In frame fibrillin-1 gene deletion in autosomal dominant Weill-Marchesani syndrome

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Weill-Marchesani syndrome (WMS) is a rare connective tissue disorder first described by Weill in 1932, and further delineated by Marchesani. The patients present with short stature, brachydactyly, joint stiffness, and characteristic eye anomalies including microspherophakia, ectopia of the lenses, severe myopia, and glaucoma. Both autosomal recessive (AR) and autosomal dominant (AD) modes of inheritance have been described and a gene for AR WMS has recently been mapped to chromosome 19p13.3-p13.2. Here, we report on the exclusion of chromosome 19p13.3-p13.2 in a large AD WMS family and show that, despite clinical homogeneity, AD and AR WMS are genetically heterogeneous entities. Because two AD WMS families were consistent with linkage to chromosome 15q21.1, the fibrillin-1 gene was sequenced and a 24 nt in frame deletion in exon 41, 5074_5097del. This in frame deletion cosegregated with the disease and was not found in 186 controls. This study strongly suggests that AD WMS and Marfan syndrome are allelic conditions at the fibrillin-1 locus and adds to the remarkable clinical heterogeneity of type I fibrillinopathies.

Patients and Methods

The members of the two AD WMS families reported by Wirtz et al. were included in this study. Family 1 has previously been reported by Gorlin et al. in 1974. Affected subjects, aged 2 to 60 years, fulfilled the inclusion criteria, namely short stature, brachydactyly, limitation of joint movements, microspherophakia and/or dislocated lenses, severe myopia, and glaucoma. In addition, two patients had carpal tunnel syndrome. Immunohistochemical staining of skin sections performed in family 1 showed an apparent decrease in fibrillin staining.

DNA extraction and microsatellite analyses were performed as previously described and primers were chosen from the Genethon map with an average spacing of 3 cM. Results were analysed by manual haplotype analysis under the assumption of autosomal dominant inheritance, with full penetrance and a 0 value for the phenocopy rate. Screening for fibrillin-1 gene mutations was performed on leucocyte DNA by direct sequencing both strands of PCR products. Exons 1 to 65 of the fibrillin-1 gene and their flanking intronic sequences were amplified as previously described. One affected subject in each family was chosen for sequencing and other affected subjects were sequenced when necessary.

Results

Fig 1 shows that family 1 was consistent with linkage to both chromosomes 19p13.2-p13.3 and 15q21.1, but linkage of the disease gene to chromosome 19p13.2-p13.3 was clearly excluded in family 2, as the six affected subjects did not share a common haplotype in this region.

Sequence analysis of the fibrillin-1 gene in family 1 showed heterozygosity for a 24 nucleotide in frame deletion in exon 41 (5074_5097del, fig 1). Eight amino acids were deleted (R-S-L-C-Y-R-N-Y). This deletion was confirmed on both strands and was also detectable when amplification products were separated on 1% agarose, 3% NuSieve gels and stained with ethidium bromide. The deletion was found in all affected subjects and cosegregated with the disease in family 1 (fig 1).

Discussion

Here, we report on a 24 nt in frame deletion of the fibrillin-1 gene in a large AD WMS family. The deleterious nature of this deletion is very likely for several reasons. This deletion cosegregates with the disease and has never been found in previous publications or controls in this study. This deletion involves one of the seven latent transforming growth factor-β1 binding protein (LTBP) motifs in a conserved region of the protein. Each motif contains eight cysteine residues and interrupts multiple stretches of calcium binding epidermal growth factor-like (cbEGF) modules. LTBP domains normally display a globular structure and comprise six antiparallel beta strands.

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and two alpha helices. The structure is stabilised by four disulphide bonds which pair the eight cysteine residues in a 1-3, 2-6, 4-7, 5-8 pattern. One of these eight cysteine residues is included in the fibrillin-1 gene deletion in family 1. Hence, heterozygosity for this partial deletion of the LTBP domain is expected to result in unstable mutant protein cleavage products that should interfere with microfibrillar assembly. Indeed, previous immunohistochemical analyses of skin sections in family 1 showed an apparent decrease in fibrillin staining when compared to controls. Why no fibrillin-1 gene mutation has been found so far in our second AD WMS family is questionable. This feature may be related to the low rate of mutation detection in another type I fibrillinopathy, Marfan syndrome (10-78%).

The fibrillin-1 gene has been previously shown to account for various conditions, including Marfan syndrome, neonatal Marfan syndrome, Shprintzen-Goldberg syndrome, familial arachnodactyly, ectopia lentis, isolated ascending aortic aneurysm and dissection, mitral valve prolapse, aortic root dilatation without dissection, skeletal and skin abnormalities (MASS phenotype), and Marfan-like syndromes. Our observation of a fibrillin-1 deletion in AD WMS adds to the striking clinical heterogeneity of type I fibrillinopathies.

It is interesting to note that mutational “hot spots” in exons 24-27 and 31-32 have been reported in neonatal Marfan syndrome and that exon skipping mutations apparently caused more severe phenotypes than mutations in the 3′ region of the gene. So far, however, the clinical heterogeneity of type I fibrillinopathies has not been accounted for by any sound genotype-phenotype correlations. Indeed, a 7 nt deletion overlapping the deleted region of our AD WMS family led to premature protein termination in a patient with Marfan syndrome. Moreover, patients with typical Marfan syndrome occasionally harboured mutations in a domain of the gene which is preferentially involved in neonatal Marfan syndrome and mutations altering the same residue in two distinct modules were found to be responsible for either autosomal dominant ectopia lentis or neonatal Marfan syndrome. Similarly, the same fibrillin-1 missense mutation (C1223Y) has been found in typical Marfan syndrome and in one patient with Shprintzen-Goldberg syndrome, although another missense mutation in a second patient was shown to be a polymorphism in subsequent papers.

This interfamilial clinical heterogeneity at the fibrillin-1 locus is even reinforced by a striking intrafamilial variability, as two patients with Marfan syndrome had children suffering from WMS. In light of this remarkable inter- and intrafamilial variability of fibrillin-1 mutations, a possible role of environmental, stochastic, or epigenetic factors has been considered but not yet demonstrated.

Mice carrying the Tight skin (Tsk) mutation are interesting regarding WMS; they have thickened skin resulting from an accumulation of extracellular matrix molecules. Other features include visceral fibrosis, increased bone and cartilage growth, lung emphysema, myocardial hypertrophy, and the presence of small tendons with tendon sheath hyperplasia. Molecular studies of the fibrillin-1 gene showed the presence of a 30 to 40 kb genomic duplication within the fibrillin-1 gene that results in a larger than normal in frame fibrillin-1 transcript. The presence of tight skin in both the Tsk mouse and WMS is particularly interesting and appears to be a distinctive feature not observed in other human conditions involving fibrillin-1 mutations.

In conclusion, we show here that despite clinical homogeneity, AD and AR WMS are genetically heterogeneous conditions. Our observation of an in frame fibrillin-1 gene deletion in an AD WMS pedigree strongly suggests that AD WMS and Marfan syndrome are allelic conditions at the fibrillin-1 locus. This study adds to the remarkable clinical heterogeneity of type I fibrillinopathies and raises the question of whether AR WMS is caused by another fibrillin gene mutation. Continuing studies will hopefully address this important question.

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