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Review article :

Marfan syndrome in the third Millenium.

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1 **Introduction:**

2 A hundred years have now elapsed since Dr. Antonin Marfan¹ 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" ². 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" ³. 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 ⁴. 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 ⁵. 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”

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

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

The gene encoding type 1 fibrillin (*FBN1*) lies on the long arm of chromosome 15 at 15q15-q21.1. This very large gene [first estimated at 110 kb, now at over 230 kb (Human Genome Sequencing Project NT_034890 sequence)] is highly fragmented into 65 exons, transcribed in a 10 kb mRNA that encodes a 2871 amino acid protein ^{10, 11, 13, 14}. Three additional

alternatively-spliced exons, likely untranslated, were found upstream of exon 1¹⁵. Conservation of nucleotide sequences within this region between human, mouse and porcine suggests that this region of the gene may harbor important regulatory elements. This region is GC-rich, contains a CpG island, and lacks conventional TATA or CCAAT boxes.

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

- The first repeated module is the **EGF-like module** that is homologous to one found in the epidermal growth factor. These modules contain six cysteine residues that form three intra-domain disulfide bonds. There are 47 of these throughout the fibrillin-1 protein. Among these, 43 contain a conserved consensus sequence for calcium binding and are called cb EGF-like modules. In these domains, the residues putatively involved in calcium binding are numbered sequentially in figure 2 as in Dietz and Pyeritz¹⁶. They include the aspartic acid at position 2, glutamic acid at position 5, asparagine at position 10 and tyrosine or phenylalanine at position 15.

- The second repeated module, found 7 times interspaced with cb EGF-like in the protein, is called **TGF β 1-binding protein-like module** (TGF β 1-BP-like module) since it is homologous to modules found in the Transforming Growth Factor β 1 binding protein. This domain appears to be limited to proteins that localize to matrix fibrils [fibrillins and latent transforming growth factor β -binding proteins (LTBPs)]. These modules contain eight cysteine residues. The fourth TGF- β 1-BP-like module contains the RGD sequence which can interact with cell receptors¹⁷. No specific function has yet been ascribed to these modules. However, some evidence suggests that these domains mediate specific protein-protein interactions¹⁸.

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

Finally the protein contains three unique regions: a **proline-rich region** that may act as a “hinge-like” region¹³ and **the amino and carboxy terminal domains**. The N- and C-terminal domains of the fibrillins display two prominent features : the presence of an even number of cysteine residues, four in the N-terminal and two in the C-terminal domains and the presence of the basic consensus sequence for processing by furin-types enzymes BXBB (B=basic amino acid residue, K or R) in each domain. The 4-cysteine domain in the N-terminus of fibrillins is homologous to similar 4-cysteine domains in the N-terminal extended forms of the LTBP_s. The C-terminal domains of the fibrillins are homologous to the C-terminal domain of all four members of the fibulin family, and thus a new type of extracellular module of approximately 120 amino acid residues in length has been proposed¹⁹. This type of homology is not shared by the LTBP_s.

When the *FBN1* gene was cloned, a second gene sharing a high degree of homology was identified and located on chromosome 5. This gene was named *FBN2* and the protein it encodes **fibrillin-2**¹⁰. *FBN2* has been genetically linked to a rare disorder that shares features of Marfan syndrome: congenital contractural arachnodactyly (CCA) (OMIM#120150). The clinical manifestations of CCA are essentially found in the skeleton and associated with distinctive manifestations including crumpled ears and campodactyly. Several mutations were identified in this gene in CCA patients²⁰.

Ikegawa et al. described the structure and chromosomal assignment to 2p16 of a “**fibrillin-like**” gene (**FBNL**), that is highly homologous to fibrillin²¹. The FBNL gene is

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

4. The fibrillin proteins.

The fibrillins are extracellular matrix glycoproteins that show a wide distribution in both elastic and non-elastic tissues and are integral components of 10 nm diameter microfibrils^{7, 23}. Fibrillin-1 is synthesized as profibrillin and proteolytically processed to fibrillin. The cleavage site has been mapped to the carboxy-terminal domain of profibrillin-1. The propeptide starts at position S²⁷³² directly C-terminal to the R²⁷²⁸KRR sequence. Wild type profibrillin is not incorporated into extracellular matrix until it is converted to fibrillin²⁴. The N-terminal region of each protein directs the formation of homodimers within a few hours after secretion and disulphide bonds stabilize the interaction²⁵. Dimer formation occurs intracellularly, suggesting that the process of fibrillin aggregation is initiated early after biosynthesis of the molecules. Fibrillin is post-translationally modified by β -hydroxylation and N-and O-linked carbohydrate formation²⁶.

1 The solution structure of the TGF- β -like module from human fibrillin-1 identified a
2 novel fold which was globular in nature ²⁷ 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 ¹⁸.

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 ²⁸. 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 ²³. 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

expression thus suggest distinct but related roles in microfibril physiology. Fibrillin-1 would provide mostly force-bearing structural support whereas fibrillin-2 would predominantly regulate the early process of elastic fiber assembly²⁹. Fibrillins would contribute to the structural and functional heterogeneity of microfibrils.

5. Role of Ca^{2+} in fibrillin,

The implication of the variable calcium binding affinities observed in fibrillin fragments is biologically significant. A number of studies have shown that the presence of calcium ions significantly protects full-length or recombinant fragments of fibrillin-1 from proteolysis by trypsin, elastase, endoproteinase Glu-C, plasmin and matrix metalloproteinases³¹⁻³⁴. Moderate to high affinities for calcium suggest that fibrillin cb EGF-like modules would be close to fully saturated in vivo. Particular regions of fibrillin may need to be rigid for appropriate function. For example, cb EGF-like#12-13, located in the neonatal Marfan syndrome region (see paragraph 8) where mutations leading to severe phenotypes cluster, may be part of a region where rigidity is required for function. Fully saturated calcium binding sites may be required for stabilization of the microfibril against proteolytic degradation, when low-affinity sites not fully saturated in vivo may contribute to flexibility of the polypeptide chain or to biomechanical function. It may be advantageous to allow some degree of extensibility of assembled microfibrils in tissues subjected to mechanical forces. The importance of domain context for modulating the structural effects of calcium binding mutations suggests an explanation why MFS phenotypes associated with apparently similar mutations may be diverse³³.

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)³⁴ (Collod-Bérout et al., In preparation). No major rearrangements have been identified except for three cases of multi-exon deletions^{35,36}. 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 lentis³⁷, isolated skeletal features of MFS³⁸, the Shprintzen-Goldberg syndrome³⁹ and, more recently, familial or isolated forms of aortic aneurysms⁴⁰. 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^{34, 41-43}. 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 syndrome⁴⁴.

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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 appearance⁴⁵. The mean life span is usually low (approximately 1 year⁴⁶). 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 sporadic⁴⁵. 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 phenotype⁴⁶. 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 domains⁴⁷. The severe phenotype associated with these specific mutations in this region of the gene represents, to date, the only genotype/phenotype

relationship established. The observed clustering of mutations enables, in a first step, direct screening of this region of the *FBN1* gene to help in diagnosis of neonatal Marfan syndrome in patients. Finally, confirmation of the sporadic nature of the mutation is important for genetic counseling since perinatal lethal Marfan syndrome can also result from compound heterozygosity⁴⁸ or potential homozygosity.

8. Pathogenic mechanisms

Fibrillins are important components of the microfibrillar system that may act as a scaffold for elastogenesis. Elastic fibers first appear in fetal development as aggregates of microfibrils. These microfibrils are arranged in parallel arrays on which elastin is deposited and appears as an amorphous material. Elastin-containing microfibrillar bundles aggregate to form true elastic fibers. These observations suggest that microfibrils determine the form and the orientation of elastic fibers, therefore directing fiber assembly as a scaffold on which elastin is deposited²⁹. This model explains the typical fragmentation and disarray of elastic fibers observed in the media of Marfan patients. However, unlike elastin, fibrillin-1 is also highly expressed in the vascular adventitia. Therefore reduction of this protein in the adventitia is very likely also involved in the mechanism for dilatation and for increased risk of aneurysm since the role of the adventitia is to maintain the vascular diameter. The pleiotropic manifestations of the disease can be explained by the observation that numerous microfibrillar aggregates devoid of elastin are found in the zonule, as well as cartilage and the extracellular matrix of many organs. However, the actual pathogenic mechanisms in these tissues still remain speculative.

At the molecular level, two different groups of mutations are distinguishable: mutations leading to a truncated protein and missense mutations. The first group correspond to one third of the mutations and is constituted of nonsense mutations (~10% of all mutations), splicing errors (~12%, only one demonstrated case of exon addition), small deletions leading to premature STOP codon (~8%), small inframe deletions (~2%), multi-exon deletions (~0.6%), and insertions leading to premature STOP codon (~4%). Mutations can be responsible for the appearance of a premature STOP codon that reduces the stability of the mutant transcript and consequently greatly reduces protein production from the mutated copy of the gene (in the affected subjects, the amount of fibrillin-1 protein produced is 50 % that of normal and is produced only from the normal gene copy), or for the production from the mutated copy of an abnormal monomere that considerably interferes with the assembly (polymerization) of fibrillin molecules (the amount of fibrillin is greatly reduced, < 35 %). The second group represent two third of mutations and correspond to missense mutation. Among them, three quarters are located in calcium binding modules. They are implicated either in creating (~3% of all mutations) or substituting (~24%) cysteine residues potentially implicated in disulfide bonding and consequently in the correct folding of the monomere. The majority of remaining mutations of this type of module affects residues of the calcium consensus sequence that play a major role in defining interdomain linkage⁵⁵. An increased protease susceptibility is a mechanism also suggested for missense mutations. Other modules are carriers of one quarter of missense mutations and pathological mechanisms have yet to be clearly demonstrated.

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

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

9. Genetic heterogeneity in Marfan syndrome

The clinical variability of Marfan syndrome is only partly explained by the great number of mutations identified in the *FBN1* gene. In effect, we have demonstrated the existence of genetic heterogeneity, i.e. the involvement, in certain cases of Marfan syndrome of mutations located in another gene named MFS2 (for Marfan syndrome type 2). Genetic heterogeneity was demonstrated through the study of a large French family in which affected individuals display an incomplete form of the syndrome: typical skeletal and cardiovascular features as well as involvement of the skin and integument. No ocular manifestations were observed until recently when one of the children developed ectopia lentis. We showed that fibrillin-1 was normal in several affected family members and excluded linkage between the *FBN1* gene and the disease in the family⁵⁰. By exclusion mapping we located the MFS2 gene on the short arm of chromosome 3⁵¹. In this area is located the gene that encodes fibuline-2 (FBLN2), another microfibrillar component. Again through a double approach (genetic and protein) we showed that MFS2 and FBLN2 were not identical⁵². We are now identifying MFS2 through positional cloning. Other teams have already identified families comparable to the French family in that they are not linked to or do not carry a mutation in the *FBN1* gene (M. Boxer, L. Peltonen and Beat Steinmann, personal communications). Clinically these families are indistinguishable from other families linked to *FBN1*. Therefore, we are also 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^{53, 54}. 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.

6 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) abnormalities⁵⁵. 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 calve⁵⁶, 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* transcript⁵⁷. *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

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

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

Finally, Jaubert et al. demonstrated the implication of type C receptor for natriuretic peptides (NPR-C) in the *strigosus (stri)* mutation⁶⁰. Homozygous mutant mice show as early as 6 days of age increased body length, longer digits, and a typical cone-shaped implantation of the tail. When older, mutant mice are exceptionally thin and have arachnodactyly, thoracic kyphosis and frequent tail and/or sacral kinks. The unexpected expression of mutations within this gene as a Marfan-like skeletal phenotype should not be overlooked in the investigation of the pathogenesis of Marfan syndrome.

11. Marfan syndrome is still an essentially clinical diagnosis,

Although no specific therapy exists for Marfan syndrome, it is of great importance to confirm or firmly exclude the diagnosis in family members at risk as early as possible because of the potential fatal complications of the disease. At present, diagnosis is still based 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 ⁸.
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 ⁶¹. 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

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

References

- 1 Marfan A. Un cas de déformation congénitale des quatre membres, plus prononcée aux extrémités, caractérisée par l'allongement des os avec un certain degré d'amincissement. Bull Mém Soc Méd Hôp Paris 1896, **13**: 220-227.
- 2 Beighton P, de Paepe A, Danks D, et al. International nosology of heritable disorders of connective tissue. Am J Med Genet 1988, **29**: 581-594.
- 3 De-Paepe A, Devereux R, Dietz H, Hennekam R, Pyeritz R. Revised diagnostic criteria for the Marfan syndrome. Am J Med Genet 1996, **62**: 417-426.
- 4 Rose P, Levy H, Ahn N et al. A comparison of the Berlin and Ghent nosologies and the influence of dural ectasia in the diagnosis of Marfan syndrome. Genetics in medicine 2000, **2**: 278-282.
- 5 Weve H. Über Arachnodaktylie (dystrophia mesodermalis congenita, Typus Marfan). Archiv Augenheilk 1931, **104**: 1-46.

- 1 6 McKusick VA. The cardiovascular aspects of Marfan's syndrome: A heritable disorder
2 of connective tissue. *Circulation* 1955, **11**: 321-341.
- 3 7 Sakai L, Keene D, Engvall E. Fibrillin, a new 350 kD glycoprotein is a compound of
4 extracellular microfibrils. *J Cell Biol* 1986, **103**: 2499-2509.
- 5 8 Hollister D, Godfrey M, Sakai L, Pyeritz R. Immunohistologic abnormalities of the
6 microfibrillar system in the Marfan syndrome. *N Eng J Med* 1990, **323**: 152-159.
- 7 9 Dietz H, Cutting G, Pyeritz R et al. Marfan syndrome caused by a recurrent de novo
8 missense mutation in the fibrillin gene. *Nature* 1991, **352**: 337-339.
- 9 10 Lee B, Godfrey M, Vitale E et al. Linkage of Marfan syndrome and a phenotypically
10 related disorder to two different fibrillin genes. *Nature* 1991, **352**: 330-334.
- 11 11 Maslen C, Corson G, Maddox B, Glanville R, Sakai L. Partial sequence of a candidate
12 gene for the Marfan syndrome. *Nature* 1991, **352**: 334-337.
- 13 12 Kainulainen K, Pulkkinen L, Savolainen A, Kaitila I, Peltonen L. Location on
14 chromosome 15 of the gene defect causing Marfan syndrome. *N Eng J Med* 1990, **323**: 935-
15 939.
- 16 13 Corson G, Chalberg S, Dietz H, Charbonneau N, Sakai L. Fibrillin binds calcium and is
17 coded by cDNAs that reveal a multidomain structure and alternatively spliced exons at the 5'
18 end. *Genomics* 1993, **17**: 476-484.
- 19 14 Pereira L, d'Alessio M, Ramirez F et al. Genomic organization of the sequence coding
20 for fibrillin, the defective gene product in Marfan syndrome. *Hum Mol Genet* 1993, **2**: 961-
21 968.
- 22 15 Biery N, Eldadah Z, Moore C, Setten G, Spencer F, Dietz H. Revised genomic
23 organization of *FBN1* and significance for regulated gene expression. *Genomics* 1999, **56**: 70-
24 77.

- 1 16 Dietz HC, Pyeritz RE. Mutations in the human gene for fibrillin-1 (*FBN1*) in the
2 Marfan syndrome and related disorders. Hum Mol Genet. 1995, 4 Spec No:1799-809. Review.
- 3 17 Sakamoto H, Brockelmann T, Cheresh D, Ramirez F, Rosenbloom J, Mecham R. Cell-
4 type specific recognition of RGD- and non-RGD-containing cell binding domains in fibrillin-
5 1. J Biol Chem 1996, **271**: 4916-4922.
- 6 18 Handford PA, Downing AK, Reinhardt DP, Sakai LY. Fibrillin: From domain structure
7 to supramolecular assembly. Matrix Biology 2000, **19**: 457-470.
- 8 19 Giltay R, Timpl R, Kostka G. Sequence, recombinant expression and tissue localization
9 of two novel extracellular matrix proteins, fibulin-3 and fibulin-4. Matrix Biol, 1999, **18**: 469-
10 480.
- 11 20 Wang M, Clericuzio CL, Godfrey M. Familial occurrence of typical and severe lethal
12 congenital contractural arachnodactyly caused by missplicing of exon 34 of fibrillin-2. Am J
13 Hum Genet 1996, **59**: 1027-34.
- 14 21 Ikegawa S, Toda T, Okui K, Nakamura Y. Structure and chromosomal assignment of
15 the human S1-5 gene (FBNL) that is highly homologous to fibrillin. Genomics 1996, **35**: 590-
16 592.
- 17 22 Stone EM, Lotery AJ, Munier FL et al. A single EFEMP1 mutation associated with both
18 malattia Leventinese and Doyne honeycomb retinal dystrophy. Nature Genetics 1999, **22**:
19 199-202.
- 20 23 Zhang H, Apfelroth S, Hu W et al. Structure and expression of fibrillin-2 a novel
21 microfibrillar component preferentially located in elastic matrices. J Cell Biol 1994, **124**: 855-
22 863.

- 1 24 Raghunath M, Putnam EA, Ritty T et al. Carboxy-terminal conversion of profibrillin to
2 fibrillin at a basic site by PACE/furin-like activity required for incorporation in the matrix. J
3 Cell Science 1999, **112**: 1093-1100.
- 4 25 Trask TM, Ritty TM, Broekelmann T, Tisdale C, Mecham RP. N-terminal domains of
5 fibrillin 1 and fibrillin 2 direct the formation of homodimers: a possible first step in
6 microfibril assembly. Biochem J. 1999, 340 : 693-701.
- 7 26 Glanville RW, Qian RQ, McClure DW, Maslen CL. Calcium binding, hydroxylation,
8 and glycosylation of the precursor epidermal growth factor-like domains of fibrillin-1, the
9 Marfan gene protein. J Biol Chem 1994, 269:26630-4
- 10 27 Yuan X, Downing AK, Knott V, Handford PA. Solution structure of the transforming
11 growth factor β -binding protein-like module, a domain associated with matrix fibrils. EMBO
12 J 1997, **16**: 6659-6666.
- 13 28 Baldock C, Koster A, Ziese U et al. The supramolecular organization of fibrillin-rich
14 microfibrils. J Cell Biol 2001, **152**: 1045-1056.
- 15 29 Zhang H, Hu W, Ramirez F. Developmental expression of fibrillin genes suggests
16 heterogeneity of extracellular microfibrils. J Cell Biol 1995, **129**: 1165-1176.
- 17 30 Reinhardt DP, Ono RN, Sakai LY. Calcium stabilizes fibrillin-1 against proteolytic
18 degradation. J Biol Chem 1997, **272**: 1231-6.
- 19 31 Reinhardt DP, Ono RN, Notbohm H, Muller PK, Bachinger HP, Sakai LY. Mutations in
20 calcium-binding epidermal growth factor modules render fibrillin-1 susceptible to
21 proteolysis. A potential disease-causing mechanism in Marfan syndrome. J Biol Chem 2000,
22 **275**: 12339-12345.

- 1 32 Ashworth J, Murphy G, Rock M et al. Fibrillin degradation by matrix
2 metalloproteinases: Implications for connective tissue remodeling. *Biochem J*, 1999, **340**: 171-
3 181.
- 4 33 McGettrick AJ, Knott V, Willis A, Handford PA. Molecular effects of calcium binding
5 mutations in Marfan syndrome depend on domain context. *Hum Mol Genet* 2000, **9**: 1987-
6 1994.
- 7 34 Collod-Bérout G, Bérout C, Adès L et al. Marfan Database (third edition): new
8 mutations and new routines for the software. *Nucleic Acids Researc* 1998, **26**: 229-233.
- 9 35 Kainulainen K, Sakai LY, Child A et al. Two mutations in Marfan syndrome resulting
10 in truncated fibrillin polypeptides. *Proc Natl Acad Sci U S A*. 1992, **89** : 5917-21.
- 11 36 Liu W, Schrijver I, Brenn T, Furthmayr H, Francke U. Multi-exon deletions of the
12 *FBN1* gene in Marfan syndrome. *BMC Med Genet*. 2001, **2**:11-19.
- 13 37 Kainulainen K, Karttunen L, Puhakka L, Sakai L, Peltonen L. Mutations in the fibrillin
14 gene responsible for dominant ectopia lentis and neonatal Marfan syndrome. *Nature*
15 *Genetics* 1994, **6**: 64-69.
- 16 38 Milewicz D, Grossfield J, Cao SN, Kielty C, Covitz W, Jewett T. A mutation in *FBN1*
17 disrupts profibrillin processing and results in isolated skeletal features of the Marfan
18 syndrome. *J Clin Invest* 1995, **95**: 2373-2378.
- 19 39 Sood S, Eldadah Z, Krause W, McIntosh I, Dietz H. Mutation in fibrillin-1 and the
20 Marfanoid-craniosynostosis (Shprintzen-Goldberg) syndrome. *Nature Genetics*, 1996, **12**: 209-
21 211.
- 22 40 Milewicz D, Michael K, Fisher N, Coselli J, Markello T, Biddinger A. Fibrillin-1 (*FBN1*)
23 mutations in patients with thoracic aortic aneurysms. *Circulation* 1996, **94**: 2708-2711.

- 1 41 Collod G, Bérout C, Soussi T, Junien C, Boileau C. Software and database for the
2 analysis of mutations in the human *FBN1* gene. Nucl Acids Res 1996, **24**: 137-140.
- 3 42 Collod-Bérout G, Bérout C, Adès L et al. Marfan database (second edition): Software
4 and database for the analysis of mutations in the human *FBN1* gene. Nucl Acids Res 1997, **25**:
5 147-150.
- 6 43 Beroud C, Collod-Beroud G, Boileau C, Soussi T, Junien C. UMD (Universal mutation
7 database): A generic software to build and analyze locus-specific databases. Hum Mutat
8 2000, **15**: 86-94.
- 9 44 Dietz HC, Pyeritz RE, Puffenberger EG et al. Marfan phenotype variability in a family
10 segregating a missense mutation in the epidermal growth factor-like motif of the fibrillin
11 gene. J Clin Invest. 1992, 89 : 1674-80.
- 12 45 Buntinx I, Willems P, Spitaels S, VanReempst P, DePaepe A, Dumon J. Neonatal
13 Marfan syndrome with congenital arachnodactyly, flexion contractures, and severe cardiac
14 valve insufficiency. J Med Genet 1991, 28: 267-273.
- 15 46 Godfrey M, Raghunath M, Cisler J et al. Abnormal morphology of fibrillin microfibrils
16 in fibroblast cultures from patients with neonatal Marfan syndrome. Am J Pathol. 1995, 146 :
17 1414-21.
- 18 47 Putnam E, Cho M, Zinn A, Towbin J, Byers P, Milewicz D. Delineation of the Marfan
19 phenotype associated with mutations in exons 23-32 of the *FBN1* gene. Am J Med Genet 1996,
20 **62**: 233-242.
- 21 48 Karttunen L, Raghunath M, Lonnqvist L, Peltonen L. A compound heterozygous
22 Marfan patient: Two defective fibrillin alleles result in a lethal phenotype. Am J Hum Genet
23 1994, **55**: 1083-1091.

- 1 49 Knott V, Downing A, Cardy C, Handford P. Calcium binding properties of an
2 epidermal growth factor-like domain pair from human fibrillin-1. *J Mol Biol* 1996, **255**: 22-27.
- 3 50 Boileau C, Jondeau G, Babron MC et al. Familial Marfan-like aortic dilatation and
4 skeletal anomalies are not linked to the fibrillin genes. *Am J Hum Genet* 1993, **53**: 46-57.
- 5 51 Collod G, Babron MC, Jondeau G et al. A second locus for Marfan syndrome maps to
6 chromosome 3p24.2-p25. *Nature Genetics* 1994, **8**: 264-268.
- 7 52 Collod G, Chu ML, Sasaki T et al. Fibuline-2: Genetic mapping and exclusion as a
8 candidate in Marfan syndrome type 2. *Eur J Hum Genet* 1996, **4**: 292-295.
- 9 53 McGookey-Milewicz D, Pyeritz R, Crawford ES, Byers P. Marfan syndrome: Defective
10 synthesis, secretion, and extracellular matrix formation of fibrillin by cultured dermal
11 fibroblasts. *J Clin Invest* 1992, **89**: 79-86.
- 12 54 Aoyama T, Francke U, Dietz H, Furthmayer H. Quantitative differences in
13 biosynthesis and extracellular deposition of fibrillin in cultured fibroblasts distinguish five
14 groups of Marfan syndrome patients and suggest distinct pathogenic mechanisms. *J Clin*
15 *Invest* 1994, **94**: 130-137.
- 16 55 Besser TE, Potter KA, Bryan GM, Knowlen GG. An animal model of the Marfan
17 syndrome. *Am J Med Genet* 1990, **37**: 159-165.
- 18 56 Potter KA, Hoffman Y, Sakai LY, Byers PH, Besser TE, Milewicz DM. Abnormal
19 fibrillin metabolism in bovine Marfan syndrome. *Am J Pathol* 1993, **142**: 803-810.
- 20 57 Kielty CM, Raghunath M, Siracusa L et al. The tight skin mouse: demonstration of
21 mutant fibrillin-1 production and assembly into abnormal microfibrils. *J Cell Biol* 1998,
22 **140**:1159-1166.
- 23 58 Pereira L, Andrikopoulos K, Tian J et al. Targetting of the gene encoding fibrillin-1
24 recapitulates the vascular aspect of Marfan syndrome. *Nature Genetics* 1997, **17**: 218-222.

- 1 59 Pereira L, Lee SY, Gayraud B et al. Pathogenetic sequence for aneurysm revealed in
2 mice underexpressing fibrillin-1. *Proc Natl Acad Sci U S A* 1999, **96**: 3819-23.
- 3 60 Jaubert J, Jaubert F, Martin N et al. Three new allelic mouse mutations that cause
4 skeletal overgrowth involve the natriuretic peptide receptor C gene (Npr3). *Proc Natl Acad*
5 *Sci USA* 1999, **96**: 10278-10283.
- 6 61 Pereira L, Levran O, Ramirez F et al. A molecular approach to the stratification of
7 cardiovascular risk in families with Marfan's syndrome. *N Eng J Med* 1994, **331**: 148-153.
- 8 62 Godfrey M, Vandemark N, Wang M et al. Prenatal diagnosis and a donor splice site
9 mutation in fibrillin in a family with Marfan syndrome. *Am J Hum Genet*, 1993, **53**: 472-480.
- 10 63 Rantamäki T, Raghunath M, Karttunen L, Lönnqvist L, Child A, Peltonen L. Prenatal
11 diagnosis of Marfan syndrome: Identification of a fibrillin-1 mutation in chorionic villus
12 sample. *Prenat Diag*, 1995, **15**: 1176-1181.

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 Pyeritz¹⁷.

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