Introduction

Marfan syndrome (MFS), is the most common inherited disorder of connective tissue with a prevelance of ~1 in 5,000 individuals. One of the main characteristics of this syndrome is the high degree of clinical variability, ranging from mild to severe phenotype. The main affected systems are the cardiovascular, the skeletal and the ocular. Skeletal system manifestations encopasses tall stature, arachnodactyly, joint laxity, pectus excavatum/carinatum and scoliosis. Common ocular system abnormalities include ectopia lentis and high myopia. However, the major morbidity in the Marfan syndrome is related to the cardiovascular system. Dilatation of the aorta , mitral valve prolapse with or without regurgitation, tricuspid valve prolapse, and enlargement of the proximal pulmonary artery are the most characteristic cardiac anomalies related to Marfan Syndrome. Until recently, its was known that MFS was caused by autosomal dominant mutations in the fibrillin-1 (FBN1) gene and the diagnosis of several cases remained based on the clinical features. In our days, with the contribution of the Next Generation Sequencing Analysis (NGS) we identify mutations in other genes of the TGF-b pathway, which are responsible for MFS and MFS-like phenotype. Some of these genes are: TGFBR1, FLNA, ADAMTSL4, CBS, SMAD3, TGFB2, MED12, FBN2, PLOD1, BMPR2.

Materials and Methods

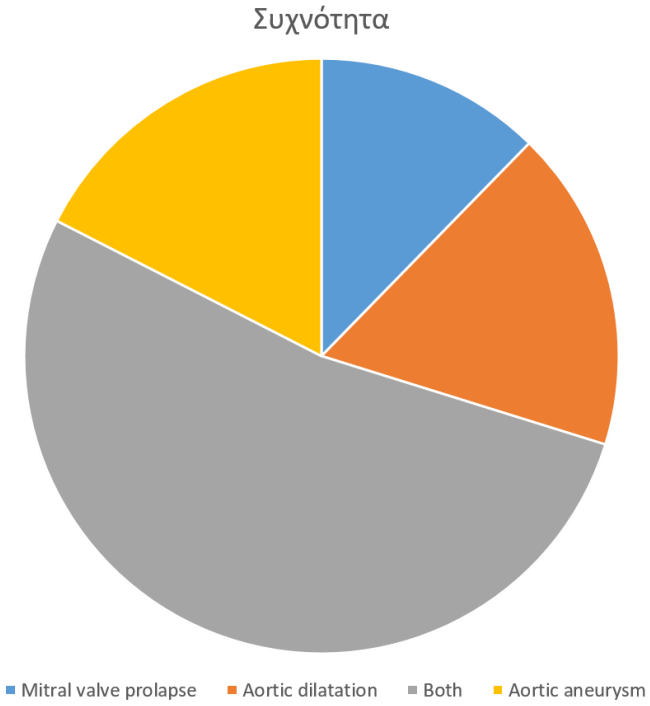
We applied Whole Exome Sequencing (WES) in 7 patiens with Marfan or Marfan-like phenotype and heart disease. Peripheral blood samples were collected, and genomic DNA was isolated from these patients. Approximately 37Mb (214.405 exons) of the Consensus Coding Sequences (CCS) were enriched from fragmented genomic DNA by >340.000 probes designed against the human genome and the generated library was sequenced on an Illumina NextSeq or HiSeq 4000 platform (Illumina). All exons and intron boundaries (+/-20bp) were analysed. Relevant variants identified by NGS were Sanger sequenced to exclude NGS artefacts.

Results

We tested 7 patients with Marfan or Marfan – like phenotype with WES. Five of them were males and 2 of them were females. We found 3 novel mutations in the FBN1 gene, 1 mutation in the BMPR2 gene and 1 mutation in the SLC2A10 gene. In 2 cases no mutation was identified by WES.

Patient	**Heart disease**	**Gene mutation**
Pat. 1	Mitral and Tricuspid valve prolapse Mild Aortic root dilatation	**FBN1**: c.3623 G >A in exon 30
Pat. 2	Ascending aortic aneurysm	**BMPR2**: c.1513dupA in exon 11 (a premature stop codon)
Pat. 3	Mitral valve prolapse	**FBN1**: c.299 G>A in exon 3

Pat. 4	Mitral valve prolapse Mild Pulmonary artery dilatation	No mutation found
Pat. 5	Mitral valve insufficiency	No mutation found
Pat. 6	Mitral valve prolapse Aortic root dilatation	**SLC2A10**: c.859 G>A (VUS)
Pat. 7	Aortic dilatation	**FBN1:** c.724_725insT in exon 6



Discussion

In the majority of the MFS cases, we have found point mutations in FBN1 gene as it was expected. In patient No 2 a pathogenic heterozygous duplication of 1 nucleotide BMPR2: c.1513dupA was identified in exon 11 of the BMPR2 gene. BMPR2 and its interacting protein SMAD1 are part of the TGF-b family genes, many of which have been associated with aortic anomalies. The same BMPR2 variant has been identifed in the father and the paternal uncle of this patient.Both have tall stature and dilatation of ascending aorta. In the undiagnosed cases, FBN1 del/dup or array-CGH analysis is recommended.

Conclusion

The contribution of the New DNA analysis Technologies are essential in MFS diagnosis and it has been proven a powerful tool in phenotype/genotype correlation. In the future Whole Genome Sequencing (WGS) may help to elucitate the undiagnosed cases.