, including cardiovascular, ophthalmological, skeletal, skin, lung and dural manifestations.

For each patient, major affected system and/or minor system involvement was defined for skeletal, ocular and cardiovascular, lung, skin and dural manifestations, according to the Ghent criteria.[5] Ghent criteria were defined as positive when an isolated patient presented with two major manifestations and involvement of at least one other body system (table 1). In familial cases, the presence of a major criterion in one organ system and the involvement of a second organ system were sufficient for the diagnosis of MFS if another family member was independently diagnosed with MFS.[5] In order to reproduce better the situation that clinicians face in clinical practice, the presence of an *FBN1* mutation was not considered as a major

feature in the first step. Thus, patients were classified into two groups: those who fulfilled international criteria on clinical grounds, and those who did not (fig 1). Clinical characteristics and prognosis were described in both groups. In the second step, we evaluated the percentage of patients requiring *FBN1* screening for the diagnosis of MFS (fig 1). Since some of the clinical or radiological features that are used as diagnostic criteria were only occasionally investigated (protrusio acetabulae, dural ectasia, flat cornea, iris or ciliary muscle hypoplasia, dilatation of the pulmonary artery, mitral annular calcification and apical blebs), an unmentioned clinical feature was considered as absent for the determination of the patient's status. For descriptive purposes, the overall population was also classified as having neonatal MFS (defined in this study by a diagnosis of type I fibrillinopathy with severe valvular involvement before 4 weeks of age),[10] severe MFS (defined by MFS diagnosis on clinical grounds with aortic dilatation before 10 years of age), probable MFS (defined by unfulfilled "clinical" criteria in childhood—that is, <18 years), other type I fibrillinopathies (defined by unfulfilled "clinical" criteria in adulthood), and classical MFS (defined by diagnosis of MFS on clinical grounds in other patients).

The Kaplan–Meier method was used to estimate the cumulative probabilities (and their 99.9% confidence interval (CI)) of diagnosing ascending aortic dilatation, aortic dissection and aortic surgery at 40 years of age in order to elucidate the impact of this clinical feature over time.[11] A log rank test was used to compare probabilities depending on whether patients fulfilled "clinical" Ghent criteria or not. The significance level was 0.001.

RESULTS

The median age at diagnosis of the probands was 20 years (interquartile range (IQR) 9–33). The ages at last follow-up ranged from birth to 72 years, with a median age of 29 years (IQR 15–40). Three hundred and twenty patients were aged <18 years (32%). Fifty-four per cent of patients were males and 46% were females. A family history of type I fibrillinopathy was found in 51% of cases (62% in the adult cohort). The population was classified as follows: 5% neonatal MFS, 7% severe MFS, 63% classical MFS, 11% probable MFS, and 14% other type I fibrillinopathies, when the presence of an *FBN1* mutation was not considered as a major feature.

Overall, the distribution of *FBN1* mutations among probands (types and locations) has been described elsewhere.[12] Briefly, 33% of mutations were predicted to produce a premature truncation codon and 67% an in-frame mutation. Seventy-four per cent of mutations were located in an epidermal growth factor (EGF) domain, 15% in a transforming growth factor-β1 binding protein (TGFβ-BP) domain, 20% in exons 24–32 (the so-called "neonatal region"), 29% in the 5′ region (exons 1 to 21), and 37% in the 3′ region (exons 43 to 65).

The percentages of clinical features that are part of the international classification are given in table 2. The percentages of major affected system and minor system involvement were determined in the overall population of probands, and also stratified in adults and in patients aged <18 years (table 3). Among probands, 32% had major skeletal affected system, 54% had major ocular affected system, and 77% had major cardiovascular affected system.

In the overall population of probands, diagnosis of MFS was possible on clinical grounds in 72%; in an additional 17% of the population, diagnosis required *FBN1* mutation screening. Analysis of adult probands only (n = 689) showed that diagnosis of MFS was possible on clinical grounds in 79%, and in an additional 11% of the population, diagnosis required *FBN1* mutation screening (fig 1, fig 2A). Since only 292 patients had been examined for dural ectasia, we also studied the percentage

Figure 1 Study population and design.

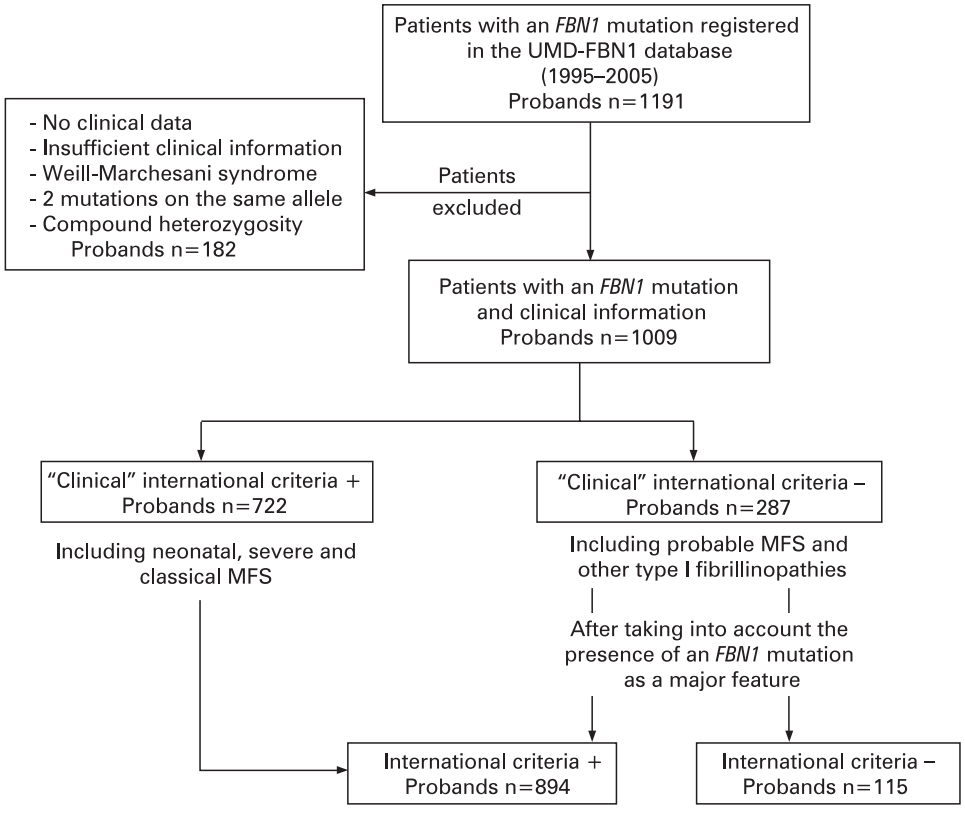

Table 2 Frequency of clinical features of the international nosology found in the 1009 probands

Clinical features	%	Number of available data
Skeletal system		
Pectus carinatum, or excavatum requiring surgery	32	958*
Arm span to height ratio >1.05, or reduced US/LS <0.86	55	943*
Positive wrist and thumb sign	78	965*
Scoliosis >20° or spondylolisthesis	53	961*
Limited elbow extension <170°	15	970*
Pes planus	47	864*
Protrusio acetabulae	23	298*
Facial appearance	49	909*
Joint hypermobility	63	952*
Moderate pectus excavatum	29	958*
High arched palate	69	928*
Major skeletal system affected	*32*	*1009**
Minor skeletal system involvement	*56*	*1009**
Ocular system		
Ectopia lentis	54	1009
Myopia	52	862
Flat cornea	9	807
Iris or ciliary muscle hypoplasia	NI	NI
Major ocular system affected	*54*	*1009*
Cardiovascular system		
Ascending aortic dilatation with or without aortic regurgitation and involving the sinuses of Valsalva	77	1009
Ascending aortic dissection	14	1009
Dilatation or dissection of descending aorta or abdominal aorta before 50 years	7	1009
Mitral valve prolapse	54	979
Mitral annulus calcification	NI	NI
Dilatation of the pulmonary artery	NI	NI
Major cardiac system affected	*77*	*1009*
Minor cardiac system involvement	*11*	*1009*
Pulmonary system		
Pneumothorax or apical blebs	7	998
Minor pulmonary system involvement	*7*	*1009*
Skin system		
Striae atrophicae	47	941
Recurrent or incisional herniae	10	984
Minor skin system involvement	*48*	*1009*
Dura		
Lumbosacral dural ectasia	53	292
Major central nervous system affected	*15*	*1009*
Family		
First degree family member independently fulfilling diagnostic criteria	51	1009
Pathogenic *FBN1* mutation	100	1009

NI, not investigated; US/LS, upper segment/lower segment.
*19 patients were classified as having minor, major, neither minor nor major skeletal features, but details on their skeletal manifestations were not available

of patients fulfilling the international criteria in this subpopulation. Eighty-two per cent of this subpopulation fulfilled the clinical international criteria and 91% fulfilled the Ghent criteria when the presence of an *FBN1* mutation was considered. Therefore, searching for dural ectasia led to an increase of 3% in the percentage of patients fulfilling the clinical international criteria.

A total of 146 adult probands (21%) could not be diagnosed on clinical grounds alone. The median age at diagnosis was 29 years (IQR 20–42) and the median age at last follow-up was 34 years (IQR 25–45). Twenty-nine per cent had a positive family history. The majority of this cohort is represented by

Table 3 System involvement according to international nosology in probands included in this study

	Overall			Adults			Children		
	Overall n = 1009 (%)	Ghent +* n = 722 (%)	Ghent –* n = 287 (%)	Overall n = 689 (%)	Ghent +* n = 543 (%)	Ghent –* n = 146 (%)	Overall n = 320 (%)	Ghent +* n = 179 (%)	Ghent –* n = 141 (%)
Skeletal system									
Major skeletal system affected	32	41	12	35	43	5	28	35	18
Minor skeletal involvement	56	52	64	53	49	64	63	62	65
Ocular system									
Major ocular system affected	54	65	24	53	60	24	55	79	25
Cardiovascular system									
Major cardiovascular system affected	77	88	49	80	87	50	71	89	48
Minor cardiovascular involvement	11	6	22	9	6	18	14	6	25
Skin system									
Minor skin involvement	48	55	28	59	64	39	23	29	16
Pulmonary system									
Minor pulmonary involvement	7	8	4	9	10	3	4	3	5
Dural system									
Major dural system affected	15	21	2	20	25	3	5	8	1
Genetic system									
Family history	51	64	21	62	71	29	29	42	13

*When the presence of the *FBN1* mutation was not considered ("clinical" criteria).

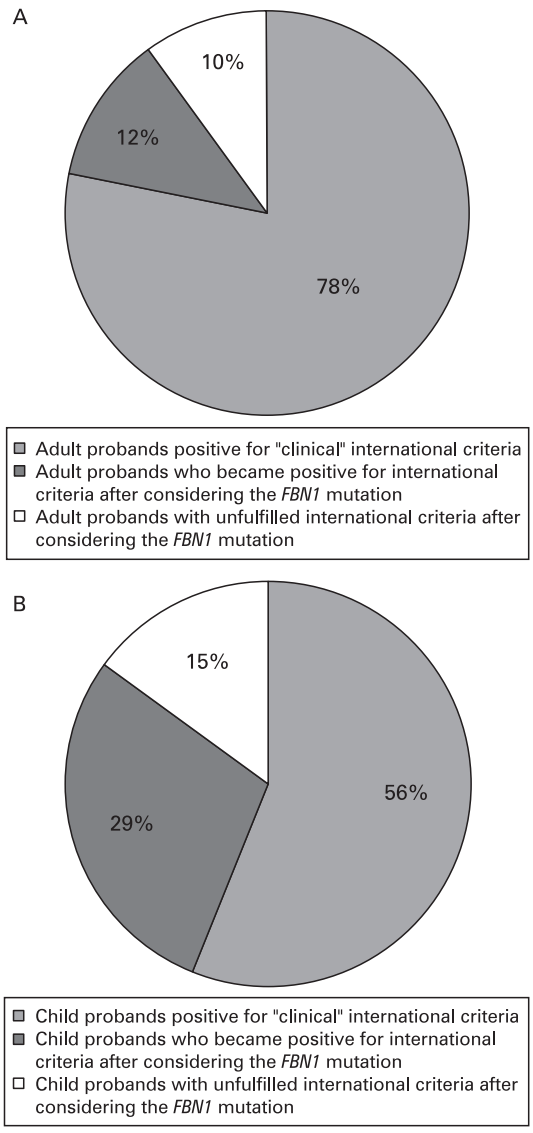

Figure 2 (A) Percentage of adult probands* fulfilling international criteria with or without knowledge of an *FBN1* mutation (n = 689). (B) Percentage of child probands fulfilling international criteria with or without knowledge of an *FBN1* mutation (n = 320). The light grey areas represent positive probands fulfilling international criteria when the presence of a *FBN1* mutation is not considered (this reflects actual clinical settings before laboratory testing). The dark grey areas represent probands fulfilling international criteria when the presence of an *FBN1* mutation is considered as a major feature (actual settings after an *FBN1* mutation has been identified in the proband). *≥18 years.

patients with one major system affected and at least one minor system involved (62%, n = 90), and patients with two major systems affected and no minor system involved (22%, n = 32). The remaining patients had either one isolated major system affected (5%, n = 8) or ⩽3 minor systems involved with no major system affected (11%, n = 16). Repartition of clinical features in the population of patients not diagnosed as MFS on clinical grounds is reported in table 3. The molecular results made it possible to reclassify 55% of this subpopulation as MFS. Cardiac manifestations were diagnosed later than when diagnosis was possible on clinical grounds (fig 3). The cumulative probability of dilatation of the ascending aorta at 40 years was 44% (99.9% CI 30% to 62%) vs 73% (99.9% CI 66% to 79%) (log rank test, p<0.001). However, the risk for aortic dissection was similar in both groups once aortic dilatation had occurred, with a cumulative probability at 40 years of 16% (99.9% CI 4% to 39%) vs 28% (99.9% CI 20% to 37%) (log rank test, p = 0.9106). Corresponding figures for the occurrence of aortic surgery were 34% (99.9% CI 16% to 59%) and 41% (99.9% CI 32% to 50%), respectively (log rank test, p = 0.9951).

Among the 320 children, the median age at last follow-up was 10 years (IQR 5;14). Only 56% fulfilled international diagnosis on clinical grounds whereas 85% were diagnosed as MFS when considering the presence of an *FBN1* mutation (fig 2B).

DISCUSSION

Early identification of individuals at risk for aortic complications is important in view of the available medical and surgical treatments that can significantly improve the life expectancy of MFS patients.[13 14] The identification of a pathogenic *FBN1* mutation confirms the presence of MFS or another type I fibrillinopathy and is a clear indicator of an aortic risk. The improvement in molecular biology and the increasing availability of *FBN1* mutation screening in clinical practice allows recognition of Marfan syndrome and its inherent aortic risk in patients with incomplete phenotypes. The aims of this study were: first, to describe the clinical characteristics of a large series of 1009 genotyped probands with *FBN1* mutation; and second, to evaluate the additional benefit of *FBN1* mutation screening for correct diagnosis, including recognition of risk for life threatening aortic complications.

The clinical approach used in our study reproduces the situations that clinicians face in their practice when they have to decide whether molecular screening is indicated: indeed, molecular data are usually lacking when patients present for initial diagnosis. *FBN1* mutation analysis adds little to the recognition of MFS patients that fulfil the clinical diagnostic criteria.[8] However, our results show that a substantial group of patients harbouring an *FBN1* mutation would not be recognised using clinical criteria alone. *FBN1* screening is helpful in patients with a mild clinical presentation. Indeed, it permitted the reclassification of 55% of adults with unfulfilled "clinical" criteria in this study as having MFS according to international nosology. This is of particular importance since, in the group

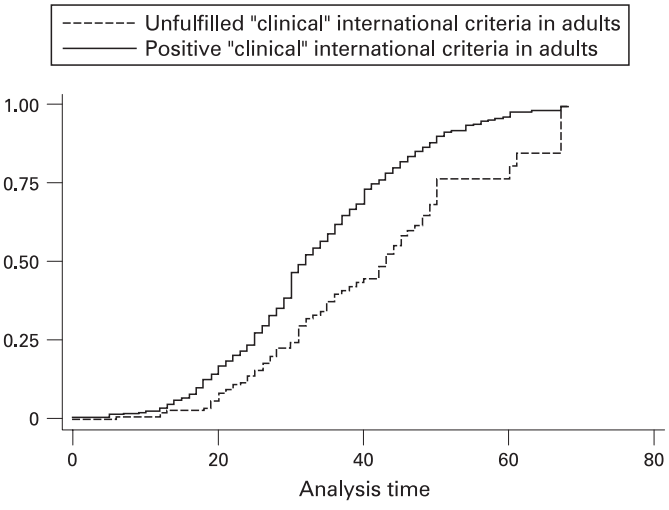

Figure 3 Cumulative probability of ascending aortic dilatation in adult probands* diagnosed on clinical grounds (n = 541, solid line) and in adult probands* with unfulfilled "clinical" international criteria (n = 146, broken line) (log rank test, p<0.0001). *≥18 years.

with unfulfilled "clinical" criteria, aortic dilatation is diagnosed later compared to patients fulfilling the "clinical" Ghent criteria ($p<0.001$), while the lifelong risk for ascending aortic dissection or surgery was not significantly different.

Also, clinical evaluation is limited by the extensive phenotypic variability of the disease.[3 15 16] Indeed, some individuals of a single family first described with "isolated" ectopia lentis developed late onset cardiovascular features,[17 18] showing that systematic cardiac follow-up should be prescribed whatever the clinical presentation associated with the *FBN1* mutation. Moreover, the recurrent c.718C>T and c.2722T>C mutations described in patients with "isolated" ectopia lentis have also been found in association with ascending aortic dilatation in other patients.[19 20] Thus, *FBN1* mutations are associated with an aortic risk, whatever the clinical presentation.

Results from the child cohort confirmed that the diagnosis of MFS is even more difficult in childhood since only 56% of children would have been diagnosed on clinical grounds alone. Therefore, when MFS or another type I fibrillinopathy is suspected in a child or adult, one should advocate systematic long term clinical follow-up searching for developing features in favour of the diagnosis of MFS. However, the clinical approach is limited by the risk of loss of motivation and therefore loss of follow-up in the absence of a definitive diagnosis given to the patient. Alternatively, one could recommend *FBN1* screening in these populations.

For the purpose of the study, we had to consider that a clinical feature which has not been mentioned in a patient was considered as absent since only 292 patients had been examined for dural ectasia. When studying the percentages of patients fulfilling the international criteria on a clinical ground in the subpopulation of 292 fully investigated patients, 82% fulfilled the clinical Ghent criteria as compared to 79% in the overall population, and 18% of patients still had not fulfilled the international criteria before the results of molecular studies. We therefore considered that the method used did not lead to a major bias of the results. Also, the authors are aware that there are problems associated with clinical evaluation in Marfan syndrome, in particular with the definitions of many skeletal features. Clinical tools and cut-off values are lacking for many of the clinical features and this could also lead to disparity in evaluation of the patients and therefore bias the results of the study. Nevertheless, the large majority of patients included in the study were evaluated by expert teams worldwide dealing with Marfan syndrome for many years.

In view of our results, screening for an *FBN1* mutation is warranted in newly suspected MFS when two systems are involved with at least one major system affected, as it would allow for a definite diagnosis in 55% of patients with *FBN1* mutation with otherwise inconclusive diagnosis. Mutation analysis may be critical to identify patients and relatives who require life long aortic follow-up in isolated ectopia lentis or isolated major skeletal involvement. Indeed, at present, for this particular group no other known genes are involved and no clinical marker of aortic risk is available. However, when no mutation is found, patients should still have aortic follow-up as false negative analyses remain possible. On the other hand, in the case of isolated aortic dilatation, systematic aortic follow-up of patients and relatives is mandatory while *FBN1* mutation screening can only be suggested for genetic counselling in view of the increasing number of genes associated with familial aortic aneurysms and overlapping syndromes.[21–25] Finally, preconceptual settings might also require *FBN1* mutation analysis as well as presymptomatic testing, in order to avoid unnecessary, cumbersome and costly clinical follow-up of unaffected relatives. We suggest that *FBN1* molecular analysis is conducted in newly suspected MFS when two systems are involved, with at least one major system affected, in order to identify patients who require aortic follow-up. These recommendations imply that diagnostic evaluation for Marfan syndrome should be performed by someone with adequate training and expertise, and must be balanced with the availability of molecular analyses and taking into account health insurance system of a given country. Alternatively, appropriate follow-up including echocardiograms must be recommended.

In conclusion, *FBN1* mutation is associated with an increased risk for aortic dilatation and dissection whatever the clinical presentation. The value of this additional risk marker when available is of particular importance when the clinical diagnosis of MFS cannot be reached, especially when aortic dilatation is not present.

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Competing interests: None declared.

Patient consent: Patients gave consent for *FBN1* mutation analysis. This study is a retrospective analysis and does not provide personal information regarding patients.

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