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Minireview

Elucidation of the gene defect in Marfan syndrome

Success by two complementary research strategies

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Marfan syndrome, which is characterized by manifestations in the skeletal, ocular and cardiovascular systems, is one of the most common inherited connective-tissue disorders. The independently performed genetic assignment of the Marfan locus and classical biochemical and immunohistochemical analyses complemented each other in the search for the Marfan gene defect and in 1991 the fibrillin gene in chromosome 15 was identified as the Marfan gene. So far, three mutations leading to the Marfan phenotype have been reported in this gene coding for a microfibrillar protein. The available data suggests a wide spectrum of different mutations of fibrillin and although mutations of the fibrillin gene account for the majority of Marfan cases, evidence also exists for locus heterogeneity in a minority of Marfan cases.

Marfan syndrome; Fibrillin; FBN1; Chromosome 15

1. INTRODUCTION

Marfan syndrome is an autosomal dominant connective-tissue disorder characterized by skeletal, cardiovascular and ocular manifestations [1]. Many of the features of the syndrome occur with variable frequencies in the general population, but when combined, constitute the systemic disorder called Marfan syndrome. Although Marfan syndrome was first observed almost 100 years ago, it was not until in the 1950's when this syndrome was identified as an inherited connective-tissue disease and precisely clinically described [2]. Two major symptoms, weakness of the wall of the aorta and dislocation of the lens were so characteristic for the syndrome that Dr McKusick wrote: 'What the suspensory ligament of the lens has in common with the media of the aorta is obscure. If known, the basic defect of syndrome might be understood' [3]. This prediction turned out to be wise and foreseeing. The independently performed genetic assignment of the Marfan locus and classical biochemical and immunohistochemical analyses of patients' fibroblasts ideally complemented each other in the search for the defective gene in Marfan syndrome and resulted in the identification of fibrillin gene as this Marfan gene (Fig. 1) [4]. A new era started for both basic scientists and clinicians interested in Marfan syndrome: specific mutations can be identified in patients, diagnostic tests can be offered for family members at the presymptomatic age and the question of genetic heterogeneity of this phenotypically highly variable syndrome can finally be answered.

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2. PHENOTYPIC FEATURES OF MARFAN SYNDROME

Marfan syndrome occurs with an estimated prevalence of 1:10000, without racial or ethnic predilection [1]. 15–30% of the patients are sporadic and thus represent new mutations [1,5]. The penetrance is considered complete, although the level of expression of the Marfan gene varies widely.

Skeletal symptoms of Marfan syndrome include increased height, disproportionally long limbs and digits and anterior chest deformity, and the typical ocular findings include myopia and subluxation of the lenses. The most serious manifestations of the disorder occur in the cardiovascular system: mitral valve prolapse and dilatation of the aortic root which may progress to aortic dissection, the cause of precocious mortality of Marfan patients [1].

3. BIOCHEMICAL STUDIES ON MARFAN SYNDROME

The fundamental defect underlying Marfan syndrome was under intensive investigation in many laboratories over the past decennia but the search proved frustratingly difficult. To explain the weakness of the

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aortic wall of the patients, the investigators studied different types of collagens, proteoglycans, elastin and elastin-associated proteins. This approach was justified because the early histological studies had demonstrated degeneration of elastic lamellae as well as an increase in collagenous tissue in samples of aortic tissue from Marfan syndrome patients [2].

3.1. *Findings on collagens*

There was much indirect evidence supporting the idea of defects in the collagen genes of Marfan patients [6]. The data included decreased amounts of type I collagen in aortic tissue, increased hydroxyproline content of the urine of Marfan patients and the increased rate of collagen synthesis by their fibroblasts suggesting an increased rate of collagen turnover in Marfan syndrome. This enhanced rate of turnover was supported by reports on a deficiency in chemically stable mature cross-links of collagen in Marfan patients.

A specific finding in type I collagen was demonstrated in one patient with somewhat atypical features of the Marfan syndrome. In her skin and aorta, together with normally migrating proα2(I)collagen chains, a population of chains with an increased apparent molecular weight was observed on SDS–PAGE [7,8]. Later, a heterozygote single base mutation converting a highly conserved Arg^{618} to Glu (R618Q) in the triple helical domain of proα2(I)collagen was established [9]. The same mutation could not be seen in the DNA of 52 control individuals or 17 other, non-related Marfan individuals. This would suggest that the nucleotide change was not a common polymorphism, but neither would it be the general cause of Marfan syndrome.

3.2. *Findings on elastin and proteoglycans*

Analogous to the search for collagen defects in Marfan syndrome, studies were carried out to search for abnormalities in other connective tissue components [6]. In two studies, exceptionally low amounts of desmosine and isodesmosine, the cross-linking amino acids of elastin, were observed in aortic samples of Marfan patients. Observations on connective tissue proteoglycans included increased biosynthesis of hyaluronic acid by Marfan fibroblasts and a drastically reduced steady state level of decorin mRNA in about one third of Marfan fibroblast cultures studied [6,10].

All these results on collagens, elastin or proteoglycans were once thought to explain the basic defect underlying Marfan syndrome but they all represented findings either secondary to the basic defect or they were found in only a few Marfan individuals and provided no explanation for the molecular defect in the majority of patients.

3.3. *Findings on a microfibrillar protein, fibrillin*

In 1986 a new extracellular matrix glycoprotein, fibrillin had been identified and characterized [11]. Its

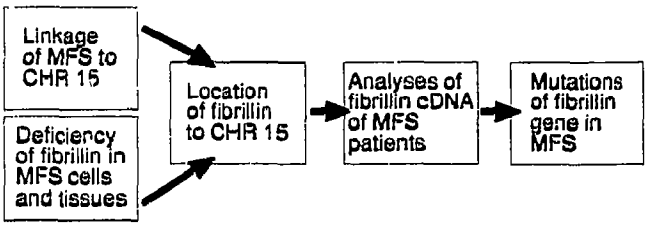

Fig. 1. The pathway taken in the search for the defect underlying Marfan syndrome. (MFS, Marfan syndrome; CHR, chromosome)

tissue distribution, including aorta and suspensory ligament of the lens, and location in elastic tissue made it an excellent candidate as the defective agent in Marfan syndrome. By using indirect immunofluorescence and several monoclonal antibodies against the fibrillin polypeptide, a deficiency of fibrillin fibers in skin sections and fibroblast cultures of Marfan patients was demonstrated [12]. Furthermore, the deficient immunofluorescence pattern of fibrillin co-segregated with the disease in nine Marfan families. In one exceptional patient with clinical findings of Marfan syndrome predominantly manifest on the left side of the body, a striking difference in the fibrillin immunofluorescence was observed both in skin sections and in corresponding fibroblast cultures derived from the left (decrease of fibrillin) and right sides (normal fibrillin) of the body [13].

To verify these results and to test the consistency of the fibrillin deficiency in Marfan syndrome, fibroblasts and skin sections of Marfan individuals, patients with other connective tissue diseases as well as controls were analyzed with indirect immunofluorescence analyses using monoclonal antibodies against fibrillin [14]. In this study, 24 out of 27 Marfan syndrome patients were correctly identified as having the syndrome. Of the three misclassified patients, two were studied only by skin immunostaining, an assay which shows fibrillin deficiency in only about 75% of Marfan patients. Interestingly, fibrillin immunofluorescence analysis of both the skin sections and fibroblasts of the third misclassified patient, with typical features of the syndrome, revealed normal fibrillin immunofluorescence staining. However, her fibroblasts produced two species of proα2(I)collagen chains: in addition to the normal chains, there were also abnormal, slowly migrating chains quite similar to those reported earlier [7,8]. All 13 normal individuals as well as 19 out of 25 patients with other connective tissue disorders were correctly classified as 'non-Marfan' in this single blind study.

To conclude, deficiency in the fibrillin immunofluorescence pattern was almost a constant finding when cultured fibroblasts and/or skin sections of Marfan patients were studied. From all the reported biochemical and immunohistochemical findings reported through the decennia, these were the most promising and strongly suggested that deficient microfibrillar fibers

were intimately coupled to the basic defect causing Marfan syndrome.

4. THE LINKAGE APPROACH

While some groups studied fibrillin in relation to Marfan syndrome, other investigators seized the possibilities of finding the Marfan locus using linkage analyses and polymorphic markers localized either in or in the vicinity of candidate genes or randomly in the human genome. The latter markers are utilized in the approach called positional cloning, the starting point of which is not a known protein and the search is not focused on defects in this protein or the gene coding for the corresponding polypeptide chain. Instead, DNA of patients and their family members is analyzed directly and the goal is to first find the chromosomal location of the disease gene. Once the locus is established and the defective gene in this region defined, the genetic code is used to decipher the normal and mutated polypeptide chain encoded by the gene.

4.1. *Linkage studies tc candidate genes*

In the case of Marfan syndrome the linkage approach was initiated by studying the inheritance pattern of candidate genes in Marfan pedigrees. Since several biochemical studies had indicated defects in one of the fibril-forming collagens, these were the first to be studied. However, several linkage studies demonstrated recombinations between Marfan syndrome and polymorphic markers in or close to both genes coding for type I procollagen chains, the gene coding for type III procollagen and, not surprisingly, the gene coding for cartilage-specific type II procollagen [15–18]. In other words, a defect in any of these genes was in practice excluded as the primary cause of the disorder.

4.2. *Random linkage approach*

The first trial towards positional cloning of the Marfan gene was an exclusion map of chromosome 2. Altogether 50 cM of the long arm of chromosome 2, including several genes coding for extracellular matrix components, was excluded as the site of the Marfan mutation [19].

In the next phase, an international collaborative study was formed to combine linkage data from nine laboratories in five countries and to produce an exclusion map of Marfan syndrome [20]. Individual laboratories had independently screened different sets of markers including both candidate loci and random polymorphisms in their own Marfan pedigrees. The linkage data combined information from 75 loci on 18 autosomes and excluded 75% of the genome as likely locations of the Marfan gene. This map suggested that the Marfan gene is located on chromosome 5, 6q, 8, 9p, 10p, 13, 15, 17p, 20p, 21, or 22.

Encouraged by this study, we began to study Finnish families for genetic linkage to random polymorphic markers on several chromosomes and in 1990 reported the linkage of Marfan syndrome to three markers on chromosome 15 in five Finnish families [21]. The linkage of Marfan syndrome to this chromosomal area was soon confirmed in American and British families suggesting that the original finding was not specific for the genetically isolated Finnish population [22,23].

The established linkage to chromosome 15 made it possible for the first time to use statistical genetic heterogeneity analyses in an international collection of Marfan families. No evidence for locus heterogeneity was found underlying the disease when classical Marfan families from England, Finland, Scotland, Switzerland and the United States were studied [24]. The studied families all had the classical type of Marfan syndrome but the material also included families with only mild ocular findings as well as families with ocular findings as the major symptom. Other groups have recently published similar data on another collection of Marfan families [25,26]. To date, the only Marfan family that is reported not to be linked to the Marfan region in chromosome 15, is a large French pedigree, in which all affected individuals fulfill the criteria set for Marfan syndrome, but do not have subluxation of the lenses [25].

5. MARFAN MUTATIONS

5.1. *Fibrillin mutations and their consequences*

The relationship between the Marfan syndrome locus on chromosome 15 and the most specific biochemical finding so far available, the fibrillin deficiency [14] was soon elucidated: after partial cloning of the fibrillin cDNA, the gene coding for fibrillin (FBN1) was, by in situ hybridization, localized to the long arm of chromosome 15 at band 21.1 [27,28]. Moreover, a gene on chromosome 5 (FBN2) demonstrating a high degree of homology to fibrillin on chromosome 15 (FBN1) was linked to the Marfan syndrome-related disorder, congenital contractural arachnodactyly in two families, this providing further support for the importance of fibrillin in the pathogenesis of Marfan syndrome [27]. Soon after that the first mutation in the FBN1 gene in a Marfan patient was reported: a missense point mutation substituting Pro for Arg^{239} (R239P) found in two unrelated severely affected 17- and 19-year-old sporadic Marfan patients [4]. The fibrillin cDNA sequence had revealed that the corresponding polypeptide chain contains at least 34 consecutive six-cysteine repeats homologous to those found in the EGF-precursor molecule and suggested to stabilize the fibrillin molecules [29]. The R239P mutation occurred in a conserved amino acid within such a six-cysteine motif suggesting that the mutation occurred at a functionally significant site of the polypeptide chain.

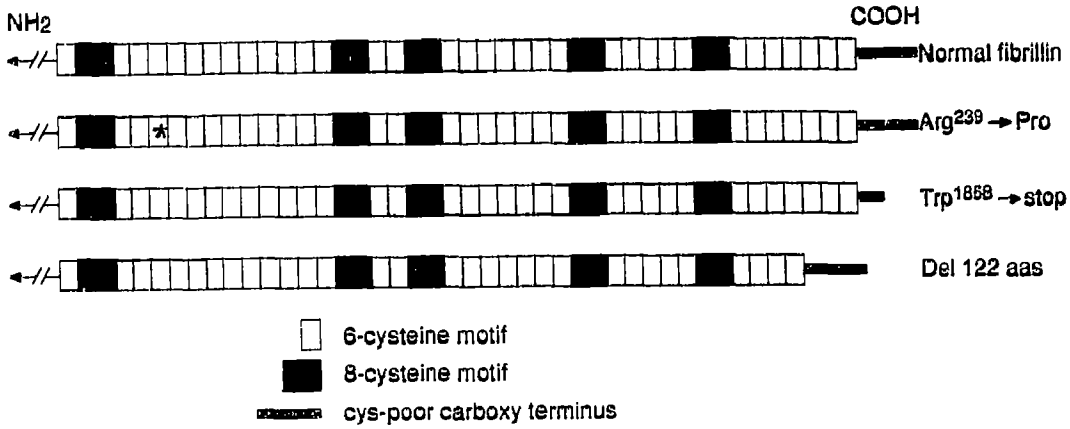

Fig. 2. A schematic representation of the normal fibrillin polypeptide chain as well as the predicted polypeptide chains caused by the three reported mutations [4,30]. Two types of repetitive motifs have been identified in the fibrillin sequence, a 6-cysteine motif homologous to that found in the EGF-precursor molecule and an 8-cysteine motif homologous to a motif seen in TGF-β1 molecules [29]. (The asterisk (*) indicates a G-to-C point mutation, Arg, arginine; Pro, proline; Trp, tryptophan; Del, deletion; aas, amino acids; Cys, cysteine). Modified form Maslen et al. [29].

To date, two other mutations of FBN1 gene have been reported in Marfan patients. We found a 366 bp deletion of fibrillin mRNA predicting a polypeptide chain 122 amino acids shorter than normal in a 48-year-old British male [30]. This mutation was shown to co-segregate with the disease in his family in which Marfan syndrome had been diagnosed in three generations. The deleted area contained three complete six-cysteine repeat motifs (see above). The consequences of a mutation in fibrillin gene were demonstrated for the first time also at the polypeptide level. The fibroblasts of this patient were shown to secrete two species of fibrillin polypeptide chains: in addition to a polypeptide chain population of normal size, there were also chains of about 15 kDa shorter, corresponding to the mutated polypeptide population.

The third detected mutation was a heterozygotic G-to-A transition at nucleotide 5574 found in the cDNA of a 55-year-old sporadic Finnish Marfan patient [30]. The consequence of this mutation was the substitution of a stop codon for Trp^{1858} (W1858) predicting the termination of the fibrillin polypeptide chain 116 amino acids too early. Unlike the protein analyses of the fibroblasts of the patient with the deletion, immunoprecipitation analyses of the medium of this patient's fibroblasts using fibrillin-specific antibodies failed to detect a shortened fibrillin polypeptide chain.

Fig. 2 shows a schematic illustration of the normal fibrillin polypeptide chains as well as the polypeptide chains predicted to result from the three mutations reported [4,30]. The true biological consequences of these mutations can only be speculated. Like most other Marfan cell lines, immunofluorescence of fibroblasts from these patients displayed reduced amounts of fibrillin fibers. Possible explanations include the reduced synthesis and fibrillogenesis of fibrillin due to a mutation in one allele or an amplified deleterious effect on fibrillogenesis due to the secretion and co-polymerization of the mutant gene product with normal fibrillin. The latter mechanism is called protein suicide and occurs in certain type I procollagen mutations resulting in osteogenesis imperfecta: the presence of abnormal proαchains interferes with normal triple helical assembly and results in the degradation of not half but three quarters of the synthesized molecules [6].

To approach the question of genetic heterogeneity of Marfan mutations, we screened DNA samples of 61 unrelated Marfan patients representing both sporadic and familial cases from Belgium, Finland, The Netherlands, Sweden, Switzerland, the United Kingdom and the United States for all three reported mutations but did not identify any of these mutations in any other patients [30]. Consequently, most Marfan mutations will probably be specific for each family excluding the possibility of a generally applicable diagnostic DNA test.

5.2. *Heterogeneity of Marfan mutations and DNA diagnostics*

The combination of biochemical and molecular genetic approaches resulted in the elucidation of the molecular defect underlying the Marfan syndrome. FBN1 and FBN2 together with a third homologous gene, fibrillin in chromosome 17 (FBN3) constitute a possibly expanding gene family [31]. It is now known that congenital contractural arachnodactyly is most probably caused by mutations in FBN2 and a definitive majority of Marfan syndrome cases are caused by mutations in FBN1. There are, however, some exceptions to this. A mutation in the proα2(I)collagen gene causes Marfan syndrome in a patient with somewhat atypical Marfan syndrome patient [9]. A finding suggesting a similar defect in proα2(I)collagen gene has been reported also in another case, a patient 'misdiagnosized' with fibrillin

immunofluorescence analysis (see above) [12]. Further, in one family in which Marfan syndrome and polycystic kidney disease are co-segregating, both disorders have been linked to polymorphic markers flanking the polycystic kidney disease locus in chromosome 16 [32]. Linkage studies have also indicated that a large French pedigree demonstrates an exclusion of linkage to chromosome 15 markers [25]. Whether these Marfan cases are caused by mutations in fibrillin genes other than FBN1 or FBN2, or whether they are caused by defects in genes coding for some other extracellular matrix proteins, will be elucidated in the future.

All existing data on the number of sporadic cases in Marfan syndrome, the degree of clinical variability in Marfan syndrome and screening of the known mutations from other patients' samples predict a spectrum of different mutations in Marfan syndrome. The current picture of Marfan syndrome is thus a variety of mutations in FBN1 with the still existing possibility of genetic heterogeneity. This does not stimulate high hopes for generally applicable DNA diagnostics for this disease. In sporadic Marfan cases, the immunofluorescence analyses detecting fibrillin fibers in fibroblast cultures is perhaps currently the most reliable diagnostic laboratory test. In the majority of Marfan families intragenic polymorphic markers of FBN1 can be used for presymptomatic diagnostics of young family members, although with caution, due to the exceptions listed above. Identification of fibrillin mutations in Marfan patients has just started and already in near future we will learn if Marfan mutations will be enriched to some regions of the large fibrillin gene, a phenomenon which would create possibilities to develop more widely applicable diagnostic test.

Identification of Marfan mutations and careful reporting of the corresponding clinical phenotypes will in the foreseeable future help us to understand the important genotype–phenotype relationships. This understanding is especially interesting in the case of Marfan syndrome. It is not only one of the most common inherited connective-tissue disorders, but several features of the phenotype are common among the general population. Consequently, the detailed knowledge of the molecular biology of Marfan syndrome and related disorders will without doubt provide new insights also into the various forms of more common connective tissue disorders, for example aortic aneurysms, myopia and mitral valve prolapse.

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NOTE ADDED IN PROOF

A fourth Marfan mutation was described during submission of this paper. It is a G-to-C point mutation substituting serine for cysteine at codon 1409 of the fibrillin gene 33.