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MARFAN SYNDROME AND RELATED FIBRILLINOPATHIES (pp761-765)

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INTRODUCTION:

Marfan syndrome (MFS, OMIM#154700) is the founding member of connective tissue disorders. It is autosomal dominant and has an estimated incidence of 1/5 000 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. It is characterized by extreme variability in phenotype severity between different affected members of a given family. At least two genes are implicated in MFS: in the majority of cases, mutations in *FBNI*, the gene encoding fibrillin-1 the major component of microfibrils, are implicated. Linkage analyses have localized a second gene in 3p24.2-p25. Since no specific clinical anomaly is associated with mutations in each gene, both must be tested for molecular diagnosis.

CLINICAL DEFINITION

In 1986, an international group of experts agreed upon diagnostic criteria to distinguish classic MFS from many related disorders. These criteria constitute what is currently referred to as the "Berlin nosology" (1). 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 revised in 1996 and referred to as the "Ghent nosology" (2). 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.

MFS AND FBN1

In 1986, Sakai and co-workers identified a new extracellular matrix protein that they named "fibrillin" (3) (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 MFS (4). Kainulainen et al. (5) demonstrated through linkage analysis that the gene involved in classic complete forms of the MFS was located on human chromosome 15. The gene encoding fibrillin-1 (*FBNI*) was cloned and located in 15q15-q21.1 (6; 7) and the first mutations in the gene were identified in MFS patients (8).

***FBNI* GENE**

The gene encoding type 1 fibrillin (*FBNI*) is very large (over 230 kb) and highly fragmented into 65 exons, transcribed in a 10 kb mRNA that encodes a 2871 amino acid protein (7; 9). Three additional alternatively-spliced exons, most likely untranslated and well conserved between human, mouse and porcine, were found upstream of exon 1. This region is GC-rich, contains a CpG island, and lacks conventional TATA or CCAAT boxes (10).

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

- **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. 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 (11).
- **TGF β 1-binding protein-like module** (TGF β 1-BP-like module), found 7 times interspaced with cb EGF-like in the protein, 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. No specific function has yet been ascribed to these modules. However, some evidence suggests that these domains mediate specific protein-protein interactions (12).

- **“hybrid module”** since it combines features of the two former. It consists of approximately 65 amino acids, and is found twice in the protein. This module is also found in LTBP, which have a single hybrid domain.

Finally the protein contains three unique regions: a **proline-rich region** that may act as a “hinge-like” region (13) and the **amino** and **carboxy terminal domains**. The N- and C-terminal domains of fibrillin 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-type enzymes BXBB (B=basic amino acid residue, K or R) in each domain. The 4-cysteine domain in the N-terminus of fibrillin is homologous to similar 4-cysteine domains in the N-terminal extended forms of the LTBP. The C-terminal domain of fibrillin is 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 proposed (14). This type of homology is not shared by the LTBP.

Other members of the fibrillin family were identified: ***FBN2*** gene in 5q23-q31 (fibrillin-2 protein) carrying mutations in congenital contractural arachnodactyly (CCA) (OMIM#120150); and ***FBNL* gene (or *EFEMP1* gene)** in 2p16 (fibrillin-like protein) carrying mutations in Doyne honeycomb retinal dystrophy (DHRD; OMIM#126600), or malattia Leventinese (MLVT).

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. Fibrillin-1 is synthesized as profibrillin and proteolytically processed to fibrillin. Wild type profibrillin is not incorporated into extracellular matrix until it is converted to fibrillin. The N-terminal region of each protein directs the formation of homodimers within a few hours after secretion and disulphide bonds stabilize the interaction (15). 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 (16). Baldock et al. (17) predicted fibrillin maturation from a parallel head-to-tail alignment. 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. 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 (18). Fibrillins could contribute to the structural and functional heterogeneity of microfibrils.

FIBRILLINOPATHIES

To date over 600 mutations have been identified in the *FBN1* gene in MFS patients and related diseases (19) (<http://www.umd.be>). *FBN1* gene mutations have been identified in complete and incomplete forms of MFS but also in various overlapping disorders: severe neonatal MFS, dominantly inherited ectopia lentis, isolated skeletal features of MFS, the Shprintzen-Goldberg

syndrome, Weil-Marchesani syndrome and familial or isolated forms of aortic aneurysms. These results define the new molecular group of “type 1 fibrillinopathies”.

PATHOGENIC MECHANISMS

Microfibrils are suggested to determine the form and the orientation of elastic fibers, therefore directing fiber assembly as a scaffold on which elastin is deposited (18). 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 adventia. Therefore reduction of this protein in the adventia is very likely also involved in the mechanism for dilatation and for increased risk of aneurysm since the role of the adventia 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 shortened protein and missense mutations. The first group corresponds to one third of the mutations. They can be responsible for a) 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 b) the production from the mutated copy of an abnormal monomer 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 corresponds to missense mutation. Among them, three quarters are located in calcium binding modules. They are implicated either in creating or substituting cysteine residues potentially implicated in disulfide bonding and consequently in the correct folding of the monomer. 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 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 *FBNI* gene explain the great variability of the disease observed not only between families but also among affected individuals in a single family.

GENETIC HETEROGENEITY

The clinical variability of MFS is only partly explained by the great number of mutations identified in the *FBNI* gene. In effect, we have demonstrated the existence of genetic heterogeneity, i.e. the involvement, in certain cases of MFS of mutations located in another gene named *MFS2* (for MFS type 2) through the study of a very 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 one of the children in the 4th generation developed ectopia lentis. We showed that fibrillin-1 was normal in several affected family members and excluded linkage between the *FBNI* gene and the disease in the family (20). By exclusion mapping we located the *MFS2* gene on the short arm of chromosome 3 (21). We are now identifying *MFS2* through positional cloning. Several families comparable to the French family in that they are not linked to or do not carry a mutation in the *FBNI* gene have been identified. Other teams, through protein studies have identified between 7 and 16 % of MFS patients with normal fibrillin metabolism (22; 23). The precise determination of this % is important for laboratories involved in diagnosis of MFS since it will give the risk associated with investigation of only the *FBNI* gene.

DIAGNOSTIC

The discovery of the involvement of fibrillin-1 has raised high hopes for a protein or DNA test applicable to MFS 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 (4). 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-MFS type 1 fibrillinopathies and of genetic heterogeneity. Therefore, an abnormal test result does not diagnose MFS, and a normal test result does not rule out MFS.

The identification of the *FBNI* 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 *FBNI* polymorphic markers to identify the mutation-bearing haplotype (24). These studies are only reliable in families in which several affected individuals are available since the involvement of a *FBNI* 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 MFS, where a clustering of mutations is found in exons 24 to 32, 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.

CONCLUSION

Although no specific therapy exists for MFS, 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. Development of preventive measures and surgery for aortic aneurysms and dissection have lead to treatment of life-threatening cardiovascular complications associated with the MFS and have considerably altered life expectancy for patients. 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.

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