

Marfan syndrome in the third Millennium

Collod-Bérout Gwenaëlle^{1*}, Boileau Catherine^{1,2}

¹ *Génétique, chromosome et cancer INSERM : U383, Université Paris Descartes - Paris V, Gh Necker - Enfants Malades 149, Rue de Sevres 75743 PARIS CEDEX 15,FR*

² *Service de biochimie, d'hormonologie et de génétique moléculaire AP-HP, Hôpital Ambroise Paré, Université Paris Descartes - Paris V, 9, avenue Charles-de-Gaulle 92100 Boulogne-Billancourt,FR*

* Correspondence should be adressed to: Gwenaëlle Collod-Bérout <gwenaelle.collod-beroud@inserm.fr>

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Introduction

A hundred years have now elapsed since Dr. Antonin Marfan¹ reported on the case of Gabrielle P. thus describing some of the skeletal features that today define the syndrome that carries his name. Since then, substantial progress has been made with respect to the description of the pleiotropic manifestations of this disease, the understanding of underlying pathophysiological mechanisms and the availability of prevention and treatment of major complications.

Nosology: What is Marfan syndrome today ?

Marfan syndrome (MFS, OMIM#154700) is an autosomal dominant connective tissue disorder that has an estimated incidence of 1/5000 with probably over 25 % of sporadic cases. The syndrome involves many systems (skeletal, ocular, cardiovascular, pulmonary, skin and integument, and dura) but its more prominent manifestations are skeletal, ocular and cardiovascular. In 1986, an international group of experts agreed upon diagnostic criteria to distinguish classic Marfan syndrome from many related disorders. These criteria constitute what is currently referred to as the "Berlin nosology"². Patients are diagnosed based on involvement of the skeletal system and two other systems with at least one major manifestation (ectopia lentis, aortic dilation/dissection, or dural ectasia). Patients with an affected first degree relative are required to have involvement of at least two other systems with one major manifestation preferred but not required.

This nosology has been found wanting in many individual cases and revised criteria were subsequently proposed that constitute the "Ghent nosology"³. This new formulation requires involvement of three systems with two major diagnostic manifestations. It provides for major skeletal manifestations and considers affected first-degree relatives or molecular data as major diagnostic criteria.

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

The continued efforts to redefine diagnostic criteria emphasize persistent shortcomings. The phenotype of the Marfan syndrome remains incompletely defined. Most manifestations are age-dependant and are difficult to quantify. The Ghent nosology has been field-tested in The National Institutes of Health⁴. Their study shows that 19% of patients diagnosed under the Berlin criteria failed to meet the Ghent standard. Molecular data are important to better characterize this subset, to study its natural history and implement relevant preventive measures.

The Marfan syndrome and FBN1

Scientists, as early as 1931, suggested that the basic defect in Marfan syndrome lay in a defect in the mesoderm⁵. In 1955, Victor McKusick considered the syndrome as a prominent 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 syndrome⁸. The following year, the gene encoding fibrillin-1 (FBN1) was cloned and the first mutations in the gene were identified in Marfan syndrome patients⁹⁻¹¹. 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.

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 1 15. 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 LTBPs, 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 LTBPs. 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 LTBPs.

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.

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 R²⁷²⁸ KRR sequence. Wild type profibrillin is not incorporated into extracellular matrix until it is converted to fibrillin 24. The N-terminal region of each protein directs the formation of homodimers within a few hours after secretion and disulphide bonds stabilize the interaction 25. 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 formation 26.

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

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

Fibrillin-1 and -2 co-distribute in elastic and non-elastic connective tissues of the developing embryo, with a preferential accumulation of the FBN2 gene product in elastic fiber-rich matrices 23. Mouse study of the developmental expression of the fibrillin genes has revealed different patterns. Except for the cardiovascular system, in which Fbn1 gene activity is early and always higher than Fbn2, Fbn2 transcripts appear earlier than Fbn1 transcripts and accumulate for a short period of time just before overt tissue differentiation i.e. a window of time immediately preceding elastogenesis. In contrast, the amount of Fbn1 transcripts increases at an apparently gradual rate throughout morphogenesis and is mainly expressed during late morphogenesis and well-defined organ structures. Furthermore, Fbn1 transcripts are predominantly represented in stress- and load-bearing structures like aortic 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 assembly 29. Fibrillins would contribute to the structural and functional heterogeneity of microfibrils.

Role of Ca²⁺ 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 metalloproteinases 31–34. 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 diverse 33.

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 lentis 37, isolated skeletal features of MFS 38, the Shprintzen-Goldberg syndrome 39 and, more recently, familial or isolated forms of aortic aneurysms 40. 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 syndrome 44.

Neonatal Marfan syndrome and FBN1 gene mutations

Neonatal Marfan syndrome is the most severe form of the disorder. Affected new-borns 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 arachnodactyly, 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 45. The mean life span is usually low (approximately 1 year 46). 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 45. 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 46. 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 47. 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 heterozygosity 48 or potential homozygosity.

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 deposited 29. 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 inter domain linkage. 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.

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 family 50. By exclusion mapping we located the MFS2 gene on the short arm of chromosome 3 51. 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 identical 52. 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 mutations in MFS2 through genetic analyses as well as their clinical spectrum. Other teams, through protein studies have identified between 7 and 16 % of Marfan syndrome patients with normal fibrillin metabolism 53, 54. The precise determination of this % is important for laboratories involved in diagnosis of Marfan syndrome since it will give the risk associated with investigation of only the FBN1 gene.

Animal model

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

Mice carrying the Tight skin (Tsk) mutation harbor a genomic duplication within the fibrillin-1 (Fbn1) gene that results in a larger than normal in-frame Fbn1 transcript 57. Tsk/+ mice exhibit a thickening of the skin with loss of elasticity, larger skeletal size because of excessive bone and cartilage growth, emphysema-like condition, myocardial hypertrophy and small tendons with tendon sheath hyperplasia. Tsk fibrillin-1 is produced, assembled, and deposited in the extracellular matrix but beaded Tsk fibrillin-1 microfibrils have a longer than normal periodicity and an altered morphology and organization in skin. Vascular 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 mg Δ 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 PGKneo-cassette without loss of endogenous sequence) 59. Homozygous mg Δ 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) mutation 60. 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.

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, echocardiography of the aorta, slit-lamp ophthalmological

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

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

The identification of the FBN1 gene has allowed the development of two types of diagnostic tests: either genetic family studies or mutation identification. Family studies can be performed with specific FBN1 polymorphic markers to identify the mutation-bearing haplotype 61. These studies are only reliable in families in which several affected individuals are available since the involvement of a FBN1 mutation (and not that of another gene) must be clearly demonstrated. However, most family structures do not comply with this requirement. Furthermore, the method is inappropriate in sporadic cases. In practice, these instances represent over 40 % of the cases referred for biological diagnosis. The second molecular test is mutation identification. Mutation identification is very costly and long. In 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.

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Figure 1

Schematic representation of the deduced primary structure of fibrillin-1.

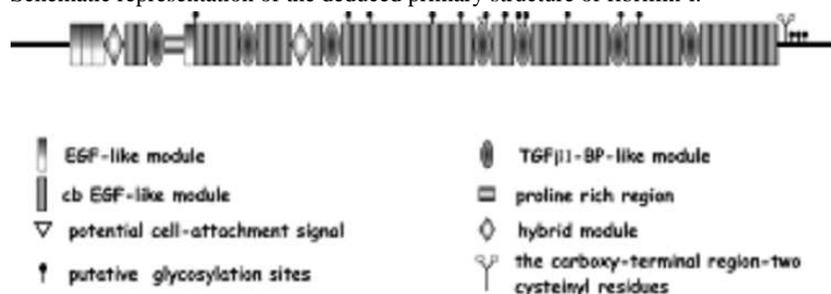


Figure 2

Schematic diagram of a normal cb EGF-like module

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

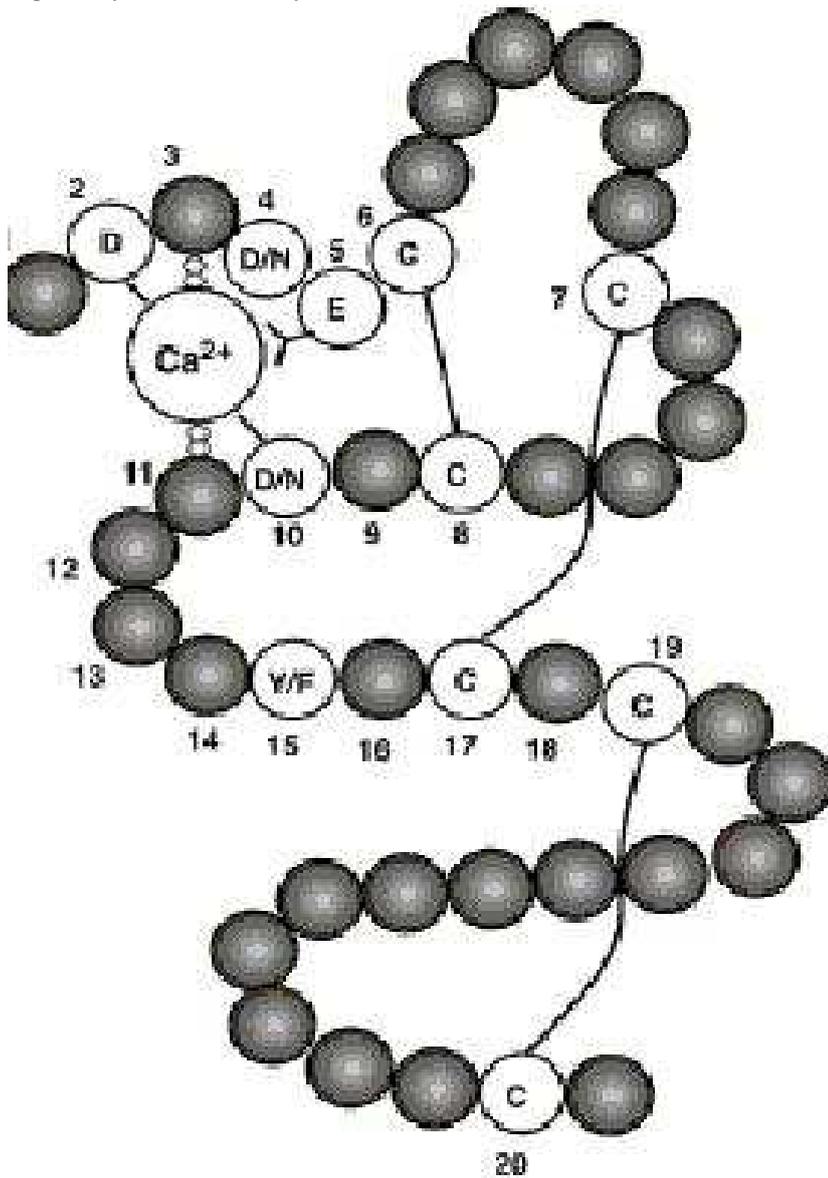


Figure 3

Distribution of the mutations identified in FBN1 gene.

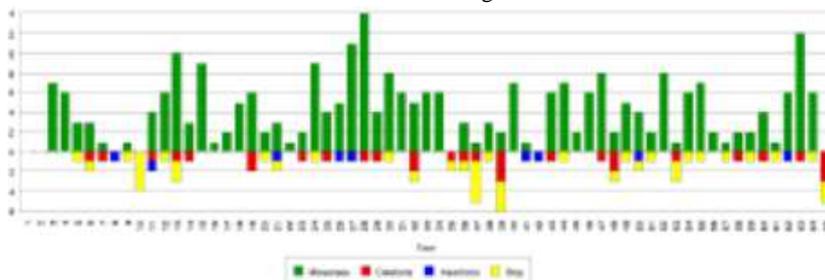


Figure 4

Distribution of mutations identified in FBN1 gene associated with a neonatal form of Marfan syndrome.

