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Fibulin-2 (FBLN2): Genetic mapping and exclusion as a candidate gene in Marfan syndrome type 2.

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Abstract

Fibulin-2 is a new extracellular matrix protein that has been considered a candidate gene for Marfan syndrome type 2 (locus MFS2) based on chromosomal colocation at 3p24.2-p25 and disease phenotype. In the absence of polymorphic markers reported for FBLN2, direct sequencing of the gene was performed and two intragenic polymorphisms were identified. Linkage was excluded between FBLN2 and the MFS2 gene. Furthermore, two point lod scores were generated between these markers and anonymous markers arrayed on the genetic map of 3p and closely linked to MFS2. These analyses placed FBLN2 at marker D3S1585.

Introduction

Marfan syndrome (MFS) is an autosomal dominant connective-tissue disorder. It involves predominantly three systems (skeletal, ocular and cardiovascular systems) and is characterized by highly variable expressivity [1]. By excluding the disease locus fibrillin-1 (FBN1) in a large French family with typical cardiovascular and skeletal anomalies of Marfan syndrome, we demonstrated genetic heterogeneity in MFS and the implication of a second locus MFS2. Linkage analyses have localized MFS2 to a region between markers D3S1293 and D3S2335 at 3 p24.2-p25 [2, 3].

Fibulin-2 (FBLN2) is a multidomain, extracellular matrix protein whose function is still unknown. It contains 10 calcium binding EGF-like motifs [4] which are also observed in fibrillin-1 (FBN1) that carries the mutations accountable for Marfan syndrome type I. Furthermore expression studies of fibulin-2 mRNA in mouse tissues have shown strong hybridization signals in the heart [4, 5, 6]. Finally, FBLN2 has been localized by *in situ* hybridization at 3p24-p25 [5]. Therefore, FBLN2 represented an excellent functional and positional candidate gene for MFS2.

Protein analyses

Indirect immunofluorescence and immunoblots were performed using affinity-purified polyclonal antibodies to fibulin-2 [4] on the networks deposited by fibroblasts from various affected family members. No significant difference was observed between patient and normal cells (figure 1). Immunoblots of cell layer extracts demonstrated in both samples the same

disulfide-bonded fibulin-2 trimers and, as mesured by radioimmuno assay, a similar content of fibulin-2 in the range 2-4 µg/mg cellular protein. This was in agreement with expression studies: Northern analysis of total RNA from cultured fibroblasts identified a unique 4.1 kb band in affected and unaffected members as well as in a control, with no significant difference in intensities. These negative results did not exclude the possible existence in affected individuals of a fibulin-2 protein with abnormal structure or function.

Genetic approach

We sequenced the gene FBLN2 and identified a polymorphic site at nucleotide 2629 of G or A (alanine 827 to threonine) [5] revealing a new *AvaII* restriction site. This new marker was tested by amplification with BUL8A : 5' AAC ACG GTG GGC TCC TAC 3' (from nucleotide 2554 to 2571), and BUL8M: 5' GGT TGT GGC ACA CTT GGC 3' (from nucleotide 2701 to 2684) under the following conditions: 94°C for 5 min, 30 cycles at 94°C for 30 sec, 53°C for 30 sec, 72°C for 40 sec followed by 72°C for 5 min. PCR products were digested with *AvaII* that cleaves the 270 bp fragment (A1, frequency of 0.70) into 195 and 75 bp (A2, frequency of 0.30). A highly informative dinucleotide repeat polymorphism was also identified in the gene and the following primers were designed for its study: F9 : 5' GTAGGTGCTCATTGGACAGTTG 3' and B9: 5' CCAAGGCTTCTGAAG GACTTGA 3' (the full description of the polymorphism will be described elsewhere). Cycling conditions were 94°C for 5 min, 30 cycles at 94°C for 30 sec, 60°C for 30 sec, 72°C for 40 sec followed by 72°C for 5 min. PCR products were then analysed as described previously [3]. Two-marker haplotype analysis identified three recombinants among clearly affected (III4) and unaffected (III44 and IV55) family members. Furthermore, 3 possible recombinants were also identified in the group of subjects having an ambiguous phenotypic status [2, 7] and carrying the unrecombined 3p marker haplotype linked to MFS2 (III13, III30 and IV53) (figure 2). These results clearly excluded linkage between MFS2 and the fibulin-2 gene. Furthermore, multipoint lod scores computed with various regional microsatellite markers (unpublished data), placed FBLN2 at marker D3S1585 (maximum location score: 63.80 (lod score: 13.86) at θ=0.0).

Conclusion

In conclusion, our results show that FBLN2 is not identical to MFS2 and place the candidate gene on the genetic map of 3p, distal to the 7 cM critical region in which MFS2 is located at present.

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Figure 1: Immunofluorescence demonstration of extracellular fibulin-2 networks in confluent fibroblast cultures. A: patient, B: unaffected control.

Figure 2: Segregation of fibulin-2 markers in the Marfan kindred.

(Note that panel A and panel B represent different parts of a single pedigree; i.e., panel B is the rightward extension of panel A). Haplotypes at "AvaII" and "(CA)n" markers are shown for each family member tested. Blackened symbols denote affected members, unblackened symbols denote unaffected spouses or unexamined family members, unblackened symbols with a dot in the center denote members considered unaffected, and hatched symbols denote members having an ambiguous phenotypic status and carrying the unrecombined 3p marker haplotype linked to MFS2. A slash denotes that the family member is deceased. An asterisk indicates the obligate and possible recombinants. As compared with previous reports, blood samples were collected for 10 new family members (III54, IV29, IV41, IV90, IV91, V1, V2, V3, V4, and V5) and 4 members for which DNA samples were depleted (II10, II13, IV32, and IV53).