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Case Report

# Undescribed mutations in FBN1 gene in two family cases of Marfan syndrome

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ABSTRACT

Marfan syndrome (MFS) is a multisystem autosomal dominant heritable disorder and, although there are over 1700 mutations that have been identified in the fibrillin-1 (FBN1) gene associated with it, there are many variants that remain unknown. Here we report two family cases of MFS with two new undescribed variations (C914S and H2426C) in FBN1 gene. Both variations produce alterations in the structural conformation of the protein resulting in pathogenic events in these patients. Finally, this case report includes both pathogenic mutations that have also been clinically and genetically confirmed to result in MFS. This clinical, genetic, and *in silico* analysis of potentially harmful variations in unrelated MFS patients provides additional evidence for the suggested causative role of the mutations c.2740T > A (C914S), c.7276_7278delCAT (p.H2426C) in FBN1 gene in MFS.

<**Learning objective:** New previously undescribed mutations in fibrillin-1 (FBN1) gene related to Marfan syndrome (MFS) have been confirmed by genetic, bioinformatics, and clinical studies. It is well known that MFS is caused by mutations in FBN1 gene; however many of them remain unknown. These data could be relevant in the screening of these patients offering a different follow-up by considering these and other genetic mutations. These types of mutations should be considered in differential diagnosis.>



## Introduction

Marfan syndrome (MFS; MIM#154700) is a multisystem autosomal dominant heritable disorder mainly caused by mutations in the fibrillin-1 gene (FBN1) at 15q21.1 [1]. Although there are over 1700 mutations that have been identified in the FBN1 gene associated with MFS, other mutations have not yet been described [2]. Fibrillins are an integral component of the extracellular matrix of connective tissue, and therefore mutations in the genes encoding them are likely to cause disruption of the connective tissue [3]. Although there are over 1000 FBN1 mutations associated with this syndrome, here we describe new ones that had not been previously described [3].

## Case reports

### *c.7276_7278delCAT (p.H2426C) variation*

A 53-year-old Caucasian male with symptomatic signs of MFS was analyzed by a specialist. Physical examination showed no alterations in head or neck, and rhythmic noise without murmurs. Other complementary examinations dismissed aortic dilated root. Clinical analysis proved that an aortic thoracic aneurysm is produced by smoking (the patient smokes around 20 cigars per day). A screening computed tomography (CT) angiography and genetic analysis in main genes of MFS (FBN1 and TGFR2) were requested by the doctor. Other data were unremarkable.

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**Table 1** Undescribed mutations in patients of Marfan syndrome.

| Nucleotide | Protein | Pathology | Gene | Location | Effect |
|---|---|---|---|---|---|
| c.2740T > A | p.C914S | MFS | FBN1 | Exon 24 | Missense |
| c.7276_7278delCAT | p.H2426C | MFS | FBN1 | Exon 59 | Missense |

MFS, Marfan syndrome.
The data were established by comparison with the reference sequence NM_000138.4 in FBN1 gene.

**Table 2** Program details of *in silico* analysis.

| Program name | URL | Prediction |
|---|---|---|
| PolyPhen-2 | http://genetics.bwh.harvard.edu/pph2/ | Based on a decision tree that combines a number of protein structural attributes with a prebuilt sequence alignment, generally including only mammalian sequences. |
| SNPs&GO | http://snps-and-go.biocomp.unibo.it/snps-and-go/ | The prediction of human disease-related single point protein mutations predicting whether a mutation at the protein level is or is not disease-related |
| MutPred | http://mutpred.mutdb.org/ | Classification of an amino acid substitution as disease-associated or neutral in human. In addition, it predicts molecular cause of disease. |
| SIFT | http://sift.jcvi.org/ | Whether an amino acid substitution affects protein function, its prediction is based on the degree of conservation of amino acid residues in sequence alignments derived from closely related sequences. |
| AlignGVGD | http://agvgd.iarc.fr/agvgd_input.php | Combines an alignment with amino acid physicochemical characteristics to calculate the range of variation present at each position in the alignment (GV) and the distance of missense substitutions from that range of variation (GD). |

After genetic analysis, the patient was discovered to have a previously undescribed mutation in the main databases such as http://www.hgmd.cf.ac.uk/ac/search.php and Genecards-Uniprot. p.His2426Cys (p.H2426C) mutation (Table 1) produces a deletion of three nucleotides in the coding region. This mutation produces a lack of histidine amino acid which affects the domain of epidermal growth factor (EGF)-like protein 41-calcium-binding. Furthermore, according to clinical references these changes are considered to produce clinical alterations due to the effects on a highly conservative region of the protein. *In silico* analysis could not be performed due to the type of this variation to confirm the pathogenetic effect of this change (Table 2). However, we have performed *in silico* analysis in other related genes (TGFBR1/2 genes) to eliminate the possibility of this being a mutation in other genes, obtaining negative results for these genes.

After the genetic analysis, due to the implications of this variation and the inheritable characteristics of MFS, the same genetic analyses were performed in his descendents (two daughters and a son). The 16-year-old son has the mutation and was also affected by MFS diagnosed by this familial analysis.

*c.2740T > A (C914S) variation*

A 32-year-old Caucasian male with symptomatic signs of MFS and several cases of ectopia lentis in his family was analyzed in FBN1 gene to confirm MFS. This patient had ocular and vascular ($z$ score 2.67) effects but no systemic effects (score <5). Genetic analysis described p.Cys914Ser (p.C914S) variation in FBN1 gene. This variation produces a change between amino acids with different size and change which produces a worse prognosis. Furthermore, this change alters disulphur bonds and in consequence the 3D structure of the extracellular protein. *In silico* analysis confirmed the pathogenicity of this mutation with high score of pathogenicity classification (99.6% with PolyPhen-2, 9/10 with SNPs&GO, 98% being deleterious in MutPred, and 0 value in SIFT). Similar to a previous case report, a genetic study was performed on other relatives and found that there were two children (5- and 4-year-olds) with the mutation and both affected by MFS, having ocular effects and aortic $z$-score of 2.5 and 2.35, respectively. The mother (59 years) with systemic, ocular, and skeletal effects and aunt (53 years) were also affected and both presented the mutation. However, a daughter was absent of the syndrome and the mutation.

## Discussion

As can be seen in this analysis, there are many new mutations discovered by genetic testing. Some of the mutations have been previously described and their effects are well known; however, some others reveal missense substitutions that are not easily classified as pathogenic or neutral. Actually, it is a real problem to define which variants of uncertain clinical significance (VUS) are deleterious/disease causing and which are neutral, and among other tools, *in silico* analysis could predict the pathogenicity or not of an undescribed mutation. Having sequence VUS makes it difficult to classify the variants into high- or low-risk in patients. These results prove that in the case of MFS, as well as in many cancers and other pathologies, the existence of VUS is a common event and we describe two new variations for MFS that can be included for the next genetic analysis in this disease.

Most mutations in exons 24–32 of FBN1 gene are predictive of a severe cardiovascular phenotype even in non-neonatal cases, and mutations leading to premature truncation codons are underrepresented in this region [4]. Mainly, this region includes the central longest stretch of $Ca^{2+}$-binding (cb) EGF repeats, which is thought to form a rigid rod-like structure that may be important for microfibril assembly, and contains a wide number of genes [4,5].

Previously reported data from patients' blood of MFS indicate that intronic variants that alter cysteine residues will have a worst prognosis due to the fact that they are usually linked to highly conservative domains and any change in them could lead to a loss of secondary structure of the protein. Furthermore, most mutations in exons 24–32 of FBN1 gene are predictive of a severe cardiovascular phenotype even in non-neonatal cases, and that mutations leading to premature truncation codons are underrepresented in this region [4]. Mainly, this region includes the central longest stretch of cbEGF repeats, which is thought to form a rigid rod-like structure that may be important for microfibril assembly [4].

## Conclusion

This case report emphasizes the important role and high diagnostic certainty of genetic tests in MFS identifying potential mutations of severe and familial cases of MFS in FBN1 gene. These data in combination with clinical information could offer better qualities of clinical treatments to the patient and advances in the detection of this syndrome. Furthermore, these data improve the

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current mutation database of this syndrome giving clinical information to VUS in FBN1.

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## Conflicts of interests

Authors declare no conflicts of interests.

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