Case Report

A Novel Fibrillin–1 Mutation in an Egyptian Marfan Family: A Proband Showing Nephrotic Syndrome Due to Focal Segmental Glomerulosclerosis

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ABSTRACT. Marfan syndrome (MFS), the founding member of connective tissue disorder, is an autosomal dominant disease: it is caused by a deficiency of the microfibrillar protein fibrillin-1 (FBN1) and characterized by involvement of three main systems: skeletal, ocular, and cardiovascular. More than one thousand mutations in FBN1 gene on chromosome 15 were found to cause MFS. Nephrotic syndrome (NS) had been described in very few patients with MFS being attributed to membranoproliferative glomerulonephritis secondary to infective endocarditis. Focal segmental glomerulosclerosis (FSGS) had been reported in NS in conjunction with MFS without confirming the diagnosis by mutational analysis of FBN1. We hereby present an Egyptian family with MFS documented at the molecular level; it showed a male proband with NS secondary to FSGS, unfortunately, we failed to make any causal link between FBN dysfunction and FSGS. In this context, we review the spectrum of renal involvements occurring in MFS patients.

Introduction

Marfan syndrome (MFS; OMIM 154700) is an autosomal dominant disease affecting fibrillin-1 (FBN1) which is encoded by fibrillin-1 gene.1,2 According to Ghent nosology, clinical diagnosis of MFS is based upon a set of “major” and “minor” manifestations affecting the skeletal, ocular, cardiovascular, pulmonary systems, dura, and skin.3 Recently, the Marfan foundation (http://www.marfan.org) utilized the published revised Ghent nosology for diagnosis of MS, and they provided an online tool for calculation of “systemic score;” several minor criteria from the old Ghent nosology had been eliminated.4

The FBN1 gene was assigned to chromosome 15q21.1 by in situ hybridization;5 it spans about 234.91 Kb of genomic DNA and com-
prises 65 Exon with an open reading frame of 8613 nucleotides.6 By means of immunohistochemistry, most MFS patients display microfibrillar abnormalities.7 It is not surprising that mutations in other genes may cause nonclassical Marfan-like phenotypes hence the necessity to apply the revised Ghent criteria.8 More than one thousand FBN1 mutations have been described giving rise to a wide clinical spectrum;8–10 the vast majority of mutations are unique to each family.8 Mutation within exons 24–32 especially those of exon 25 cause severe and neonatal forms (cardiovascular and ectopia lentis), nonsense mutations of terminal exons 59–65 may cause milder forms of large-joint hypermobility but multi-exon deletions usually cause severe cardiovascular manifestations.11–13

A wide spectrum of renal involvements had been described in MFS; among them, nephrotic syndrome (NS) with focal segmental glomerulosclerosis (FSGS) had been described in 18-year-old Indian MFS patient whose mutation type was not documented.14 We hereby present an Egyptian family with 4 cases of MFS; a 12.5 years old proband with three major criteria (aortic root dilatation more than 2 Z score, ectopia lentis and FBN1 mutation). Mother and the two other sibs were diagnosed by the positive family history and FBN1 mutation as well as the nontypical skeletal system affection. Proband presented to Mansoura University Children’s Hospital (MUCH) with NS that had been classified pathologically as FSGS. Up to the best of our knowledge, this is the first Egyptian report for an MFS family diagnosed at the molecular level with some emphasis on the link between MFS and FSGS.

Case Report

The proband is the 3rd child of a nonconsanguineous Egyptian couple; he was born full term (38 weeks gestation) with a history of mild developmental delay. He was diagnosed as a case of NS at the age of five years and had been renal biopsied at the age of seven years because of the steroid dependence. Renal biopsy revealed minimal change disease followed by multiple courses of steroid due to the frequent relapses. He was started on a protocol of mycophenolate mofetil for one year with partial remission then lost his follow-up for three years during which he received multiple interrupted courses of steroids.

He first presented to Nephrology Unit of MUCH at the age of 12.5 years with both clinical and laboratory relapses of NS. Examination revealed pectus carinatum, high arched palate, dolichocephaly, enophthalmos, malar hypoplasia, mid-diastolic click, and pansystolic murmur over apex. Inguinal hernia, puffy eyelids, edema of lower limbs, shifting dullness for ascites with positive wrist and thumb signs (Figure 1). Height was 160 cm (90th percentile), weight 41 kg (25th–50th percentile), according to the growth parameters in Egyptian children,15 Span 178 cm, upper segment/lower segment (US/LS) ratio 0.84 and head circumference 58 cm. Figure 1 shows some clinical signs used for calculation of “systemic score” suggestive for MFS: (a) thumb sign, (b) wrist sign, (c) slit lamp examination: ectopia lentis (subluxated lens), and (d) acutabular protrusion and hence, the patient has a “systemic score” of nine (http://www.marfan.org/dx/score).

Echocardiography revealed mitral valve prolapse with Grade II mitral regurgitation, mild dilatation of the left atrium, aortic root dilatation more than 2 Z score (Figure 2). According to the revised Ghent nosology for diagnosis of MFS, a “systemic score” of more than seven is considered positive which serves as a major criterion for diagnosis. The other three major criteria include aortic root dilatation Z-score more than two, ectopia lentis, and FBN1 mutation. In the absence of family history, there must be two major criteria with mandatory aortic root dilatation. Positive family history of MFS (as defined above) is considered as another major criterion, hence only one additional major criterion is needed,4 our proband fulfilled two major criteria of MFS. Laboratory data revealed heavy proteinuria (urinary protein more than 2 g/dL), urinary protein creatinine ratio 3.2, serum albumin 1.6 g/dL, serum creatinine 0.5 mg/dL. Renal biopsy done in our center revealed FSGS (there are
three glomeruli; one is collapsed, and the other two showed mild mesangial hypercellularity, interstitial tissue showed mild inflammation). Electron microscopic examination revealed vacuolar degeneration of epithelial cells with effacement of their foot processes. Focal glomerular basement membrane wrinkling and collapse were noted. No electron dense deposits could be detected. The glomerular basement membrane is of normal texture and electron density (Figure 3). After biopsy, the patient started cyclosporine therapy, captopril,
valsartan, steroids, with partial remission till present.

All family members were clinically screened for the systemic signs; the 45-year-old mother 1102 was relatively tall (height 182 cm, span 195), her body weight 68 kg, US/LS 82/100 (0.82), height/span 1.07, history of intraocular lens implantation at the age of 35 years. Echocardiography revealed mitral valve prolapse, mitral regurgitation Grade II. Urine analysis was free. The oldest 20-years sister 1201 was clinically asymptomatic like her father 1101, her weight 63 kg, height 162 cm, span 164 cm, US/LS ratio 1.04, no audible murmur, normal ECG, urinalysis was free. The second 1202 and youngest 1204 sisters looked like their mother, i.e., marfanoid; 1202 was 16 years, height 178 cm (>95th percentile), weight 61.5 kg (25th–50th), span 195, US/LS 0.85. History of intraocular lens implantation at the age of 12 years, echocardiography showed mitral valve prolapse without incompetence in 1202. Urinalysis was free, serum albumin 4.2g/dL, serum creatinine 0.4 mg/dL. Girl 1204 was 5 years old, weight 20 kg (50th percentile), height 112 cm (75th–90th percentile), US/LS ratio 0.9, no audible murmur, normal echocardiography, and normal renal function. Based on the positive family history (clinically diagnosed proband), mother 1102 and second daughter 1202 have one major feature if the lens implantation is considered to be done for ectopia lentis; however, the systemic score for individuals 1102, 1202, and 1204 was less than seven. Hence, FBN1 mutation analysis was recommended to document the mutant form among this Egyptian family and to have an explanation, if any, for the renal involvement in the proband.

**Mutation analysis of FBN1**

After obtaining an informed consent from all members of the Egyptian family, genomic DNA was extracted from 5 mL blood on EDTA for the mutation analysis of FBN1 gene. Amplification using polymerase chain reaction for all coding exons and their flanking introns had been done. Consequently, these

![Figure 3. Electron microscopy of the second renal biopsy: (a) Epithelial cells showed vacuolar epithelial degeneration with effacement of their foot processes, (b) focal glomerular basement membrane wrinkling and collapse is noted, (c and d) no electron dense deposits could be detected, glomerular basement membrane is of normal texture and electron density.](http://www.sjkdt.org)
amplicons were analyzed using the Illumina’s sequencing by synthesis technology (MiSeq personal sequencer). The presence of mutation was then confirmed by Sanger sequencing starting from the original genomic DNA.

Sequence NM_000138.4 (http://www.ncbi.nlm.nih.gov/nuccore/NM_000138) was used as a reference, while the location of the mutation at the DNA or protein level was numbered according to the Universal Mutation Database (http://www.umd.be/FBN1/). In the proband 1203, a heterozygous disease-causing missense mutation c.6388G>A (p.Glu2130Lys) was found in exon 52 that presented also in his mother 1102 and sisters 1202 and 1204, hence, this family is considered as having four cases of MFS. However, the father 1101 and his oldest daughter 1201 did not carry that mutation (Figure 4). This mutation had been previously identified in our laboratory in three MFS cases (data not published).

Discussion

The importance of FBN1 in the kidney was emphasized by Kanwar et al who showed the tight regulation FBN1 during renal development of rats; administration of FBN-1 antisense oligonucleotides to embryonic metanephron revealed that FBN1 is essential for metanephric development in organ culture. The microfibrillar protein FBN1 is a component of the mesangial matrix; its defect predisposes MFS individuals to vascular damage, but the role of FBN1 in kidney disease is still unknown. Regarding its localization around the glomerular capillaries, it seems likely that FBN1 could contribute to the elastic strength and anchorage of the glomerular capillary tuft to maintain the high ultrafiltration rate.

Several reports showed different types of renal disease in MFS, however, it is still unknown whether these lesions were coincidental or due to a systemic renal defect caused by FBN1 mutation. Bilateral renal vein thrombosis and NS in a patient with MFS had been reported. Both the structural renal changes (hepatorenal polycystosis) and functional renal abnormalities (aminoaciduria and phosphaturia) had been described with MFS but still are not completely understood.
with unclear pathogenetic explanations.\textsuperscript{24-27} In four MFS cases presented with microhematuria and proteinuria; renal biopsies revealed segmental increase in mesangial matrix with some sclerotic lesions in two cases, thus suggesting the microfibrillar disarrangement to be the cause of glomerular basement membrane alterations.\textsuperscript{28} In the current report, nephrotic range proteinuria has been described in the 12.5-year-old boy that started at the age of five years; there was no preceding systemic illnesses, urinary hues or evidence of previous skin or upper respiratory tract infections. The presence of focal glomerular basement membrane wrinkling and collapse on electron microscopy should consider the microfibrillar dysfunction as a plausible pathogenetic factor.

A mutant mouse model that exhibits a 5-fold underexpression of \textit{FBN1} was established by Pereira et al. These mice showed some hallmarks of MFS, including cardiovascular complications due to mechanical collapse and dissecting aneurysms of the aortic wall; however, no obvious renal involvement was noticed.\textsuperscript{29} Hartner et al. studied the renal phenotype in the MFS mouse model; they found the glomerular function in mice with a 5-fold underexpression of \textit{FBN1} not different from the wild type. Moreover, there were only subtle changes in glomerular volume and mesangial area, thus concluding that \textit{FBN1} underexpression in mice did not seem to lead to defects in renal function.\textsuperscript{30} The reason that MFS mouse models do not show the same renal alterations that occurs in MFS patients might be explained by the fact that mouse still had enough normal \textit{FBN1} that could maintain the glomerular structure and function; whereas, in MFS patients, \textit{FBN1} mutations generally lead to the expression of altered \textit{FBN1} microfibrils and a lack of normal \textit{FBN1}.\textsuperscript{31} Lack of any clinical renal disease in the older mutation-positive MFS girl 1202 should deny the role of age in \textit{FBN1}-underexpression, unless there are some additional epigenetic factors that can modify the gene expression like gender and/or any other modifier genes. As the cardiovascular system is one of the primary sites for \textit{FBN1} dysfunction, secondary renal involvements are not uncommon in MFS patients. A case of membranoproliferative glomerulonephritis had been reported from an underlying undetected bacterial endocarditis in a 79-year-old patient with MFS and mitral valve prolapse, 4.7 cm ascending aortic aneurysm with resultant aortic regurgitation, and mildly decreased ejection fraction.\textsuperscript{32} An 18-year-old boy with MFS and NS secondary to FSGS had been described as a novel association, however, no mutation studies had been done, but authors speculated that the glomerular damage could be secondary to \textit{FBN1} mutations.\textsuperscript{14} Our current case with an \textit{FBN1} missense mutation showed FSGS for the second time in literature. An interesting point, in this case, is that despite the mutation that involves a terminal exon, it causes a classical MFS like those involving 24–32 exons.\textsuperscript{11,12} Whether the renal pathology is secondary to or coincidental with \textit{FBN1} dysfunction is unclear at the moment. Immuno-histochemical staining of a renal biopsy, which unfortunately is not usually done, could solve that issue.

Recently, a genetic interaction between polycystic kidney disease (\textit{PKD1}) and \textit{FBN1} genes has been postulated through the implication transforming growth factor beta (TGF-\textbeta) signaling in the pathogenesis of vascular complications in autosomal dominant \textit{PKD} (ADPKD). They investigated the overlap between ADPKD and MFS by breeding a mouse model with targeted mutations in \textit{PKD1} and \textit{FBN1} genes. Double heterozygotes displayed an exacerbation of the typical \textit{FBN1} heterozygous aortic phenotype.\textsuperscript{33}

To sum up, renal involvements in MFS patients had been widely studied both in human at the clinical, histopathological and therapeutic levels, and in the experimental animal model at pathogenetic and molecular levels. Early diagnosis of MFS phenotype in renal patients could preclude the necessity for repeated renal biopsies and save many unneeded courses of immunosuppressive therapy if treatment is directed towards \textit{FBN1}-TGF-\textbeta signaling pathway.
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References

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