Original Article
Two novel mutations of *FBN1* in Jordanian patients with Marfan syndrome

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Abstract: Marfan syndrome is an autosomal dominant inheritance disorder with a 1/5000-live-birth prevalence. More than 3000 mutations have been characterized thus far in the *FBN1* gene. The goal of this study is to facilitate Marfan syndrome diagnosis in Jordanian patients using a molecular genetic testing. All of the 65 coding exons and flanking intronic sequences of the *FBN1* gene were amplified using polymerase chain reaction and were subjected to sequencing in five unrelated Jordanian patients suspected of having Marfan syndrome. Four different mutations were identified, including two novel mutations: the c.1553dupG frame-shift (p.Tyr519Ilefs*14) and the c.6650G>A (p.Cys2217Tyr) missense mutations. Two other missense mutations, c.2243G>A (p.Cys748Tyr) and c.2432G>A (p.Cys811Tyr), have been previously detected. Patient number five was heterozygous for the synonymous substitution variant c.1875T>C (p.Asn625Asn; rs#25458). Additionally, eight variants in the intronic sequence of the *FBN1* gene were identified, of which the c.2168-46A>G mutation was a new variant. The data provide molecular-based evidence linking Marfan syndrome to pathogenic mutations in the *FBN1* gene among Jordanians for the first time. Thus, our results will contribute to the better management of the disease using molecular tools and will help in genetic counseling of the patients' families.

Keywords: Cysteine, Marfan syndrome, *FBN1* gene, mutation, Jordan

Introduction

Marfan syndrome (MFS) is an autosomal dominant inheritance disorder with a 1/5000-live-birth prevalence. MFS affects males and females equally and has been described in most ethnic groups [1-3].

Microsatellite DNA markers were used in linkage analysis in DNA samples from five different Marfan syndrome families and linked the causative gene in these MFS families to chromosome 15q15-q21.1 [4, 5]. Interestingly, in parallel works, the *FBN1* gene was cloned by both positional and functional approaches and the first missense substitution (Arg to Pro) was reported [5-7].

More than 3000 mutations have been characterized thus far in the *FBN1* gene, with over 50% of these mutations being missense mutations and mostly involving cysteine substitutions [8]. Thus far, no report of a founder mutation in the *FBN1* gene has been described. In contrast, most of the mutations identified were unique or private for single families or in sporadic cases [1-3].

Patients with the same or different mutations in the *FBN1* gene showed variable phenotype expressivities of the disease, in which the clinical symptoms ranged from mild to severe [9]. Although confirmed *FBN1* mutations identified to date was highly penetrant, they expressed a pleiotropic effect, where the symptoms were manifested in one or more of the body systems in different patients [1-3]. Thus, due to the variable expressivities, pleiotropic nature of the *FBN1* mutations and age dependent clinical manifestations, a phenotype-genotype correla-

tion is inconsistent for most of the mutations identified [1-3].

MFS clinical symptoms primarily manifest in the connective tissue of the skeletal, ocular and cardiovascular systems, where aorta dilatation and rupture in direct consequence to aortic aneurysm and dissections progressiveness is the primary cause of death in MFS patients [1-3].

The clinical diagnostic criteria of Marfan syndrome are still the golden standard for patient diagnosis. Thus, several diagnostic criteria were proposed, in which skeletal system manifestation, family history and *FBN1* mutations were acknowledged [10, 11]. This is because *FBN1* mutations were also characterized in several other genetic disorders collectively named as type 1 fibrillinopathies [1-3].

The *FBN1* gene consists of 65 exons and encodes a 2871 amino acid fibrillin-1 monomeric protein [12]. Fibrillin monomers with other extracellular matrix proteins polymerize into microfibrils and functions in providing structural support to connective tissues. Fibrillin-1 constitutes a repeat of 47 epidermal growth factor-like domains (EGF-like domains). Of these domains, 43 have specific residues for calcium binding (cbEGF-like domains). Fibrillin-1 also has seven transforming growth factor beta (TGF-β) binding protein-like domains [13]. Each EGF-like domain contains six conserved cysteine residues forming 3 disulfide bonds, which are necessary for the correct folding of each of these domains. Moreover, there are 8 cysteine amino acids in each of the TGF-β like domains [13].

In this study, DNA was collected from 5 unrelated MFS Jordanian patients and their available family members. All of the DNA samples were screened for the 65 exons of the *FBN1* gene using direct DNA sequencing. The results of this study are expected to provide better genetic counseling for the patient's families and improve their medical care depending on a confirmed MFS diagnosis.

Materials and methods

Patients

This study was approved by the joint institutional review board committee of the Jordan University of Science and Technology and King Abdullah University Hospital. Consent was obtained from the patients and from all family members tested for the mutations.

Patient 1 was a 30-year-old male who was abnormally tall and referred to the cardiologist to rule out Marfan syndrome. His cardiological assessment was: prolapse mitral and tricuspid valve and dilated aortic root at 4.1 cm. In addition to the above findings, he was found to have severe myopia, flat feet, and a scar from surgery for an inguinal hernia. His father died from a heart attack at the age of 49 years, and his sister has scoliosis and is tall. Additionally, one close relative on his father's side has aortic dilation. DNA samples from his sister and mother were available for testing.

Patient 2 was a 30-year-old female who underwent plastic surgery for her nose and was referred to our clinic for further evaluation because of features that suggested Marfan syndrome. She was found to be abnormally tall and her arm span was greater than her height span. In addition, she manifested scoliosis, joint hyper mobility, high arch palate, and archaea dactyl. Her eye examination also showed hypermetropia with an astigmatism. DNA samples from her 7 sisters and brothers, mother and from several tissues, including the blood, hair, mouth and nasal, of her father were available for testing.

Both Patient 3 and 5 were referred to our laboratory for molecular diagnosis because of their tall stature and were suspected of having MFS. No clinical data were available for them at our clinic.

Patient 4 was a 13-year-old male referred by his general practitioner because he noticed some abnormal movements in his eyes. On examination he was not abnormally tall, but both eyes showed iridodenesis. DNA samples from his mother, father and his sisters were available for testing.

Fifty DNA samples from control individuals were collected to confirm the absence of the missense mutations identified in this study.

Sequence analysis

Polymerase chain reaction (PCR) was carried out to amplify the 65 exons of the *FBN1* gene from the genomic DNA of the 5 patients. The forward and reverse PCR primers for the 65
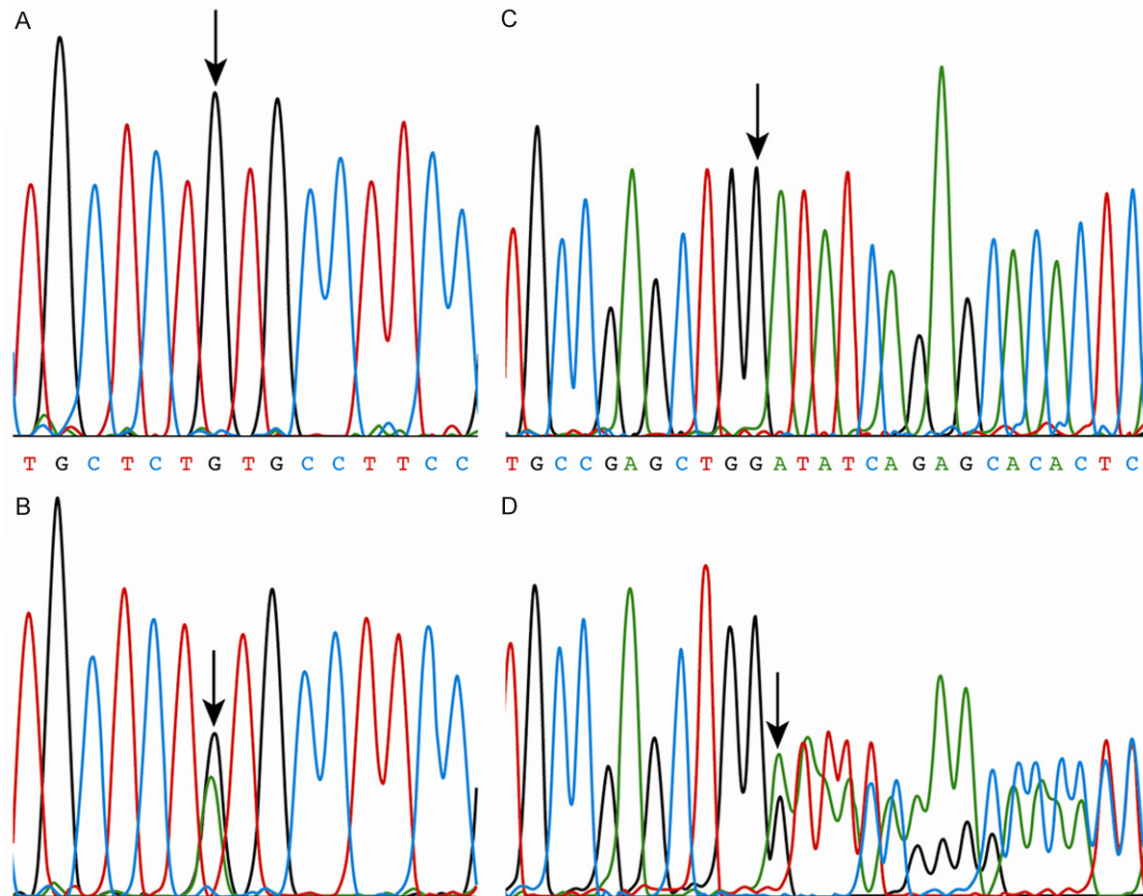
Figure 1. Sequencing chromatograms encompassing the *FBN1* mutations. c.6650G>A; p.Cys2217Tyr in exon 54 (A) homozygous wild type (patient 1, mother); (B) heterozygous (patient 1); c. 1553dupG ; p.Tyr519Ilefs*14 in exon 12 (C) homozygous wild type (patient 2, sibling); (D) heterozygous (patient 2). The arrows indicate the locations of the mutation.

exons were designed to cross the exons-intron junctions using the Primer3 algorithm (http://frodo.wi.mit.edu/primer3) according to genomic GenBank accession number NG_008805.2. All of the primer sequences and PCR reaction conditions are available upon request.

All of the PCR products were directly sequenced using the Big-Dye Terminator v3.1 Cycle Sequencing Kit and analyzed using an ABI PRISM 310 Genetic Analyser (Applied Biosystems, Foster City, CA, USA). The patient sequencing data were aligned against the normal *FBN1* c-DNA sequence, accession number: L13923.1, using the ChromasPro 1.34 software (Technelysium Pty Ltd, Australia). Sequence nomenclatures for the coding and noncoding variants are described in accordance with the Human Genome Variation Society Nomenclature standards, where the A

nucleotide of the initiation is the +1 nucleotide. The pathogenicity prediction of the Tyr2217, Tyr748 and Tyr811 was performed using the PolyPhen program (http://genetics.bwh.harvard.edu/pph/).

Results

Five different Jordanian patients were suspected of having MFS because at least one Marfanic manifestation in the skeletal, cardiac and eye systems was found. DNA sequencing of the coding exons (1-65) of the *FBN1* gene in the 5 patients revealed four different mutations and nine variants. Two of these mutations were novel, including a missense and frame-shift mutation. Patient 1 and his sister were affected and heterozygous for the transition point mutation c.6650G>A (**Figure 1A**, **1B**), which is in exon 54 and results in the substitution of
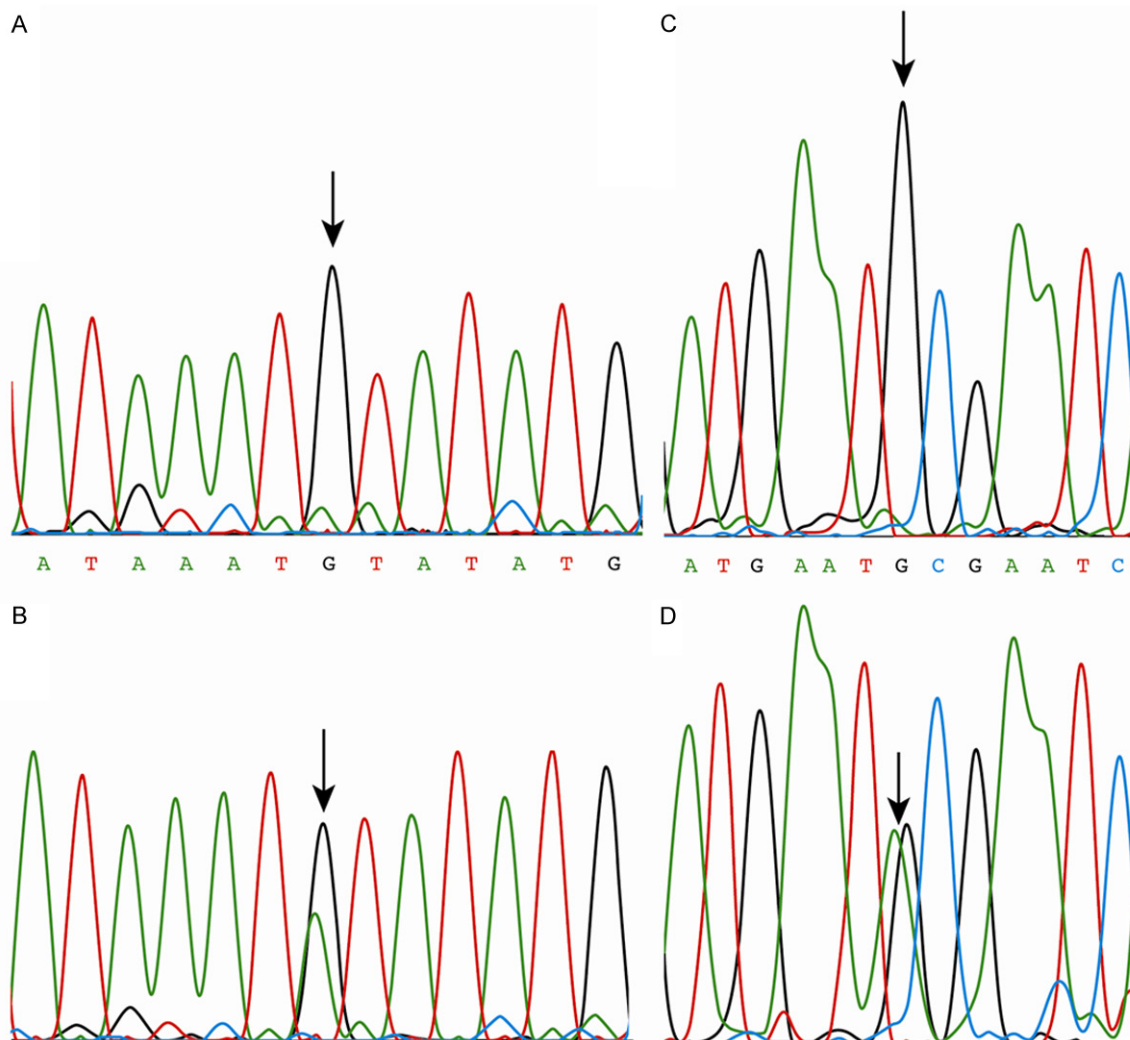
Figure 2. Sequencing chromatograms encompassing the *FBN1* mutations. c.2243G>A; p.Cys748Tyr in exon 18 (A) homozygous wild type (normal control); (B) heterozygous (patient 3); c.2432G>A; p.Cys811Tyr in exon 20 (C) homozygous wild type (patient 4, mother); (D) heterozygous (patient 4). The arrows indicate the locations of the mutation.

Cys2217 with a Tyr. The mother was homozygous for the c.6650G wild-type allele. This mutation was not found in the 50 control patients.

The c. 1553dupG (**Figure 1C**, **1D**) identified in patient 2 in exon 12 of the *FBN1* gene is a novel frame-shift mutation resulting in an amino acid substitution for residues 519 (Tyr519Ilefs*14) to 531 and a premature stop codon at residue 532 (Asp532X). This mutation was not found in any of brothers and sisters nor in the mother or the four DNA samples extracted from different tissues of the father of patient 2.

Patient 3 had a recurrent c.2243G>A mutant allele (**Figure 2A**, **2B**), which is in exon 18 and replaces Cys748 with a Tyr. Patient 4 was het-

erozygous for the c.2432G>A mutant allele (**Figure 2C**, **2D**) in exon 20, even though the patient's parents and unaffected sister were homozygous wild type. This mutation converts Cys811 to a Tyr. The mutated allele was not detected in the 50 control individuals. All three Tyr mutated residues at positions 2217, 748 and 811 were predicted by the PolyPhen Program to be damaged with a score of 0.997, 0.997 and 0.999, respectively.

We also identified several recurrent single nucleotide polymorphisms (SNPs) in the *FBN1* gene (http://www.ncbi.nlm.nih.gov/snp). These SNPs included: c.1875T>C (p.Asn625Asn; rs#25458), c.1960+98A>T (rs#35464791), c.3208+55_60delTCTTTA (rs#143836403), c.5224+111A>G (rs#9806595), c.6037+54T>A

(rs#2303502), c.6496+128A>G (rs#363820), c.6997+17C>G (rs#363832) and c.7570+ 153C>A (rs#1820488). Additionally, we identified one novel (c.2168-46A>G) variant. The synonymous substitution variant c.1875T>C (p.Asn625Asn; rs#25458) was found to be heterozygous in patient 5. The patient was also heterozygous for the rs35464791, rs14383-6403, rs363820 and rs1820488 polymorphisms.

Discussion

This is the first molecular *FBN1* gene study on Jordanian patients with MFS. In this study, we identified three missense mutations in exons 18, 20 and 54 of the *FBN1* gene in 3 different patients, including a novel Cys2217Tyr mutation and a novel frame-shift, c.1553dupG, in exon 12 in patient number 2.

The novel missense mutation was located in the cbEGF-like #34 domain of the protein. This domain is composed of 41 amino acids (UniProtKB accession number: P35555) and harbors 6 Cys residues that are necessary for 3 disulfide bonds formations (Cys2210 to Cys2221, Cys2217 to Cys2230, and Cys2232 to Cys2245). The c.6650A mutant allele was presumably transmitted from the father, who died of heart complications at a younger age and was not investigated at that time. There are several missense mutations in the cbEGF-like #34 domain, 4 of which resulted in Cys2221 substitutions for Arg, Gly, Ser and Phe and one missense mutation, resulting in a Cys2232 substitution for Tyr [14-18]. Thus, the mutation that we report is the first to affect Cys2217, which is involved in the second disulfide bond of this domain. This mutation is associated with classical major MFS criteria, which include complications in the heart, skeletal system, and eyes as well as a strong family history.

The other two recurrent missense mutations, Cys748Tyr and Cys811Tyr, were located in cbEGF #7 and #9, disrupting the second and the first disulfide bonds of these two domains, respectively. The Tyr748 mutant residue was originally identified in a 9-year-old patient with both ocular and cardiac system complications [19]. Notably, Cys811 is considered to be one of the most mutable residues in the *FBN1* gene. Cys811 (TGC) substitutions for a Tyr (TAC), Stop codon (TGA) or Trp (TGG) have been reported in

several classical MFS patients from various nationalities [20, 21]. In this study, Patient 4, a carrier for the Tyr811 mutation, manifested MFS symptoms in the eyes at the early age of 13.

Although the mRNA stability of several cysteine residue substitutions in the cbEGF-like domain was comparable to the wild-type allele, the protein stability, processing and secretion were impaired in two-thirds of the different mutations investigated [14]. Thus, by disrupting the disulfide bonding formation, the fibrillin monomer misfolds and is more susceptibility to proteases, which might partially explain the mechanism of the missense mutation pathogenicity in MFS [23, 24].

The frame-shift mutation that results in a truncated fibrillin-1 protein, if stable, lacks 2339 amino acids and occurs in the cbEGF-like #03 domain. In our study, the patient with the mutation demonstrated a severe disease phenotype. Although, we cannot fully exclude the possibility of germ-line mosaicism in this family, the absence of this mutation in 7 siblings, in mother and in several tissue samples from the father suggested that the mutation is a *de novo*, which is estimated to have a prevalence of approximately 25% in the *FBN1* gene [1-3, 25]. Similarly, patient 4 presumably had a new *de novo* mutation.

Mutation types, such as splicing, nonsense and frame-shift, result in a shorter protein that might cause MFS by two mechanisms: either haploinsufficiency, where the 50% of the protein that translated from the wild-type allele is not enough to support the fibrillin-1 structural function, or by a dominant negative effect, where the mutant short protein interferes with the polymerization of normal fibrillin molecules. Similar haploinsufficiency and loss-of-function mechanisms have been proposed for some of the missense mutations [1-3].

A genotype-phenotype study of 1013 MFS patients demonstrated that ocular complications occur in most of the MFS patients who are carriers for Cys substitutions in cbEGF-like domains, while mutations that introduce early termination codons by direct nucleotide substitution or a frame-shift resulted in severe skeletal and skin manifestations [26]. Both patients in this study were heterozygous for Cys substi-

tutions showed myopia and iridodenesis of their eyes, while the patients with a frame-shift manifested severe skeletal deformities. Moreover, the mutations identified in exons 24-32 of the *FBN1* gene were found to be associated with the severe form of FMS in both neonates and adults [27].

Even though a mutation was identified in the *FBN1* gene by DNA sequencing the coding exons and their flanking intronic regions in over 90% of MFS patients, no mutation was identified in patient 5. However, the finding of four heterozygous intronic variants and one synonymous substitution (Asn625Asn) was intriguing. The c.1875C allele of Asn625 was more frequently associated with tall stature males compared to males of normal height [28]. An unidentified mutation in this patient might be within the deep intronic regions, which are extremely large in the *FBN1* gene and were not sequenced in this study [29].

In conclusion, we detected four *FBN1* mutations in four Jordanian patients. Two of these mutations were previously identified and two are reported for the first time, one is *de novo* and the other is familial. These results will facilitate the molecular genetic diagnosis of MFS in Jordan and potentially prevent disease complications on the basis of molecular diagnosis.

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Disclosure of conflict of interest

None.

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