JOURNAL OF BIOMEDICINE AND TRANSLATIONAL RESEARCH

Marfan syndrome, a review

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Abstract

Background: Marfan syndrome is an inherited condition, that affects many organ systems. The most obvious features are the skeletal abnormalities: tall stature, long limbs, arachnodactyly (spider hands). Ocular symptoms include severe myopia and lens luxation. The clinically most severe symptoms are cardiovascular: mitral valve insufficiency, aortic root enlargement and thoracic aortic aneurysms and dissections. Marfan syndrome is usually inherited in an autosomal dominant fashion, but in some families a recessive mode of inheritance has been described. Most cases are caused by mutations in the FBN1 gene. The phenotype is highly variable, also within families. The absence of the skeletal symptoms often leads to a failure to recognize the syndrome. The prevalence of Marfan syndrome is estimated to be 1 in 2500, in all populations, including Indonesia. There is some controversy in the literature regarding the involvement of transforming growth factor beat (TGFβ) in the etiology of Marfan syndrome, due to the fact that phosphorylation of the signal molecules smad2 and smad3 in some publications has been wrongly interpreted as exclusively caused by TGFβ, which is only one of the many ligands that can induce smad2/3 phosphorylation.

Clinical trials, testing the effect of losartan on aortic root dilatation and the growth of aneurysms, have shown conflicting results. In one study, the effect of losartan was only found in a subgroup of patients with a specific type of mutations, that lead to reduced amount of normal fibrillin 1 protein (haploinsufficiency). The distribution of this type of mutations in different population may explain the conflicting results of the trials.

Keywords: Marfan syndrome; *FBN1*; aortic aneurysm, losartan.
Permalink/ DOI: https://doi.org/10.14710/jbtr.v4i2.3560

INTRODUCTION

Marfan syndrome is named after the French pediatrician Antoine Bernard-Jean Marfan who described in 1896 a girl with arachnodactyly and long limbs[1]. The patient also had congenital contractures of the elbows and would not fulfill the current criteria for Marfan syndrome. She probably was suffering from a condition that we now call Contractural arachnodactyly, caused by mutations in the *FBN2* gene.

The clinical features of Marfan syndrome affect many systems of the body. The most obvious are the skeletal features, long limbs, tall stature, long thin fingers (arachnodactyly or spider fingers). The skeletal features can be scored objectively as: arm span more than 1.05 x body length; wrist sign (thumb and index finger can encircle the wrist of the other hand with at least one digit overlap) and thumb sign (when making a fist around the thumb, one digit of the thumb sticks out). The main neurological symptom is dural ectasias. The most severe symptoms are cardiovascular: mitral valve prolapse, aortic dilatation and thoracic aortic aneurysms and dissections, which may lead to sudden death[2]. However, I noticed in discussions with patients that they often consider the ocular symptoms, severe myopia and lens luxation, the worst for themselves, because the latter may lead to blindness.

It is important to realize that the obvious skeletal features are not always present. Within families we often see carriers of the same pathogenic mutation, that have very different phenotypes[3,4,5]. This means that Marfan syndrome can be present without the typical "Marfan habitus". The multitude of symptoms have led to defining clinical criteria for Marfan syndrome, named the Berlin nosology[6], Ghent criteria[7] and the revised Ghent nosology or criteria[8], named after the symposia where the criteria were formulated and discussed. In the revised criteria, the major and minor criteria were replaced by a scoring system. Dural ectasias play a less important role in the criteria, because they are also very common in non-Marfan patients[8,9]. In Asian Marfan patients, the phenotype is different from patients with a European background[10,11], so the criteria may have to be adapted for the Asian population.

One of the most important criteria for Marfan syndrome nowadays is the presence of a pathogenic mutation in the *FBN1* gene. However, assessing the pathogenic effect of DNA variants is not a simple matter, as will be discussed below. Mutations in *FBN1* have also been demonstrated in patients that do not meet clinical criteria for Marfan syndrome[12]. The first mutation in *FBN1* found in Marfan syndrome was published in 1991[13]. There is still discussion on the subject of locus heterogeneity, but it is clear that the majority of Marfan syndrome patients have a pathogenic variant in the *FBN1* gene[14,15]. In many countries, however, genetic testing is not readily available, so the use of the clinical criteria remains important in assessing Marfan syndrome and related phenotypes[16,17].
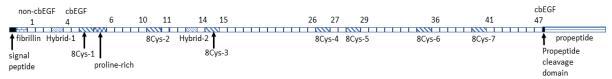
In most cases, Marfan syndrome is inherited in an autosomal dominant fashion. However, autosomal recessive inheritance of mutations in *FBN1* has also been demonstrated and should be taken into account in genetic counseling[18,19].

THE PROTEIN

The fibrillin 1 protein is a glycoprotein and is an important constituent of the elastic fibers in the large blood vessels (www.uniprot.org/uniprot/P35555). Fibrillin is important for tensile strength, whereas elastin is important for elasticity and extensibility. Consequently, the ratio of fibrillin and elastin has a large impact on the properties of the vessel wall. The cells that produce these structural proteins have no possible way of determining the amount of protein that has been produced, so the production of the proteins is not subject to any feedback regulation. Consequently, the gene dosage effect is very strong and null-alleles of the encoding genes have a strong effect on protein production and the ratio between elastin and fibrillin.

Apart from a function in structural properties of the vessel wall, fibrillin is also important as docking site for a number of inactive protein complexes, such as the latent TGF-β1, -2 and -3[20,21] and several matrix proteases, such as ADAMTS10 and ADAMTS17[22]. So fibrillin plays an important role in regulating the bioavailability of these growth factors and proteases. Other proteins that bind to fibrillin 1, such as the glycoproteins ADAMTSL2 and ADAMTSL4 probably play a role in fibril formation[22,23].

The fibrillin 1 protein has many repeated motifs (figure 1). The most common motifs are the epidermal growth factor-like domains (EGF-like domains). There are 47 EGF-like domains, 44 of these are calcium-binding and 3 are non-calcium binding. Other repeated domains are the seven 8-cys domains (containing 8 cysteine residues) and the two hybrid domains. Most of these domains function as docking sites for other proteins, as mentioned above. The loss function of these binding site probably leads to Mafan syndrome symptoms, but no clear genotype-phenotype correlations have been described, except for the domains involved in Weill-Marchesany syndrome or ectopia lentis[22, 22].

Figure 1. Functional domains in fibrillin 1. cbEGF = calcium binding EGF-like domains. The signal peptide and propeptide are removed during transport and fibril formation. The domains are drawn to scale.

THE *FBN1* GENE

The *FBN1* gene is a very large gene, consisting of 65 coding exons and one non-coding 5'exon, that cover 237,482 nucleotides of genomic DNA and encode a messenger RNA of 8616 coding nucleotides, 395 5'UTR and 2684 nucleotides 3'UTR. The *FBN1* gene has a single large translated transcript (www.ensemble.org). We refer to transcript NM_000138.4 in this paper, when describing DNA variants on the cDNA.
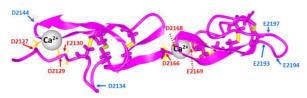
VARIANTS IN FBN1 AND THEIR INTERPRETATION

Since the publication of the first mutation in *FBN1*, pathogenic DNA variants have been found in all 65 coding exons of this gene, which reflects the structural and docking functions of fibrillin 1, that can be disturbed by changes almost anywhere in the protein. This is also apparent from the extremely high level of evolutionary sequence conservation of fibrillin 1 (Figure 2). In our diagnostic laboratory at Amsterdam University medical centers, 869 unique pathogenic DNA variants have been found in *FBN1*. Some 65% of

these mutations are missense mutations, that usually have a dominant effect, because the aberrant protein is incorporated in the extracellular matrix (ECM) and disturbs structure and function of the fibrils and interactions with other proteins.

disulfide bridges, that determine the three-dimensional structure of these domains (figure 3). Therefore, the missense mutations involving loss or gain of a cysteine in these domains are considered to be pathogenic. The same can be said for the 8-cys and hybrid domains.

Figure 2. Example of evolutionary sequence conservation of the fibrillin 1 protein, using single letter amino acid code, showing cbEGF domains #36 and #37. Differences are marked with grey background. The proposed cbEGF consensus sequence is shown at the bottom. The essential amino acid residues in the consensus sequence are boxed. The numbers at the top refer to amino acid residues in the protein sequence.

The international database lists 1847 unique variants in *FBN1* (http://www.umd.be/FBN1/)[24]. Mutations in *FBN1* range from single base substitutions to large genomic deletions or duplications[25,26,27,28]. Only 1.7% of the records in the database show large deletions or duplications ($\geq$ 1 exon), whereas In our laboratory, 12% of the pathogenic mutations are large deletions or duplications. This probably reflects the fact that our lab, as co-developers of the multiplex ligation dependent probe amplification (MLPA)[30] technique that is used to detect this type of mutations, has tested all patients. Deletion of the entire gene is a recurrent and not a founder mutation, because deletions of different lengths, ranging from 1 Mb to 10 Mb, have been found in different families. Only the largest deletions were associated with additional features in the phenotype, such as mental retardation[25,29].

Figure 3. Three dimensional representation of cbEGF-domains #36 and #37, created by iCn3D[31,32]. Disulfide bonds (yellow bars) and negatively charged amino acid residues are indicated. D = aspartic acid; E = glutamic acid. The N-terminal acidic residues of each domain are involved in calcium binding and are shown in red. Other negatively charged residues are shown in blue. These are pointed outward and are probably involved in interactions with other proteins.

The large number of missense mutations (65%) makes interpretation of variants difficult. In some cases the conclusions are not complicated. For example, in the EGF-like domains, the six cysteine residues play an important role in the formation of intramolecular three

Unfortunately, in the publication concerning the revised Ghent criteria (Dietz et al. 2010), the paragraphs about mutation interpretation contain several errors, which will lead to false assumptions about the pathogenic or benign effects of variants. For example, the consensus amino acid sequence motif of the EGF domains is incorrectly represented as: ((D/N)X(D/N)(E/Q)Xm(D/N)Xn(Y/F) with m and n representing variable numbers of residues; D aspartic acid, N asparagine, E glutamic acid, Q glutamine, Y tyrosine, F phenylalanine). This would mean that the first amino acid residue can be either aspartic acid (D) or asparagine (N) and the fourth residue can be either glutamic acid (E) or glutamine (Q), whereas in the calcium binding EGF-like domains, the first and fourth amino acids are always the negatively charged aspartic acid and glutamic acid. These residues are 100% conserved in all 44 calcium binding EGF domains of fibrillin 1. These negatively charged residues play an essential role in binding of the positively charged calcium ions, Ca^{2+}, that are necessary for stabilization of the protein, as has been shown by NMR analysis and 3d-imaging (figure 3)[31,32]. In Marfan patients the effect of mutations of the first aspartic acid of cbEGF domains has been shown by Hilhorst et al.[33] The position and relative distance of the cysteine residues is also an essential feature of the EGF domains. The consensus amino acid sequence of the calcium binding EGF domains should be represented as:

$$DX(D/N)ECX_{n(4-7)}CX_{n(3-6)}CXNXX(G/S)X(F/Y)XCXCX_{n(10-13)}CX,$$

where D is aspartic acid, N is asparagine, E is glutamic acid, C is cysteine, G is glycine, S is serine and X is any amino acid residue and n is a number in the indicated ranges. Any deviation from this consensus sequence should be considered pathogenic.

Another doubtful point in the publication of Loeys et al.[8] is this: "Other missense mutations: segregation

in family if possible + absence in 400 ethnically matched control chromosomes, if no family history: absence in 400 ethnically matched control chromosomes." The problem here is the threshold. For a dominantly inherited mutation it can be assumed that a carrier frequency in the population of >0.1% can be considered as evidence that the variant is not pathogenic. Probability calculation tells us that testing of 400 ethnically matched controls, a variant with a frequency of 0.1% can be found with a probability of $1-0.999^{400} = 0.33$, or 33% chance of finding a variant with a population frequency of 0.1% in 400 control samples. Moreover, the presence of undiagnosed Marfan syndrome patients in the control population cannot be excluded, so even if a variant is found once in the control samples, it is still not clear weather that is evidence of a non-pathogenic variant.

Furthermore, the most common recurrent pathogenic mutation, deletion of the entire *FBN1* gene[25], is not mentioned by Loeys et al.[8] nor are multi-exon deletions and duplications.

pathogenic nature of a variant, absence of the variant in the databases or in control samples cannot be used as conclusive evidence for pathogenic variants. On the other hand, presence of a variant at low frequency (<0.001) in the databases is not conclusive evidence of a benign variant.

INDONESIAN MARFAN CASES

When discussing Marfan syndrome with MD's in East Asian countries, I always hear that Marfan syndrome is extremely rare in East Asia. However, I am convinced that Marfan syndrome is very much underestimated in East Asia. As discussed before, in many Marfan families it is clear that within the families, not all carriers of the same pathogenic mutation have the clearly recognizable Marfan habitus with long limbs and long fingers[2,3,4]. However, they all have the high risk of aortic aneurysms and dissections, often leading to sudden death. The gnomAd (or ExAc) database[33] contains exome sequences of some 9,000 East Asian individuals without obvious disease.

Table 1. Frequencies of pathogenic (class 5) and likely pathogenic (class 4) variants in *FBN1* in East Asian DNA samples, according to the Exome Aggregation database[34]. MAF is minor allele frequency. A MAF of 0.0178% means a carrier frequency of 1 in 2800. (cDNA numbers according to transcript NM_000138.4)

Variant (DNA)	Variant (protein)	MAF East Asia %	Pathogenicity class
c.3128A>G	p.(Lys1043Arg)	0.0120	5
c.3172G>C	p.(Gly1058Arg)	0.0058	4
c.5546A>G	p.(Asp1849Gly)	0.0058	5
c.8123A>G	p.(Asn2708Ser)	0.0640	4
	Total	0.0876	Class 4+5
	subtotal	0.0178	Class 5

In the currently available exome databases, the population frequencies of variants can be easily checked in many populations. The frequencies in these databases are usually given as minor allele frequencies (MAF), which is not the carrier frequency of the variant, because every individual has two alleles, so the carrier frequency of rare variants is close to 2x the MAF.

In my experience, it is a very common error in the interpretation of mutations to think that it is necessary to look at the frequency in the same ethnic group as the patient, so it is assumed that the online databases cannot be used for patients from a population that is not represented. This is certainly not true for conclusions regarding the benign nature of variants that have a high frequency in ANY population. If, for example, a variant has a frequency of 5% in the Dutch population, this is sufficient evidence that the variant is NOT pathogenic, because we know that the frequency of Marfan syndrome in that population is nowhere near 5%. If, on the other hand, a new variant in a patient is not found in any of the exome databases, it can still be a common neutral variant in the ethnic group to which the patient belongs, or it can be a rare benign variant. So, the reasoning presented by Dietz et al. when using 400 control samples can only be used for a negative conclusion: if a variant is found in the control group with a frequency of >1% this is conclusive evidence of a benign variant. Without additional evidence of

Checking this database for variants in *FBN1,* shows that the carrier frequency of known pathogenic mutations in the East Asian population is 0.000356, equaling 1 in 2800 individuals. This is a low estimate, because we only looked at variants that have been listed in CLINVAR as pathogenic, so pathogenic mutations that are specific for the East Asian population cannot be seen. The occurrence of 1 in 2800 is very close to the known incidence in other populations and would mean a total of around 100,000 patients in Indonesia. My point here is, that we don't see the patients in East Asian countries such as Indonesia, because no one is looking.

In Semarang five families with clinical features of Marfan syndrome have been tested and 3 pathogenic variants were found, 2 of which were novel (publication in preparation). This shows that 1) Marfan syndrome is found in Indonesia and that 2) unique mutations are indeed present. This supports my thesis that 1 in 2800 is a low estimate.

GENOTYPE PHENOTYPE CORRELATIONS

Despite the large phenotypic variation within families among carriers of the same mutation, some genotype – phenotype correlations have been found. Neonatal Marfan syndrome, a very severe form which is apparent at birth, is often caused by single exon deletions or exon skipping mutations, or missense mutations in the central part of the protein (encoded by

exons 24-36)[35,36]. Nearly all exons of *FBN1* consist of a multiple of 3 bases, so exon skipping or deletion do not lead to frameshift and nonsense mediated decay (NMD) of the mutant mRNA. This has probably played an important role during evolution, when the gene acquired a large number of almost identical functional domains, that necessitated a continuous reading frame. Truncating mutations and exon skipping or deletion outside exon 24-36 lead to a more severe phenotype than other mutations, probably because the short protein interferes with correct fibril formation[37]. Grouping patients based on effect of the *FBN1* mutations on the protein resulted in improved genotype-phenotype correlation[38,39,40,41], with a striking effect on the response to medication[42], as discussed below. One group was defined as "haploinsufficiency" (HN), with reduced production of normal fibrillin 1 due to deletion of the gene or null allele caused by nonsense mediated decay. The other group was defined as "dominant negative" (DN), which indicates that the production of mutant protein leads to a negative effect on the extracellular matrix.

MEDICATION

The life-threatening symptoms of Marfan syndrome are the aortic root dilatation and thoracic aortic aneurysms and dissections, which may lead to sudden death. Therefore, prevention of aortic root dilatation and aneurysm growth is the target of developing drug therapy.

Habashi et al.[43] have shown in a Marfan rat model, that the angiotensin 1 receptor antagonist losartan has a reducing effect on the growth of aortic diameter. This effect was stronger than blood pressure reduction by beta-blockers. It was concluded that losartan has a side-effect on the TGF-β receptors. The involvement of TGF-β was based on the finding of increased nuclear phospho-smad2 in a (one!) patient with Marfan syndrome by Loeys et al.[44], which led to the assumption that increased TGF-β activity is the cause of aortic aneurysms in Marfan syndrome and familial thoracic aortic aneurysms and dissections TAAD). This has led to the so-called TGF-β paradox in aortic aneurysms. However, in my opinion, the paradox is the result of an extremely simplified interpretation of the findings. Smad2 is a signal molecule that can be phosphorylated by at least 4 different receptors in response to different ligands, such as angiotensin II, myostatin, TGF-β1, -2 and -3 and activin-A[45]. So increased psmad2 is not evidence of increased TGF-β activity, as has been suggested[46]. A more logical candidate for the increased p-smad2 and for the effect of losartan is angiotensin II, through the effect of the drug on angiotensin II receptor type 1[42,47,48]. The aggravating effect that has been found for inflammation in Marfan syndrome may be a result of local production of activin-A by mast cells and macrophages[49].

Clinical trials with losartan have shown variable results. Studies in the Netherlands[50] and the USA[51] showed a significant reduction in growth of aortic diameter in Marfan patients that used losartan, compared to patients that used beta blockers, whereas this was not confirmed by studies in France[52] and Spain[53]. A meta analysis of clinical trials with losartan, comprising 1398 subjects, revealed no significant effect of losartan[54].

We re-analyzed the data of the Netherlands study after grouping the patients based on effect of the *FBN1* mutations on the protein[42]. The groups were defined as described above The HN group comprised 35% of the patients and the DN group 65%.

Interestingly, a significant effect of losartan was found only in the HN group (p<0.001). This is in contrast with the idea that TGF-β is responsible for the growth of aortic diameter in Marfan patients. It has been assumed that TGF-β is easily released from mutant fibrillin 1, causing activation of the TGF-β receptors and smad2 phosphorylation, which supposedly could be inhibited by a side effect of losartan. If that were the case, losartan would be only effective in the DN group, whereas we demonstrated the opposite. Our study so far has not been confirmed by others, probably because in the other clinical trials no complete mutation analysis of all patients had been performed and no fibroblasts were available for RNA studies that are necessary to prove NMD. The discrepancy in the results of the clinical trials might be a result of distribution of DN and HN mutations. A relatively small number of HN mutations in the population would lead to a lack of statistical significance in the overall results.

We concluded from the finding that losartan is only effective in patients with HN mutations, that a more likely explanation for the effect of losartan is through angiotensin II[42]. HN mutations lead to a reduced production of fibrillin 1, which will cause a lower ratio of fibrillin to elastin and increased extensibility of the aorta. This will cause low blood pressure, which will induce activation of angiotensin II. This will cause phosphorylation of smad2 and-3 and changes in gene regulation, affecting the balance between proliferation and differentiation of smooth muscle cells, which is ultimately the cause of growth of the aortic diameter. Losartan, in this scenario, exerts an effect by inhibiting the angiotensin II receptor type 1, as it is supposed to do.

We have, as yet, no working hypothesis that explains the growth of aortic diameter in patients with a DN mutation.

CONCLUSIONS

Marfan syndrome is an under-diagnosed condition in East Asia. The awareness of this condition is very low, even among health care professionals. Mutation testing of *FBN1* is important for 1) Genetic counseling (recurrence risk; options for prevention). 2) Pre-symptomatic diagnosis in families to assess individuals at risk. 3) Medication: the effect of losartan probably depends on the type of causative mutation in *FBN1* (HN or DN). Therefore, mutation testing may be important in choice of therapy.

Further studies are needed to elucidate the effect of losartan, especially in relation to the type of mutation. The interpretation of data from losartan research is obscured by the so-called TGF-β paradox in aortic

aneurysms. This paradox is the result of oversimplified interpretation of observations concerning smad2/3 phosphorylation. There is no need for a paradox if the data are interpreted correctly.

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