European Journal of Human Genetics (2010) 18; doi:10.1038/ejhg.2010.42

www.nature.com/ejhg

CLINICAL UTILITY GENE CARD

Clinical utility gene card for: Marfan syndrome type 1 and related phenotypes [*FBN1*]

Mine Arslan-Kirchner*[,1], Eloisa Arbustini[2], Catherine Boileau[3], Anne Child[4], Gwenaelle Collod-Beroud[5], Anne De Paepe[6], Jörg Epplen[7], Guillaume Jondeau[8], Bart Loeys[6] and Laurence Faivre[9]

This Clinical Utility Gene Card has been corrected since first published online on 7 April 2010. The Publisher was advised that seven author names had been omitted from the submitted article and these have now been incorporated as above.
European Journal of Human Genetics (2010) **18,** doi:10.1038/ejhg.2010.42; published online 7 April 2010

1. DISEASE CHARACTERISTICS

1.1 Name of the disease (synonyms)

Marfan syndrome type 1 and related phenotypes.

1.2 OMIM# of the disease

154700.

1.3 Name of the analysed genes or DNA/chromosome segments

FBN1.

1.4 OMIM# of the gene(s)

134797.

1.5 Mutational spectrum

Over 1700 different disease-causing mutations have been described (UMD database[1]; Collod-Béroud *et al*[2]; Collod-Beroud G, personal communication).

All types of mutations have been reported. From a study of 1013 probands with a pathogenic *FBN1* mutation, the distribution was as follows: 56% missense mutations; 17% frameshift mutations; 14% nonsense mutations; 11% splice mutations; 2% in-frame deletions.[3]

1.6 Analytical methods

Two different strategies for *FBN1* mutation screening procedures are currently applied:

(1) direct sequencing of genomic exonic DNA with flanking intronic sequences;
(2) or DHPLC or high-resolution melting with confirmation by direct sequencing

When no mutation is identified, a search for *FBN1* genomic rearrangements by MLPA or related techniques could be proposed in clinically convincing cases. Indeed, this search seems to increase the *FBN1* mutation uptake number by a significant percentage. From a study of 101 patients with Marfan syndrome or related phenotypes but with an absence of *FBN1* mutation after direct sequencing, two *FBN1* genomic deletions (2%) were found using MLPA.[4] Similarly, Liu *et al*[5] identified two *FBN1* genomic deletions using RT-PCR out of a series of 60 patients (3.3%), 55 of whom met diagnostic criteria for MFS.

SSCP analysis does not seem to be a satisfying technique for *FBN1* mutation screening, as it has been shown that it was less efficient than direct sequencing. Indeed, Loeys *et al*[6] detected 73 sequence variants in 95 patients after screening by SSCP. They identified 13 additional mutations by performing direct sequencing in patients with normal SSCP.

1.7 Analytical validation

Sequencing of both strands was carried out. When a mutation is identified, validation of the results using a second primer set is recommended, +/− using a second technique (PCR with restriction enzyme digestion, high-resolution melting or DHPLC) when possible.

1.8 The estimated frequency of the disease (incidence at birth ('birth prevalence') or population prevalence)

There was a population prevalence of approximately 3 out of 10 000.[7]

1.9 If applicable, prevalence in the ethnic group of the investigated person

Not applicable.

1.10 Diagnostic setting

	Yes	No
A. (Differential) diagnostics	☒	☐
B. Predictive testing	☒	☐
C. Risk assessment in relatives	☒	☐
D. Prenatal	☒	☐

Comment:
FBN1 mutation screening does not seem to be useful for the positive diagnosis of Marfan syndrome in patients fulfilling international Ghent criteria.[8] However, it seems useful in the following situations,

[1]Institute of Human Genetics, Hannover Medical School, Hannover, Germany; [2]Laboratorio di Diagnostica Molecolare, Patologia Cardiovascolare e dei Trapianti, Centro Malattie Genetiche Cardiovascolari, Policlinico San Matteo, Pavia, Italy; [3]Département de Génétique, Institut de Recherche Necker Enfants Malades, Hopital Necker – Enfants Malades, Paris, France; [4]Sonalee Laboratory, Cardiac and Vascular Sciences, St George's University of London, London, UK; [5]Laboratoire de Génétique Moléculaire, CHU de Montpellier, Institut Universitaire de Recherche Clinique, Montpellier, France; [6]Center for Medical Genetics, Ghent University Hospital, Gent, Belgium; [7]Department of Human Genetics, Ruhr University Bochum, Bochum, Germany; [8]Service de Cardiologie, CHU Hôpital Xavier Bichat – Claude Bernard, Paris, France; [9]Centre de Génétique, Centre Hospitalier Universitaire de Dijon, Dijon, France
*Correspondence: Dr M Arslan-Kirchner, Institute of Human Genetics, Hannover Medical School, Carl-Neuberg-Str. 1, D-30625 Hannover, Germany.
Tel: +49 511 532 6532; Fax: +49 511 532 8533; E-mail: arslan.mine@mh-hannover.de

to determine whether follow-up and preventive treatment for aortic dilatation is indicated:[9,10]

(1) patients not fulfilling international Ghent criteria, in particular patients with isolated ectopia lentis and patients with suggestive cardiovascular features combined with skeletal findings, or in sporadic cases of young age[11]
(2) predictive testing in young children (offspring of an affected parents) or relatives (large clinical heterogeneity)[12]

The decision with regard to searching for an *FBN1* gene mutation in such cases will vary and depend on specific family and individual circumstances. The indications of genotyping could be extended to all cases/families in which the proven genetic diagnosis could influence the lifestyle (athletes), the initiation of treatment, or the rate of clinical controls/monitoring.

FBN1 mutation screening can also be indicated in an affected patient with reproductive issues. A prenatal test for Marfan syndrome is rarely requested, but it is expected that the greater availability of mutation testing of the *FBN1* gene will increase requests for prenatal diagnosis. Prenatal diagnosis is technically possible by analysis of DNA extracted from foetal cells obtained by chorionic villus sampling at about 10–12 weeks of gestation.[13] Prenatal diagnosis is possible when the disease-causing mutation has been identified in the family with careful exclusion of maternal DNA contamination when the mother is the affected parent. In a few cases, when a family can be sampled at large and the disease-causing mutation has not been identified, linkage analysis can be performed. Prenatal diagnosis can be then offered only if conclusive linkage has been obtained and an unambiguous disease-associated haplotype has been identified. A careful analysis of intra- and extragenic *FBN1* markers is required.

Prenatal diagnosis can be discussed case by case, with couples requesting it in the framework of a genetic clinic, especially in families with severe cardiac manifestations. Practical use of prenatal diagnosis remains difficult because of the extremely broad variability of clinical expression, even within families, and our inability, at present, to predict the severity of the disease in a given individual. However, it is unlikely that a neonatal MFS will occur in newborns of an adult affected parent. Neonatal MFS cases are always caused by *de novo* FBN1 mutations.

Alternatively, preimplantation genetic diagnosis (PGD) can be offered for families in which the disease-causing mutation has been identified in an affected family member. However, rules, laws and regulations vary in different European countries, and PGD is illegal in some countries.

2. TEST CHARACTERISTICS

	Genotype or disease		A: True positives B: False positives	C: False negative D: True negative
	Present	Absent		
Test				
Positive	A	B	Sensitivity:	A/(A+C)
			Specificity:	D/(D+B)
Negative	C	D	Positive predictive value:	A/(A+B)
			Negative predictive value:	D/(C+D)

2.1 Analytical sensitivity
(proportion of positive tests if the genotype is present)
The proportion is practically 100%.

The possibility of preferential amplification of one allele if primers are localized on an SNP or because of deletion exists, although these events are exceptional.
Classical criteria for determining the pathogenicity of an *FBN1* mutation are the following:

(1) Nonsense mutation
(2) Splice-site mutations affecting canonical splice sequence or shown to alter splicing at mRNA/cDNA level
(3) Out-of-frame and in-frame deletion or insertion
(4) *De novo* missense mutation (with proven paternity and absence of disease in parents)
(5) Missense mutation previously shown to segregate in a Marfan family
(6) Missense replacing/creating cysteine (42% of missense mutations)
(7) Missense mutation affecting cbEGF consensus sequence (22% of missense mutations)
(8) Missense mutation involving a highly conserved amino acid (6% of missense mutations)

For other missense mutations, the search for segregation in the family should be performed if possible, as well as in the absence of the variant in 400 ethnically matched control chromosomes.

2.2 Analytical specificity
(proportion of negative tests if the genotype is not present)
The proportion was practically 100%.

2.3 Clinical sensitivity
(proportion of positive tests if the disease is present)
Clinical sensitivity can be dependent on variable factors such as age or family history. In such cases, a general statement should be given, even if a quantification can only be made case by case.
A few studies have addressed the question of clinical sensitivity for *FBN1* mutation recognition. Results are variable depending on the method used for mutation screening, but also depending on the set of clinical criteria required for molecular diagnosis. Indeed, a high variable expressivity has been reported in *FBN1* mutations and the clinical sensibility is higher when patients fulfilled the Ghent criteria. Results of the more recent studies, including a reasonable number of patients, are as follows:

(1) Identification of *FBN1* mutations in 86 out of 93 individuals presenting with classic Marfan syndrome, all fulfilling the Ghent criteria (93%), using SSCP and direct sequencing in negative cases[6]
(2) Identification of *FBN1* mutations in 74 out of 81 individuals presenting with MFS or Marfan-like phenotypes (91.35%), using DHPLC[14]
(3) Identification of *FBN1* mutations in 69 out of 105 individuals with suspected MFS, all fulfilling the Ghent criteria (76%), using direct sequencing[15]
(4) Identification of *FBN1* mutations in 90 out of 110 individuals fulfilling the Ghent criteria (82%), in 84 out of 315 individuals with incomplete MFS (27%), in 19 out of 38 individuals with EL (50%) and in none of the 45 individuals with isolated ascending aortic aneurysm using SSCP or DHPLC. The mutation rate was higher with DHPLC. For example, in individuals with classical MFS, the mutation detection rate was 91% using DHPLC *vs* 75% using SCCP[16]
(5) Identification of *FBN1* mutations in 80 out of 85 individuals fulfilling the Ghent criteria (88%) and in 36% of patients with other fibrillinopathies type I using DHPLC[17]

(6) Identification of *FBN1* mutations in 193 out of 266 individuals fulfilling the Ghent criteria (72.5%), in 61 out of 105 with incomplete Ghent criteria (58%) and in 3 out of 21 (14.3%) patients referred as possible MFS but with no major diagnostic criterion in any organ system.[18]

Some explanations can be given accounting for the imperfect clinical sensitivity for *FBN1* mutation screening in MFS:

(1) Genetic heterogeneity: mutations within the *TGFBR1* and *TGFBR2* genes have been reported in patients with MFS or suspected MFS.[19] Sakai *et al* found one patient with a *TGFBR1* mutation out of a series of 49 patients (2%) and two *TGFBR2* mutations (4%);[20] Mátyás *et al*[21] reported 10 *TGFBR1* or *TGFBR2* mutations in 70 unrelated individuals with MFS-like phenotypes who previously tested negative for mutations in *FBN1*; Singh *et al*.[22] found two *TGFBR1* and five *TGFBR2* mutations in 41 unrelated patients fulfilling or not fulfilling the diagnostic criteria of Ghent nosology, in whom mutations in the *FBN1* coding region were not identified; Stheneur *et al*[23] found six mutations in the *TGFBR2* gene and one in the *TGFBR1* gene in 105 MFS patients and nine mutations in the *TGFBR2* gene and two mutations in the *TGFBR1* gene in 247 patients with incomplete or probable MFS who were negative for an *FBN1* gene mutation. Screening for *TGFBR1/2* should be indicated in the first step when one of the following clinical or imaging features is encountered: hypertelorism, bifid uvula, cleft palate, craniosynostosis, clinical features of vascular Ehlers–Danlos syndrome, arterial tortuosity and aneurysms.
(2) Incomplete detection of mutations with the method used: mutations in the 5′ upstream regions[24] or intronic mutations.[25]

2.4 Clinical specificity
(proportion of negative tests if the disease is not present)
Clinical specificity can be dependent on variable factors such as age or family history. In such cases, a general statement should be given, even if quantification can only be made case by case.
The proportion is probably 100%, but no data were available for this measure.

2.5 Positive clinical predictive value
(lifetime risk of developing the disease if the test is positive)
The possibility is nearly 100%.
Exceptional cases of incomplete penetrance have been reported.[26]
It is noteworthy that a large number of MFS manifestations are age dependent. A child with an *FBN1* mutation can be identified as at-risk but only presents MFS features at a later age.
Although all patients with an *FBN1* pathogenic mutation will present a clinical feature at some time during life, it is possible that some patients will not fulfil international criteria for MFS throughout life.

2.6 Negative clinical predictive value
(probability not to develop the disease if the test is negative)
Assume an increased risk based on family history for a nonaffected person. Allelic and locus heterogeneity may need to be considered.
When the index case in that family had been tested, the proportion was found to be nearly 100%
When the index case in that family had not been tested, it was concluded that predictive testing for family members should only be proposed when a pathogenic mutation has been identified in an index case.

3. CLINICAL UTILITY

3.1 (Differential) diagnosis: the tested person is clinically affected
(To be answered if in 1.10, 'A' was marked).

3.1.1 Can a diagnosis be made other than through a genetic test?

No	☐ (Continue with 3.1.4)	
Yes	☒	
	Clinically	☒
	Imaging	☒
	Endoscopy	☐
	Biochemistry	☐
	Electrophysiology	☐
	Other (please describe) family history (Ghent criteria[8])	

3.1.2 Describe the burden of alternative diagnostic methods to the patient.
Cardiological (including echocardiography), orthopaedic (including X-rays) and ophthalmological investigations can altogether establish a diagnosis (but not always).
MRI to diagnose or exclude dural ectasia is occasionally necessary to establish the diagnosis, in patients not fulfilling the international criteria with the previously cited investigations. Dural ectasia is present in many other connective tissue disorders, such as Ehlers Danlos or Loeys–Dietz syndrome, hence this will not, on its own, allow the establishment of a diagnosis.

3.1.3 How is the cost effectiveness of alternative diagnostic methods to be judged?
Unknown.

3.1.4 Will disease management be influenced by the result of a genetic test?

No	☐	
Yes	☒	
	Therapy (please describe)	Indication of drug therapy or replacement of dilated aortic segments is similar in patients diagnosed with Marfan syndrome with or without identification of the molecular *FBN1* defect.[27] Indeed, as the mutation detection rate is not 100% and the availability of *FBN1* screening is different from country to country, appropriate treatment should be prescribed for all patients with a clinical diagnosis of MFS. As the presence of a mutation in the *FBN1* gene is a major criterion of the international nosology, the genetic result may lead to diagnosis of Marfan syndrome that could have consequences in terms of regular cardiological follow-up, and prescription of drug therapy for preventing or limiting aortic dilatation.
	Prognosis (please describe)	Similarly, the identification of a *FBN1* mutation in an MFS patient will not lead to a different prognosis when compared with patients with MFS but in whom a mutation has not been sought or identified. Nevertheless, there is evidence that patients with *TGFBR1/2* mutation need more extensive imaging of the aorta, and in some series, have increased risk for dissection at smaller aortic diameters.[28] Therefore, identification of either an *FBN1* compared with a *TGFBR1/2* mutation could influence prognosis, management and therapy.

(Continued)

Management (please describe)	The results of genetic tests will influence genetic counselling by permitting predictive testing of children or paucisymptomatic family members and determining accurate recurrence risk. Rare cases of somatic or germline mosaicism have been reported.[29–31] The identification of a *FBN1* mutation might also be helpful in patients not fulfilling the clinical Ghent criteria and without aortic manifestations to reduce the risk of loss to follow-up.[10,32] All cases should be integrated in a multidisciplinary clinic. Preventive medical treatment for aortic dilatation are recommended in patients with the clinical diagnosis of MFS and patients with an *FBN1* mutation, even in the absence of aortic manifestations,[10,27] but attitudes could vary between countries through Europe. Indeed, some teams propose to install medical therapy only when regular echocardiograms do demonstrate some definite progressive involvement, arguing that some families with ocular and skeletal manifestations only do not demonstrate cardiac involvement.[33]

CONFLICT OF INTEREST

The authors declare no conflict of interest

ACKNOWLEDGEMENTS

This work was supported by EuroGentest, an EU-FP6-supported NoE, contract number 512148 (EuroGentest Unit 3: 'Clinical genetics, community genetics and public health', Workpackage 3.2).

1 UMD database, http://www.umd.be/FBN1/.
2 Collod-Béroud G, Le Bourdelles S, Ades L, *et al*: Update of the UMD-FBN1 mutation database and creation of an FBN1 polymorphism database. *Hum Mutat* 2003; **22**: 199–208.
3 Faivre L, Collod-Beroud G, Loeys BL *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**: 454–466.
4 Mátyás G, Alonso S, Patrignani A *et al*: Large genomic fibrillin-1 (FBN1) gene deletions provide evidence for true haploinsufficiency in Marfan syndrome. *Hum Genet* 2007; **122**: 23–32.
5 Liu W, Schrijver I, Brenn T, Furthmayr H, Francke U: Multi-exon deletions of the FBN1 gene in Marfan syndrome. *BMC Med Genet* 2001; **2**: 11.
6 Loeys B, De Backer J, Van Acker P *et al*: Comprehensive molecular screening of the FBN1 gene favors locus homogeneity of classical Marfan syndrome. *Hum Mutat* 2004; **24**: 140–146.
7 Orphanet database, http://www.orpha.net/consor/cgi-bin/OC_Exp.php?lng=EN&Expert=558.
8 De Paepe A, Devereux RB, Dietz HC, Hennekam RC, Pyeritz RE: Revised diagnostic criteria for the Marfan syndrome. *Am J Med Genet* 1996; **62**: 417–426.
9 De Backer J, Loeys B, Leroy B, Coucke P, Dietz H, De Paepe A: Utility of molecular analyses in the exploration of extreme intrafamilial variability in the Marfan syndrome. *Clin Genet* 2007; **72**: 188–198.
10 Faivre L, Collod-Beroud G, Child A *et al*: Contribution of molecular analyses in diagnosing Marfan syndrome and type I fibrillinopathies: an international study of 1009 probands. *J Med Genet* 2008; **45**: 384–390.
11 Faivre L, Collod-Béroud G, Callewaert BL *et al*: Pathogenic *FBN1* mutations in 146 adults not meeting clinical diagnostic criteria for Marfan syndrome: further delineation of Type 1 fibrillinopathies and focus on patients with an isolated major criterion. *Am J Med Genet* 2009; **149A**: 854–860.
12 Faivre L, Masurel-Paulet A, Collod-Béroud G *et al*: Clinical and molecular study of 320 children with Marfan syndrome and related type I fibrillinopathies in a series of 1009 probands with pathogenic FBN1 mutations. *Pediatrics* 2009; **123**: 391–398.
13 Loeys B, Nuytinck L, Van Acker P *et al*: Strategies for prenatal and preimplantation genetic diagnosis in Marfan syndrome (MFS). *Prenat Diagn* 2002; **22**: 22–28.
14 Arbustini E, Grasso M, Ansaldi S *et al*: Identification of sixty-two novel and twelve known FBN1 mutations in eighty-one unrelated probands with Marfan syndrome and other fibrillinopathies. *Hum Mutat* 2005; **26**: 494.
15 Tjeldhorn L, Rand-Hendriksen S, Gervin K *et al*: Rapid and efficient FBN1 mutation detection using automated sample preparation and direct sequencing as the primary strategy. *Genet Test* 2006; **10**: 258–264.
16 Comeglio P, Johnson P, Arno G *et al*: The importance of mutation detection in Marfan syndrome and Marfan-related disorders: report of 193 FBN1 mutations. *Hum Mutat* 2007; **28**: 928.
17 Attanasio M, Lapini I, Evangelisti L *et al*: FBN1 mutation screening of patients with Marfan syndrome and related disorders: detection of 46 novel FBN1 mutations. *Clin Genet* 2008; **74**: 39–46.
18 Stheneur C, Collod-Béroud G, Faivre L *et al*: Identification of the minimal combination of clinical features in probands for efficient mutation detection in the *FBN1* gene. *Eur J Hum Genet* 2009; **17**: 1121–1128.
19 Mizuguchi T, Collod-Beroud G, Akiyama T *et al*: Heterozygous TGFBR2 mutations in Marfan syndrome. *Nat Genet* 2004; **36**: 855–860.
20 Sakai H, Visser R, Ikegawa S *et al*: Comprehensive genetic analysis of relevant four genes in 49 patients with Marfan syndrome or Marfan-related phenotypes. *Am J Med Genet A* 2006; **140**: 1719–1725.
21 Mátyás G, Arnold E, Carrel T *et al*: Identification and in silico analyses of novel TGFBR1 and TGFBR2 mutations in Marfan syndrome-related disorders. *Hum Mutat* 2006; **27**: 760–769.
22 Singh KK, Rommel K, Mishra A *et al*: TGFBR1 and TGFBR2 mutations in patients with features of Marfan syndrome and Loeys-Dietz syndrome. *Hum Mutat* 2006a; **27**: 770–777.
23 Stheneur C, Collod-Béroud G, Faivre L *et al*: Identification of 23 TGFBR2 and 6 TGFBR1 gene mutations and genotype-phenotype investigations in 457 patients with Marfan syndrome type I and II, Loeys-Dietz syndrome and related disorders. *Hum Mutat* 2008; **29**: E284–E295.
24 Singh KK, Shukla PC, Rommel K, Schmidtke J, Arslan-Kirchner M: Sequence variations in the 5′ upstream regions of the FBN1 gene associated with Marfan syndrome. *Eur J Hum Genet* 2006b; **14**: 876–879.
25 Guo DC, Gupta P, Tran-Fadulu V *et al*: An FBN1 pseudoexon mutation in a patient with Marfan syndrome: confirmation of cryptic mutations leading to disease. *J Hum Genet* 2008; **53**: 1007–1011.
26 Buoni S, Zannolli R, Macucci F *et al*: The FBN1 (R2726W) mutation is not fully penetrant. *Ann Hum Genet* 2004; **68**: 633–638.
27 Keane MG, Pyeritz RE: Medical management of Marfan syndrome. *Circulation* 2008; **117**: 2802–2813.
28 Loeys BL, Schwarze U, Holm T *et al*: Aneurysm syndromes caused by mutations in the TGF-beta receptor. *N Engl J Med* 2006; **355**: 788–798.
29 Tekin M, Cengiz FB, Ayberkin E *et al*: Familial neonatal Marfan syndrome due to parental mosaicism of a missense mutation in the FBN1 gene. *Am J Med Genet A* 2007; **143A**: 875–880.
30 Rantamäki T, Kaitila I, Syvänen AC, Lukka M, Peltonen L: Recurrence of Marfan syndrome as a result of parental germ-line mosaicism for an FBN1 mutation. *Am J Hum Genet* 1999; **64**: 993–1001.
31 Collod-Béroud G, Lackmy-Port-Lys M, Jondeau G *et al*: Demonstration of the recurrence of Marfan-like skeletal and cardiovascular manifestations due to germline mosaicism for an FBN1 mutation. *Am J Hum Genet* 1999; **65**: 917–921.
32 Pepe G, Lapini I, Evangelisti L *et al*: Is ectopia lentis in some cases a mild phenotypic expression of Marfan syndrome? Need for a long-term follow-up. *Mol Vis* 2007; **13**: 2242–2247.
33 Lönnqvist L, Child A, Kainulainen K, Davidson R, Puhakka L, Peltonen L: A novel mutation of the fibrillin gene causing ectopia lentis. *Genomics* 1994; **19**: 573–576.