

1 **Review article :**

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## **Marfan syndrome in the third Millenium.**

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## 1 **Introduction:**

2 A hundred years have now elapsed since Dr. Antonin Marfan<sup>1</sup> reported on the case of  
3 Gabrielle P. thus describing some of the skeletal features that today define the syndrome that  
4 carries his name. Since then, substantial progress has been made with respect to the  
5 description of the pleiotropic manifestations of this disease, the understanding of underlying  
6 pathophysiological mechanisms and the availability of prevention and treatment of major  
7 complications.

### 9 **1. Nosology: What is Marfan syndrome today ?**

10 Marfan syndrome (MFS, OMIM#154700) is an autosomal dominant connective tissue  
11 disorder that has an estimated incidence of 1/5 000 with probably over 25 % of sporadic  
12 cases. The syndrome involves many systems (skeletal, ocular, cardiovascular, pulmonary,  
13 skin and integument, and dura) but its more prominent manifestations are skeletal, ocular  
14 and cardiovascular. In 1986, an international group of experts agreed upon diagnostic criteria  
15 to distinguish classic Marfan syndrome from many related disorders. These criteria constitute  
16 what is currently referred to as the "Berlin nosology" <sup>2</sup>. Patients are diagnosed based on  
17 involvement of the skeletal system and two other systems with at least one major  
18 manifestation (ectopia lentis, aortic dilation/dissection, or dural ectasia). Patients with an  
19 affected first degree relative are required to have involvement of at least two other systems  
20 with one major manifestation preferred but not required.

21 This nosology has been found wanting in many individual cases and revised criteria  
22 were subsequently proposed that constitute the "Ghent nosology" <sup>3</sup>. This new formulation  
23 requires involvement of three systems with two major diagnostic manifestations. It provides

1 for major skeletal manifestations and considers affected first-degree relatives or molecular  
2 data as major diagnostic criteria.

3 Finally, development of preventive measures and surgery for aortic aneurysms and  
4 dissection have lead to treatment of life-threatening cardiovascular complications associated  
5 with the Marfan syndrome and have considerably altered life expectancy for patients.  
6 Interestingly, the review of the medical problems of surviving patients has revealed possible  
7 unidentified pleiotropic manifestations of the Marfan syndrome or manifestations that could  
8 be related to aging of this population. These medical problems include the onset of arthritis  
9 at an early age, varicose veins, ruptured or herniated discs, and prolapse of the uterus or  
10 bladder in women. These medical problems now need to be properly investigated and  
11 monitored.

12 The continued efforts to redefine diagnostic criteria emphasize persistent  
13 shortcomings. The phenotype of the Marfan syndrome remains incompletely defined. Most  
14 manifestations are age-dependant and are difficult to quantify. The Ghent nosology has been  
15 field-tested in The National Institutes of Health <sup>4</sup>. Their study shows that 19% of patients  
16 diagnosed under the Berlin criteria failed to meet the Ghent standard. Molecular data are  
17 important to better characterize this subset, to study its natural history and implement  
18 relevant preventive measures.

## 20 **2. The Marfan syndrome and *FBN1*.**

21 Scientists, as early as 1931, suggested that the basic defect in Marfan syndrome lay in a  
22 defect in the mesoderm <sup>5</sup>. In 1955, Victor McKusick considered the syndrome as a prominent  
23 member of the new nosologic group he named “the heritable disorders of connective tissue”

1 <sup>6</sup>. The Marfan syndrome was long considered to be due to a defect either in one of the  
2 collagens or elastin since their abnormalities are prominent features of the disease. However,  
3 protein and gene studies conclusively demonstrated that neither was involved. In 1986, Sakai  
4 and co-workers identified a new extracellular matrix protein that they named "fibrillin" <sup>7</sup>  
5 (OMIM#134797). This protein is the major component of microfibrils that are structures found  
6 in the extracellular matrix either as isolated aggregates or closely associated with elastin  
7 fibers. Ultrastructurally, microfibrils display a typical "beads-on-a-string" appearance  
8 consisting of a long series of globules connected by multiple filaments. In 1990, Hollister et al.  
9 using a monoclonal antibody against fibrillin, reported abnormalities of the microfibrillar  
10 system in the Marfan syndrome <sup>8</sup>. The following year, the gene encoding fibrillin-1 (*FBN1*)  
11 was cloned and the first mutations in the gene were identified in Marfan syndrome patients <sup>9</sup>  
12 <sup>11</sup>. Interestingly, the year before the *FBN1* gene was cloned, Kainulainen et al. <sup>12</sup> demonstrated  
13 through linkage analysis that the gene involved in classic complete forms of the Marfan  
14 syndrome was located on human chromosome 15 precisely where the *FBN1* gene was later  
15 located. Therefore the identification of the gene defect in Marfan syndrome is a rare example  
16 in which both positional and functional cloning strategies converged rapidly to identify a  
17 disease gene.

### 19 **3. The *FBN1* gene and other members of the fibrillin family.**

20 The gene encoding type 1 fibrillin (*FBN1*) lies on the long arm of chromosome 15 at  
21 15q15-q21.1. This very large gene [first estimated at 110 kb, now at over 230 kb (Human  
22 Genome Sequencing Project NT\_034890 sequence)] is highly fragmented into 65 exons,  
23 transcribed in a 10 kb mRNA that encodes a 2871 amino acid protein <sup>10,11,13,14</sup>. Three additional

1 alternatively-spliced exons, likely untranslated, were found upstream of exon 1<sup>15</sup>.

2 Conservation of nucleotide sequences within this region between human, mouse and porcine  
3 suggests that this region of the gene may harbor important regulatory elements. This region  
4 is GC-rich, contains a CpG island, and lacks conventional TATA or CCAAT boxes.

5 The deduced primary structure reveals a highly repetitive protein that contains  
6 essentially three repeated modules (figure 1):

7 • The first repeated module is the **EGF-like module** that is homologous to one found in the  
8 epidermal growth factor. These modules contain six cysteine residues that form three intra-  
9 domain disulfide bonds. There are 47 of these throughout the fibrillin-1 protein. Among  
10 these, 43 contain a conserved consensus sequence for calcium binding and are called cb EGF-  
11 like modules. In these domains, the residues putatively involved in calcium binding are  
12 numbered sequentially in figure 2 as in Dietz and Pyeritz<sup>16</sup>. They include the aspartic acid at  
13 position 2, glutamic acid at position 5, asparagine at position 10 and tyrosine or  
14 phenylalanine at position 15.

15 • The second repeated module, found 7 times interspaced with cb EGF-like in the protein, is  
16 called **TGF  $\beta$ 1-binding protein-like module** (TGF  $\beta$ 1-BP-like module) since it is homologous  
17 to modules found in the Transforming Growth Factor  $\beta$ 1 binding protein. This domain  
18 appears to be limited to proteins that localize to matrix fibrils [fibrillins and latent  
19 transforming growth factor  $\beta$ -binding proteins (LTBPs)]. These modules contain eight  
20 cysteine residues. The fourth TGF- $\beta$ 1-BP-like module contains the RGD sequence which can  
21 interact with cell receptors<sup>17</sup>. No specific function has yet been ascribed to these modules.  
22 However, some evidence suggests that these domains mediate specific protein-protein  
23 interactions<sup>18</sup>.

1 • Finally, the protein contains a third module consisting of approximately 65 amino acids,  
2 and found twice in the protein. These are called “**hybrid modules**” since they combine  
3 features of the EGF-like and the TGF- $\beta$ 1-BP-like modules. This module is also found in  
4 LTBPs, which have a single hybrid domain.

5 Finally the protein contains three unique regions: a **proline-rich region** that may act as  
6 a “hinge-like” region <sup>13</sup> and **the amino and carboxy terminal domains**. The N- and C-  
7 terminal domains of the fibrillins display two prominent features : the presence of an even  
8 number of cysteine residues, four in the N-terminal and two in the C-terminal domains and  
9 the presence of the basic consensus sequence for processing by furin-types enzymes BXBB  
10 (B=basic amino acid residue, K or R) in each domain. The 4-cysteine domain in the N-  
11 terminus of fibrillins is homologous to similar 4-cysteine domains in the N-terminal extended  
12 forms of the LTBPs. The C-terminal domains of the fibrillins are homologous to the C-  
13 terminal domain of all four members of the fibulin family, and thus a new type of  
14 extracellular module of approximately 120 amino acid residues in length has been proposed  
15 <sup>19</sup>. This type of homology is not shared by the LTBPs.

16 When the *FBN1* gene was cloned, a second gene sharing a high degree of homology  
17 was identified and located on chromosome 5. This gene was named *FBN2* and the protein it  
18 encodes **fibrillin-2** <sup>10</sup>. *FBN2* has been genetically linked <sup>10</sup> to a rare disorder that shares features  
19 of Marfan syndrome: congenital contractural arachnodactyly (CCA) (OMIM#120150). The  
20 clinical manifestations of CCA are essentially found in the skeleton and associated with  
21 distinctive manifestations including crumpled ears and campodactyly. Several mutations  
22 were identified in this gene in CCA patients <sup>20</sup>.

23 Ikegawa et al. described the structure and chromosomal assignment to 2p16 of a  
24 “**fibrillin-like**” gene (**FBNL**), that is highly homologous to fibrillin <sup>21</sup>. The FBNL gene is

1 expressed in many tissues but it is not expressed in brain and lymphocytes. The amino acid  
2 sequence of the FBNL gene is 36.3% identical to *FBN1* (OMIM#134797) and 35.4% identical to  
3 *FBN2*. FBNL contains 1 EGF-like module and 5 repeated cb EGF-like modules. The gene  
4 spans approximately 18 kb of genomic DNA and contains 12 exons. The FBNL gene was  
5 thought to possibly be involved in Marfan-like conditions such as hypermobility syndrome  
6 or mitral valve prolapse. In 1999, Stone et al. identified a single nonconservative mutation in  
7 the FBNL gene, also named **EFEMP1** (EGF-containing fibulin-like extracellular matrix  
8 protein 1) in 5 families with Doyme honeycomb retinal dystrophy (DHRD; OMIM#126600), or  
9 malattia Leventinese (MLVT)<sup>22</sup>. This autosomal dominant disease is characterized by yellow-  
10 white deposits known as drusen that accumulate beneath the retinal pigment epithelium.

#### 12 **4. The fibrillin proteins.**

13 The fibrillins are extracellular matrix glycoproteins that show a wide distribution in  
14 both elastic and non-elastic tissues and are integral components of 10 nm diameter  
15 microfibrils<sup>7, 23</sup>. Fibrillin-1 is synthesized as profibrillin and proteolytically processed to  
16 fibrillin. The cleavage site has been mapped to the carboxy-terminal domain of profibrillin-1.  
17 The propeptide starts at position S<sup>2732</sup> directly C-terminal to the R<sup>2728</sup>KRR sequence. Wild type  
18 profibrillin is not incorporated into extracellular matrix until it is converted to fibrillin<sup>24</sup>. The  
19 N-terminal region of each protein directs the formation of homodimers within a few hours  
20 after secretion and disulphide bonds stabilize the interaction<sup>25</sup>. Dimer formation occurs  
21 intracellularly, suggesting that the process of fibrillin aggregation is initiated early after  
22 biosynthesis of the molecules. Fibrillin is post-translationally modified by  $\beta$ -hydroxylation  
23 and N-and O-linked carbohydrate formation<sup>26</sup>.

1 The solution structure of the TGF- $\beta$ -like module from human fibrillin-1 identified a  
2 novel fold which was globular in nature <sup>27</sup> and appears to break up linear regions within  
3 fibrillin-1 molecules after rotary shadowing electron microscopy. If these linker regions are  
4 effectively flexible, the kinks and bends observed in fibrillin-1 molecules would be required  
5 for proper alignment of molecules within the assembled microfibril <sup>18</sup>.

6 Baldock et al. have derived a model of fibrillin alignment in microfibrils based on  
7 automated electron tomography, immunolocalization in directionally orientated untensioned  
8 microfibrils, mass changes on microfibril extension, immunofluorescence studies and  
9 published observations <sup>28</sup>. Their model predicts maturation from a parallel head-to-tail  
10 alignment to an approximately one-third stagger that is stable as a 56-nm folded form, but  
11 not as an ~100-nm form. This model accounts for all microfibril structural features, suggests  
12 that inter- and intramolecular interactions drive conformation changes to form extensible  
13 microfibrils, and defines the number of molecules in cross section.

14 Fibrillin-1 and -2 co-distribute in elastic and non-elastic connective tissues of the  
15 developing embryo, with a preferential accumulation of the FBN2 gene product in elastic  
16 fiber-rich matrices <sup>23</sup>. Mouse study of the developmental expression of the fibrillin genes has  
17 revealed different patterns. Except for the cardiovascular system, in which *Fbn1* gene activity  
18 is early and always higher than *Fbn2*, *Fbn2* transcripts appear earlier than *Fbn1* transcripts  
19 and accumulate for a short period of time just before overt tissue differentiation i.e. a  
20 window of time immediately preceding elastogenesis. In contrast, the amount of *Fbn1*  
21 transcripts increases at an apparently gradual rate throughout morphogenesis and is mainly  
22 expressed during late morphogenesis and well-defined organ structures. Furthermore, *Fbn1*  
23 transcripts are predominantly represented in stress- and load-bearing structures like aortic  
24 adventitia, suspensory ligament of the lens, and skin. Spatio-temporal patterns of gene

1 expression thus suggest distinct but related roles in microfibril physiology. Fibrillin-1 would  
2 provide mostly force-bearing structural support whereas fibrillin-2 would predominantly  
3 regulate the early process of elastic fiber assembly <sup>29</sup>. Fibrillins would contribute to the  
4 structural and functional heterogeneity of microfibrils.

## 6 **5. Role of Ca<sup>2+</sup> in fibrillin,**

7 The implication of the variable calcium binding affinities observed in fibrillin  
8 fragments is biologically significant. A number of studies have shown that the presence of  
9 calcium ions significantly protects full-length or recombinant fragments of fibrillin-1 from  
10 proteolysis by trypsin, elastase, endoproteinase Glu-C, plasmin and matrix  
11 metalloproteinases <sup>31-34</sup>. Moderate to high affinities for calcium suggest that fibrillin cb EGF-  
12 like modules would be close to fully saturated in vivo. Particular regions of fibrillin may  
13 need to be rigid for appropriate function. For example, cb EGF-like#12-13, located in the  
14 neonatal Marfan syndrome region (see paragraph 8) where mutations leading to severe  
15 phenotypes cluster, may be part of a region where rigidity is required for function. Fully  
16 saturated calcium binding sites may be required for stabilization of the microfibril against  
17 proteolytic degradation, when low-affinity sites not fully saturated in vivo may contribute to  
18 flexibility of the polypeptide chain or to biomechanical function. It may be advantageous to  
19 allow some degree of extensibility of assembled microfibrils in tissues subjected to  
20 mechanical forces. The importance of domain context for modulating the structural effects of  
21 calcium binding mutations suggests an explanation why MFS phenotypes associated with  
22 apparently similar mutations may be diverse <sup>33</sup>.

## 6. *FBN1* gene mutations in Marfan syndrome and related disorders,

To date over 500 mutations have been identified in the *FBN1* gene in Marfan syndrome patients and related diseases (Figure 3)<sup>34</sup> (Collod-Bérout et al., In preparation). No major rearrangements have been identified except for three cases of multi-exon deletions<sup>35,36</sup>. Three categories of mutations have been described: 1) missense mutations, 2) small insertions or deletions, mutations causing premature termination of translation and 3) exon-skipping mutations.

*FBN1* gene mutations have been identified in complete and incomplete forms of Marfan syndrome but also in various disorders: severe neonatal Marfan syndrome, dominantly inherited ectopia lentis<sup>37</sup>, isolated skeletal features of MFS<sup>38</sup>, the Shprintzen-Goldberg syndrome<sup>39</sup> and, more recently, familial or isolated forms of aortic aneurysms<sup>40</sup>. These results define the new molecular group of “type 1 fibrillinopathies” that comprises a spectrum of overlapping diseases. Presently no genotype/phenotype correlations have been identified except for neonatal mutations (see paragraph 8). To facilitate their identification, a “Marfan database” has been developed that includes not only molecular but also clinical data. The database is attached to a software that provides various tools for its analysis and allows optimized multicriteria research<sup>34, 41-43</sup>. It is only through a large collaborative international effort that genotype/phenotype correlations will be eventually identified.

No case of incomplete penetrance has ever been demonstrated for families in which patients carrying fibrillin-1 mutations are associated with Marfan syndrome. However, patients with the same mutation can show a wide degree of phenotypic variability. This has been exemplified in large pedigrees with sharp differences in clinical severity of musculoskeletal and cardiovascular features of the syndrome<sup>44</sup>.

## 7. Neonatal Marfan syndrome and *FBN1* gene mutations

Neonatal Marfan syndrome is the most severe form of the disorder. Affected newborns display severe cardiac valve regurgitation and dilatation of the proximal aorta which usually lead to heart failure and death in the first year of life. Skeletal manifestations such as arachodactyly, dolichostenomelia, and pectus deformities are typically present. Such infants may also display congenital flexion contractures, crumpled ears, loose redundant skin, and a characteristic "senile" facial appearance<sup>45</sup>. The mean life span is usually low (approximately 1 year<sup>46</sup>). The primary cause of death is congestive heart failure associated with mitral and tricuspid regurgitation. Family investigation usually reveals that the Marfan patients with the severe neonatal phenotype are sporadic cases: Buntinx et al. reported that 37 of 44 cases with neonatal manifestations were sporadic<sup>45</sup>. For a longtime it was generally thought that the neonatal phenotype could be explained by mutations in a distinct gene than that involved in the classic "adolescent-adult" form of the syndrome as the observed symptoms were extremely severe and overlapped with congenital contractural arachnodactyly. Godfrey et al. showed an abnormal morphology of fibrillin microfibrils in fibroblast cultures from patients with the neonatal phenotype<sup>46</sup>. As in the classic "adolescent-adult" form, there was an apparent decrease in accumulation of immunostainable fibrillin, but they appeared shorter, fragmented and frayed. Molecular analyses revealed that the neonatal Marfan syndrome was also due to mutations within the *FBN1* gene. Furthermore a clustering of mutations in the protein region encoded by exons 24 to 32 was observed (figure 4), suggesting an unknown but critical function of these domains<sup>47</sup>. The severe phenotype associated with these specific mutations in this region of the gene represents, to date, the only genotype/phenotype

1 relationship established. The observed clustering of mutations enables, in a first step, direct  
2 screening of this region of the *FBN1* gene to help in diagnosis of neonatal Marfan syndrome  
3 in patients. Finally, confirmation of the sporadic nature of the mutation is important for  
4 genetic counseling since perinatal lethal Marfan syndrome can also result from compound  
5 heterozygosity<sup>48</sup> or potential homozygosity.

## 7 **8. Pathogenic mechanisms**

8 Fibrillins are important components of the microfibrillar system that may act as a  
9 scaffold for elastogenesis. Elastic fibers first appear in fetal development as aggregates of  
10 microfibrils. These microfibrils are arranged in parallel arrays on which elastin is deposited  
11 and appears as an amorphous material. Elastin-containing microfibrillar bundles aggregate  
12 to form true elastic fibers. These observations suggest that microfibrils determine the form  
13 and the orientation of elastic fibers, therefore directing fiber assembly as a scaffold on which  
14 elastin is deposited<sup>29</sup>. This model explains the typical fragmentation and disarray of elastic  
15 fibers observed in the media of Marfan patients. However, unlike elastin, fibrillin-1 is also  
16 highly expressed in the vascular adventitia. Therefore reduction of this protein in the adventitia  
17 is very likely also involved in the mechanism for dilatation and for increased risk of  
18 aneurysm since the role of the adventitia is to maintain the vascular diameter. The pleiotropic  
19 manifestations of the disease can be explained by the observation that numerous  
20 microfibrillar aggregates devoid of elastin are found in the zonule, as well as cartilage and  
21 the extracellular matrix of many organs. However, the actual pathogenic mechanisms in  
22 these tissues still remain speculative.

1 At the molecular level, two different groups of mutations are distinguishable:  
2 mutations leading to a truncated protein and missense mutations. The first group correspond  
3 to one third of the mutations and is constituted of nonsense mutations (~10% of all  
4 mutations), splicing errors (~12%, only one demonstrated case of exon addition), small  
5 deletions leading to premature STOP codon (~8%), small inframe deletions (~2%), multi-exon  
6 deletions (~0.6%), and insertions leading to premature STOP codon (~4%). Mutations can be  
7 responsible for the appearance of a premature STOP codon that reduces the stability of the  
8 mutant transcript and consequently greatly reduces protein production from the mutated  
9 copy of the gene (in the affected subjects, the amount of fibrillin-1 protein produced is 50 %  
10 that of normal and is produced only from the normal gene copy), or for the production from  
11 the mutated copy of an abnormal monomere that considerably interferes with the assembly  
12 (polymerization) of fibrillin molecules (the amount of fibrillin is greatly reduced, < 35 %).  
13 The second group represent two third of mutations and correspond to missense mutation.  
14 Among them, three quarters are located in calcium binding modules. They are implicated  
15 either in creating (~3% of all mutations) or substituting (~24%) cysteine residues potentially  
16 implicated in disulfide bonding and consequently in the correct folding of the monomere.  
17 The majority of remaining mutations of this type of module affects residues of the calcium  
18 consensus sequence that play a major role in defining interdomain linkage<sup>55</sup>. An increased  
19 protease susceptibility is a mechanism also suggested for missense mutations. Other modules  
20 are carriers of one quarter of missense mutations and pathological mechanisms have yet to be  
21 clearly demonstrated.

22 What is still unknown are the multiple consequences triggered by the various  
23 mutations and the effect of unknown modifier (enhancing or protecting) genes on the clinical

1 expression. These mechanisms and the great number of mutations identified in the *FBN1*  
2 gene explain the great variability of the disease observed not only between families but also  
3 among affected individuals in a single family.  
4

## 5 **9. Genetic heterogeneity in Marfan syndrome**

6 The clinical variability of Marfan syndrome is only partly explained by the great  
7 number of mutations identified in the *FBN1* gene. In effect, we have demonstrated the  
8 existence of genetic heterogeneity, i.e. the involvement, in certain cases of Marfan syndrome  
9 of mutations located in another gene named MFS2 (for Marfan syndrome type 2). Genetic  
10 heterogeneity was demonstrated through the study of a large French family in which affected  
11 individuals display an incomplete form of the syndrome: typical skeletal and cardiovascular  
12 features as well as involvement of the skin and integument. No ocular manifestations were  
13 observed until recently when one of the children developed ectopia lentis. We showed that  
14 fibrillin-1 was normal in several affected family members and excluded linkage between the  
15 *FBN1* gene and the disease in the family<sup>50</sup>. By exclusion mapping we located the MFS2 gene  
16 on the short arm of chromosome 3<sup>51</sup>. In this area is located the gene that encodes fibuline-2  
17 (FBLN2), another microfibrillar component. Again through a double approach (genetic and  
18 protein) we showed that MFS2 and FBLN2 were not identical<sup>52</sup>. We are now identifying  
19 MFS2 through positional cloning. Other teams have already identified families comparable to  
20 the French family in that they are not linked to or do not carry a mutation in the *FBN1* gene  
21 (M. Boxer, L. Peltonen and Beat Steinmann, personal communications). Clinically these  
22 families are indistinguishable from other families linked to *FBN1*. Therefore, we are also  
23 trying to determine the percentage of Marfan syndrome cases that are associated with

1 mutations in MFS2 through genetic analyses as well as their clinical spectrum. Other teams,  
2 through protein studies have identified between 7 and 16 % of Marfan syndrome patients  
3 with normal fibrillin metabolism<sup>53, 54</sup>. The precise determination of this % is important for  
4 laboratories involved in diagnosis of Marfan syndrome since it will give the risk associated  
5 with investigation of only the *FBN1* gene.

## 7 **10. Animal model.**

8 The first animal model described was a limousine calve which presented with skeletal  
9 (kyphosis, long, thin limbs), integuments (severe joint and tendon laxity), ocular  
10 (microspherophakia, ectopia lentis) and cardiovascular (heart murmurs, aortic dilatation,  
11 sudden death at a young age due to aortic rupture) abnormalities<sup>55</sup>. The similarities between  
12 the human and the bovine diseases suggest that similar metabolic defects could be  
13 responsible. To date, although reduced immunostained fibrillin in cultured aortic smooth  
14 muscle cells in this limousine calve<sup>56</sup>, no mutation in the corresponding bovine *FBN1* gene or  
15 in another gene was yet identified in this model.

16 Mice carrying the *Tight skin (Tsk)* mutation harbor a genomic duplication within the  
17 fibrillin-1 (*Fbn1*) gene that results in a larger than normal in-frame *Fbn1* transcript<sup>57</sup>. *Tsk/+*  
18 mice exhibit a thickening of the skin with loss of elasticity, larger skeletal size because of  
19 excessive bone and cartilage growth, emphysema-like condition, myocardial hypertrophy  
20 and small tendons with tendon sheath hyperplasia. *Tsk* fibrillin-1 is produced, assembled,  
21 and deposited in the extracellular matrix but beaded *Tsk* fibrillin-1 microfibrils have a longer  
22 than normal periodicity and an altered morphology and organization in skin. Vascular  
23 complications were thought to be absent in these animals because the level of functional

1 microfibrils does not drop below the critical threshold. The heterozygous mice have a normal  
2 life span contrary to the human counterpart.

3 Gene-targeting experiments in mice resulted in two mutant lines in mice: the mg $\Delta$   
4 mutant from the J1 lines of ES cells (deletion of exons 19 to 24)<sup>58</sup> and the mgR mutant from  
5 R1 lines of ES cells (integration of the PGK*neo*-cassette without loss of endogenous sequence)  
6<sup>59</sup>. Homozygous mg $\Delta$  mice begin life with a drastic reduction in protein (5%) and die early  
7 because of structural failure of the vascular system. Homozygous mgR mice produce a  
8 quarter of the normal amount of fibrillin-1 and display phenotypic features in the skeleton  
9 and the aorta similar to those of patients with classic Marfan syndrome. The mgR/mgR mice  
10 support the notion that microfibrils control bone overgrowth negatively.

11 Finally, Jaubert et al. demonstrated the implication of type C receptor for natriuretic  
12 peptides (NPR-C) in the strigosus (*stri*) mutation<sup>60</sup>. Homozygous mutant mice show as early  
13 as 6 days of age increased body length, longer digits, and a typical cone-shaped implantation  
14 of the tail. When older, mutant mice are exceptionally thin and have arachnodactyly, thoracic  
15 kyphosis and frequent tail and/or sacral kinks. The unexpected expression of mutations  
16 within this gene as a Marfan-like skeletal phenotype should not be overlooked in the  
17 investigation of the pathogenesis of Marfan syndrome.

## 18 19 **11. Marfan syndrome is still an essentially clinical diagnosis,**

20 Although no specific therapy exists for Marfan syndrome, it is of great importance to  
21 confirm or firmly exclude the diagnosis in family members at risk as early as possible  
22 because of the potential fatal complications of the disease. At present, diagnosis is still based  
23 on thorough clinical examination, including measurements of body proportions,

1 echocardiography of the aorta, slit-lamp ophthalmological evaluation and radiographs. A  
2 complete family history is also an essential part of the diagnosis. However in some cases the  
3 manifestations are not evident until adolescence and the clinical expression of the disease  
4 varies greatly between affected members of a single family. Therefore, there is an absolute  
5 need for an accurate diagnostic test.

6 The discovery of the involvement of fibrillin-1 has raised high hopes for a protein or  
7 DNA test applicable to Marfan syndrome patients. Immunofluorescence studies of cultured  
8 fibroblasts and skin sections of patients using monoclonal antibodies against fibrillin have  
9 revealed that the amount of fibrillin deposition or of fibrillin microfibrils is greatly reduced<sup>8</sup>.  
10 Therefore, immunofluorescence analysis could be helpful in diagnosis. However the method  
11 has proven to be insufficiently sensitive and specific because of the existence of non-Marfan  
12 syndrome type 1 fibrillinopathies and of genetic heterogeneity. Therefore, an abnormal test  
13 result does not diagnose Marfan syndrome, and a normal test result does not exclude Marfan  
14 syndrome.

15 The identification of the *FBN1* gene has allowed the development of two types of  
16 diagnostic tests: either genetic family studies or mutation identification. Family studies can  
17 be performed with specific *FBN1* polymorphic markers to identify the mutation-bearing  
18 haplotype<sup>61</sup>. These studies are only reliable in families in which several affected individuals  
19 are available since the involvement of a *FBN1* mutation (and not that of another gene) must  
20 be clearly demonstrated. However, most family structures do not comply with this  
21 requirement. Furthermore, the method is inappropriate in sporadic cases. In practice, these  
22 instances represent over 40 % of the cases referred for biological diagnosis. The second  
23 molecular test is mutation identification. Mutation identification is very costly and long. In  
24 effect, there is no quick and 100 % reliable method to investigate a large (~ 230 kb) and

1 highly fragmented (10 kb of coding sequence fragmented in 65 exons) gene, knowing that  
2 almost each family has its own specific defect and that the mutations are essentially point  
3 mutations. Finally, this very costly analysis may fail to identify a mutation since only the  
4 coding sequence and closely surrounding regions are investigated. However, in the case of  
5 neonatal Marfan syndrome, where a clustering of mutations is found in a specific region,  
6 molecular diagnosis can be performed. In all other instances and until better molecular tools  
7 are available, mutation identification cannot be performed on a systematic basis. However, in  
8 a few cases where the family mutation had been identified, it was possible to perform  
9 prenatal diagnosis on chorionic villus samples or offer presymptomatic diagnosis in children  
10 at risk of affected subjects<sup>62,63</sup>.

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1 **Legends to figures**

2

3 **Figure 1: Schematic representation of the deduced primary structure of fibrillin-1.**

4

5 **Figure 2: Schematic diagram of a normal cb EGF-like module.**

6 The cysteine residues which are disulfide-bonded and stabilize the native fold of the domain  
7 are represented in white. Other highly conserved residues are designated by their single-  
8 letter amino acid code. Residues with putative significance for calcium binding are  
9 numbered sequentially as in Dietz and Pyeritz<sup>17</sup>.

10

11 **Figure 3: Distribution of the mutations identified in *FBN1* gene.**

12

13 **Figure 4: Distribution of mutations identified in *FBN1* gene associated with a  
14 neonatal form of Marfan syndrome.**

15