Family-based whole-exome sequencing identifies novel lossof-function mutations of *FBN1* for Marfan syndrome

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Background. Marfan syndrome (MFS) is an inherited connective tissue disorder involving the ocular, skeletal and cardiovascular systems. Previous studies of MFS have demonstrated the association between genetic defects and clinical manifestations. Our purpose was to investigate the role of novel genetic variants in determining MFS clinical phenotype.

Methods. We applied whole-exome sequencing for 19 individuals derived from three Han Chinese families. The sequence data were analyzed by a standard pipeline. Variants were further filtered against the public database and in-house database. Then, we performed pedigree analysis under different inheritancepatterns according to American College of Medical Genetics guidelines. Results were confirmed by Sanger sequencing.

Results. Two novel loss-of-function indels (c.5027_5028insTGTCCTCC, p.D1677Vfs*8; c.5856delG, p.S1953Lfs*27) and one nonsense variant (c.8034C>A, p.Y2678*) of *FBN1* were identified in Family 1, Family 2 and Family 3, respectively. All affected members carried pathogenetic mutations, whereas other unaffected family members or control individuals did not. These different kinds of LOF (loss of function) variants of *FBN1* were located in the cbEGF region and conservative domain across species was not reported previously.

Conclusions. Our study extended and strengthened the vital role of *FBN1* LOF mutations in the pathogenesis of MFS with autosomal dominant inheritance pattern. Genetic testing by next-generation sequencing of blood DNA could be fundamental in helping clinicians conduct mutation-based pre- and postnatal screening, genetic diagnosis and clinical management for MFS.

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15 Abstract

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Methods. We applied whole-exome sequencing for 19 individuals derived from three Han Chinese families. The sequencing data were analyzed by a standard pipeline. Variants were further filtered against the public database and in-house database. Then, we performed pedigree analysis under different inheritance patterns according to American College of Medical Genetics guidelines. Results were confirmed by Sanger sequencing.

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39 Introduction

Marfan syndrome (MFS) is an inherited connective tissue disorder with autosomal dominant 40 transmission. The clinical manifestations of MFS vary from individuals to individuals. More than 41 30 different signs and symptoms are variably associated with Marfan syndrome. The most 42 43 prominent of these affect the skeletal, cardiovascular, and ocular systems, but all fibrous connective tissue throughout the body can be affected (Pyeritz & McKusick 1979). Clinically, 44 aortic dilatation and dissection are the most important and life-threatening manifestations of MFS 45 (Biggin et al. 2004). The estimated prevalence is 1 in 5,000 individuals and there is no predilection 46 for gender (Sponseller et al. 1995; von Kodolitsch & Robinson 2007). An epidemiological study 47 in Taiwan revealed that the overall prevalence of MFS in Chinese population was 10.2 (95% CI, 48 9.8-10.7) per 100,000 individuals, with peaks at the age of 10 to 24 years. The minimal birth 49 incidence of 23.3 (95% CI, 21.7-23.3) per 100,000 individuals was estimated in those aged 20 to 50 51 29 years (Chiu et al. 2014).

Most of MFS patients are believed to be caused by a defect in the genes that make fibrillin which 52 results in abnormal connective tissue (https://www.nhlbi.nih.gov/health/health-topics/topics/mar). 53 54 Mutations in FBN1 (OMIM #134797, encoding fibrillin-1) account for 70%-80% of MFS (Pinard et al. 2016; Stheneur et al. 2009). In addition to FBN1, there are other genes related to MFS, such 55 56 as TGFBR1, TGFBR2, ACTA2, SMAD3, MYH11 and MYLK. Habashi et al. showed that aortic 57 aneurysm in a mouse model of MFS is associated with increased TGF-beta signaling (Habashi et al. 2006) and Holm et al. concluded that noncanonical (Smad-independent) TGF-beta signaling is 58 59 a prominent driver of aortic disease in Marfan syndrome mice (Holm et al. 2011). Traditionally, 60 the discovery of pathogenic genes for MFS depends on locus mapping using candidate-gene

strategy with family-based designs, and the heritability of all known genes for MFS is about 90% 61 (Li et al. 2017). However, inconclusive results concerning their association with MFS are still 62 present, and the pathogenesis is complex. Most cases inherit it from their parents due to 63 its autosomal dominant nature (Wieczorek et al. 1996) and approximately 15-30% of all cases are 64 due to de novo genetic mutations (Cotran et al. 1999). Marfan syndrome is also reported to relate 65 to the dominant negative mutation and haploinsufficiency (Hilhorst-Hofstee et al. 2011; Judge et 66 al. 2004; Judge & Dietz 2005). Therefore, more pathogenic genes or atypical mutation types in 67 specific populations are warranted to be identified. Here, we performed a family-based study using 68 whole-exome sequencing (WES) in 19 individuals, which were derived from three Han Chinese 69 MFS families. We identified three novel LOF mutations in FBN1 causing MFS. Systematical 70 evaluations and experimental replications were conducted to validate our findings. 71

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73 Materials and Methods

74 Study Subjects

A total of 19 voluntary family members (11males and 8 females, 8 cases and 11 controls) from 75 three Han Chinese families were recruited from the First Affiliated Hospital of Nanjing Medical 76 77 University during 2012 to 2016. The average age of onset of cases from 3 families was 21.75 (Family 1: I-1 loss to follow-up). All probands were diagnosed with MFS by medical record 78 review, physical examination, and family history following the clinical criteria (revised 79 80 Ghent nosology) which include: (i) Ectopia lentis; (ii) Systemic score \geq 7; (iii) Aortic root Z-score \geq 2, when there is history of Marfan syndrome in a primary relative (Loeys et al. 2010). The study 81 82 was approved by the institutional ethical committee of Nanjing Medical University and complied 83 with the principles of the declaration of Helsinki. All subjects included in this study were given



84 written informed consents.

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86 Whole-exome sequencing

Genomic DNA was isolated from peripheral blood using the OIAamp[™] DNA and Blood Mini 87 kit (QiagenTM) according to the protocol. Total DNA concentration and quantity were assessed by 88 measuring absorbance at 260 nm with NanoDrop 2000c Spectrophotometer (Thermo Scientific[™]). 89 WES library construction and sequencing were performed as follows: 300ng genomic DNA was 90 fragmented in Covaris® M220 Focused-ultrasonicator[™] to 100-500bp fragments followed by end 91 repair, adding A-tailing, adaptor ligation, and 11 PCR cycles according to the manufacturer's 92 protocols. After hybridization, exome enrichment was conducted with the Agilent XT SureSelect 93 Human All Exon v5 Kit, which targets ~50 Mb of the human exonic regions. Five DNA libraries 94 were multiplexed on every lane and 101 base paired-end sequencing was performed on Illumina 95 HiSeq1500 (Illumina, Inc). 96

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98 Quality control, mapping and variant calling

Raw sequencing reads were filtered to trim adapters and low quality reads using Trimmomatic-99 100 0.3.2 under PE module (ILLUMINACLIP: adapter.fa:2:30:10; LEADING: 3; TRAILING: 3; SLIDINGWINDOW: 4:15; MINLEN: 20). All the qualified reads were processed with an in-house 101 bioinformatics pipeline, which followed the best practice steps suggested by Genome Analysis 102 103 Toolkit (GATK v3.5) (DePristo et al. 2011). Briefly, we first aligned the clean sequence reads to the human reference genome (UCSC Genome Browser hg19) using Burrows-Wheeler Aligner 104 (BWA-MEM v0.7.12 with default parameters) (Li & Durbin 2010). PCR duplicates were removed 105 106 by Picard v1.141. After initial quality control (QC), all eligible sequences were determined for

regional realignment and base quality recalibration with GATK v3.5. Then variations including
single nucleotide variants (SNVs), insertions and deletions were called using GATK v3.5
HaplotypeCaller module.

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111 Gene Classification and Functional annotation

We used Annovar (2016Feb01) (Wang et al. 2010) for functional annotation with Online 112 Mendelian Inheritance in Man (OMIM), the Exome Aggregation Consortium (ExAC) Browser, 113 MutationTaster2 and The Combined Annotation Dependent Depletion (CADD). Based on OMIM 114 and MFS-related literature reported previously, genes were classified into three categories 115 according to American College of Medical Genetics (ACMG) standards and guidelines (Richards 116 et al. 2015): Category I: 8 MFS-causing genes reported directly; Category II: 125 MFS-related 117 genes from GeneCards; Category III: Unknown genes not reported previously (Supplementary 118 Table 1). 119

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121 Manually review and replication using Sanger sequencing

All remaining mutations were manually inspected using the Integrated Genome Viewer (IGV 2.3.80) (Thorvaldsdottir et al. 2013) before Sanger sequencing. PCR Primers were designed for the target regions and were used to amplify these regions by PCR for Sanger sequencing. Primers were listed in the **Supplementary Table 2**. Mutations were validated according to the resulting data screened through Chromas 2.4.1 and Dnaman 6.0.

127

- 128 **Results**
- 129 One 18-year-old male (the proband, II-1, age of onset was 10) of Han Chinese ancestry from

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Family 1 was diagnosed with MFS presented with an acute, anterior chest pain, like tearing on 130 admission. His father (I-1) also had MFS (Fig. 1A). Computed Tomography (CT) revealed that 131 the proband had an aortic aneurysm with dissection (type III), ascending aortic root dilatation with 132 the diameter of 4.9 cm. Family 2 is a large three-generation family with five family members 133 affected (I-2, I-5, I-6, II-1 and III-1) (Fig. 1B). The proband (II-1, age of onset was 30), a 31-year-134 135 old male, presented with the elongated digits but no pectus excavatum. CT showed an aortic aneurysm with dissection (type I). His mother (I-2), one aunt (I-5) and one uncle (I-6) all had MFS 136 with aortic aneurysms. His mother underwent surgery for aortic dissection in 1993 and died in 137 2015. Family 3 spanned three generations with two family members affected (I-2 and II-2). The 138 proband (II-2, age of onset was 27) was a 28-year-old male with a history of MFS. He underwent 139 thoracic surgery because of pectus excavatum when he was 2 years old. Although he had no 140 clinical symptoms of cardiovascular system, CT showed ascending aortic dilatation, aortic 141 regurgitation and mitral regurgitation. His mother (I-2) was also confirmed to have MFS (Fig. 1C). 142 In addition, although I-3 presented elongation of fingers mild pectus excavatum, the systemic score 143 = 4 and a ortic root Z-score < 2 and we had no sufficient clinical features necessary to perform the 144 diagnosis (Table 1). 145

Quality summary of sequencing for the 19 samples was summarized in **Supplement Table 3**. Every sample had an average of 69.46M raw reads, and about above 99.60% of them were successfully mapped to the reference genome. The average of median insert size was 201 bp and duplicates percent of reads ranged from 1.44% to 8.11%. Totally 237,252 variants were kept for following evaluation. To identify qualified pathogenic mutations, stringent criteria according to ACMG guidelines were performed (**Supplement Fig. 1**). Firstly, we removed these variants under following criteria: (i) untranslated region, synonymous, intronic variants (except variants

considered to be splicing variants and located at exon-intron junctions ranging from -5 to +5; (ii) 153 variants with minor allele frequency (MAF) \geq 1% based on 1000 Genomes (1KG) and ExAC 154 databases; (iii) variants present in our in-house whole genome sequencing (WGS) database of 100 155 non-Marfan controls. Then, we classified these rare genetic variants into three categories: a. MFS-156 causing genes; b. MFS-related genes; c. Unknown genes. Then we assessed whether these variants 157 158 were loss-of-function (nonsense, frameshift and essential splice-site variants). Three inheritance patterns were evaluated for the qualified mutations including autosomal dominant, autosomal 159 recessive and compound heterozygotes manners. Finally, we manually reviewed and selected 160 variants in Category I and II genes for technological validation. After replication by Sanger 161 sequencing, three LOF mutations in FBN1 were eventually identified in three families 162 respectively. For Family 1, the insertion (c.5027 5028insTGTCCTCC) was detected in both I-1 163 and II-1 individuals, which resulted in a frameshift (p.D1677Vfs*8). For Family 2, a heterozygous 164 deletion (c.5856delG) in exon 48 (NM 000138) was found in four patients (I-5, I-6, II-1 and III-165 1), also resulting in a frameshift (p.S1953Lfs*27). I-2 and II-2 individuals from family 3 were 166 heterozygous for the nonsense variant (c.8034C>A), which gained an immediate stop codon 167 (p.Y2678*). All mutations (c.5027 5028insTGTCCTCC, c.5856delG and c.8034C>A) identified 168 in three families were predicted to be disease-causing using MutationTaster2 and CADD. In 169 addition, all mutation sites were located in highly conserved amino acids region (Calcium-binding 170 EGF domain) across different species (Fig. 2). A summary of these mutations was presented in 171 172 Table 2. All healthy family members and other 100 non-MFS controls did not carry the FBN1 variants. It is worth noting that these mutations in FBNI have not been reported previously, but 173 the variant (c.5857dupT), nearby c.5856delG, was collected by the Human Gene Mutation 174 175 Database (HGMD). In addition to FBN1, We could only find co-segregation missense mutation in

- MFS associated Gene *FBN3*. The mutation (c.6722G>A, p.R2241Q) was identified in Family 3
 (I-2, II-2 and III-1) (Supplement Table 4).
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179 Discussion

MFS is a systemic disorder of connective tissue with a high degree of clinical variability that involves skeletal, ocular, and cardiovascular systems (Dietz 1993). In our study, massively parallel sequencing was performed to identify genetic abnormalities in three MFS families, and three functional variations in the three families have been detected in *FBN1*. All rare mutations have not been reported previously.

Fibrillins are the major components of microfibrils in the extracellular matrix of elastic and non-185 elastic tissues. Fibrillin-1 serves as a structural component of calcium-binding microfibrils and is 186 encoded by FBN1 gene. FBN1 is mapped to chromosome 15g21.1 and encodes a 2,871 amino acid 187 protein. More than 1,800 different mutations have been identified in FBN1, most of which are 188 associated with MFS from the UMD-FBN1 mutations database (Collod-Beroud et al. 1997; 189 Collod-Beroud et al. 1998; Collod et al. 1996) using a generic software called Universal Mutation 190 Database (UMD) (Collod-Beroud et al. 2003). Similar to UMD, approximately 1,700 variants in 191 FBN1 are associated with MFS according to the professional version of The Human Gene 192 Mutation Database (HGMD), which accounts for more than 90% of related variants. In our study, 193 a similar phenomenon was observed that all affected individuals could be attributed to FBN1 LOF 194 195 mutations (c.5027 5028insTGTCCTCC, c.5856delG and c.8034C>A).

Karina *et al.* reviewed the effects of mutations in fibrillin-1 on both a structural and functional
level (Zeyer & Reinhardt 2015). Calcium-binding epidermal growth factor (cbEGF)-like domains
are important for homotypic interaction (Lin et al. 2002), protection against proteolysis (Reinhardt

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et al. 1997), mediating ligand interactions (Cain et al. 2005; Reinhardt et al. 1996; Tiedemann et 199 al. 2001) and structural stabilization. The three identified mutations all located in the cbEGF and 200 the conservative domain. Accordingly to the study of Schrijver I et al. in the majority of PTC 201 (premature termination codon) samples, synthesis of normal-sized fibrillin protein was $\sim 50\%$ of 202 control levels, but matrix deposition was disproportionately decreased. They concluded that PTC 203 mutations have a major impact on the pathogenesis of type 1 fibrillinopathies. In our study, the 204 mutations (p.S1953Lfs*27 and p.Y2678*) are near the mutations (p.Q1955X and p.I2681X) 205 reported by Schrijver I et al (Schrijver et al. 2002). Istva'n et al. found that the relative amounts 206 of PTC-containing FBN1 transcripts in fresh and PAXgene-stabilized blood samples were 207 significantly higher (33.0+/-3.9%) to 80.0+/-7.2%) than those detected in affected fibroblasts with 208 inhibition of nonsense-mediated mRNA decay (NMD) (11.0+/-2.1% to 25.0+/-1.8%), whereas in 209 fibroblasts without NMD inhibition no mutant alleles could be detected (Magyar et al. 2009). So 210 mutated mRNAs could be stable and the mutant proteins could be detected in our study. We would 211 212 plan to conduct protein truncation test to confirm the activation of nonsense mediated decay or the production of truncated proteins (Tjeldhorn et al. 2015). 213

Further, we tend to focus on the genotype-phenotype correlation. Schrijver I et al. concluded 214 215 that PTC mutations have a major impact on the pathogenesis of type 1 fibrillinopathies and convey a distinct biochemical, clinical, and prognostic profile (Schrijver et al. 2002). Faivre et al. found 216 217 that patients with an FBN1 premature termination codon had a more severe skeletal and skin phenotype than did patients with an inframe mutation. Mutations in exons 24-32 were associated 218 219 with a more severe and complete phenotype (Faivre et al. 2007). In our study, the LOF mutations located in exon 41, 48 and 64 and patients did not have complete phenotype such as ectopia lentis 220 and skin striae. 221

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We have to note that III-1 in Family 2 and III-1 in Family 3 had no sufficient clinical features 222 necessary to early diagnosis due to the age. Fortunately, our pedigree analysis showed an 223 autosomal dominant pattern with high penetrance for FBN1 gene. In family 2, one 4-year-old boy 224 (III-1) with longer finger and anterior chest deformity (pectus excavatum) slightly was taller than 225 the peers as a suspected case. It was difficult to make clinical diagnosis owing to the age and 226 227 uncertain clinical status following the clinical criteria, but our WES-based screening may ease this process of disease definition for III-1. In family 3, we detected that clinically unaffected subject 228 I-3 had anterior chest deformity (pectus excavatum) slightly. In spite that she had Marfan 229 Syndrome-related symptom, they were clinically considered as healthy individuals, which is 230 consistent with the gene-based diagnosis result that she had no FBN1 or other pathogenic 231 mutations. Similarly, III-1 would be healthy. 232

The fibrillin-3 gene (*FBN3*) on human chromosome 19p13 is fragmented into 63 exons, and encodes a 2,809 amino acid protein. *FBN3* has overall homology of greater than 60% with either *FBN1* or *FBN2*, and contains multiple EGF-like domains (Nagase et al. 2001). Tomomi *et al.* indicated that disease-causing mutations in exon regions of the *FBN3* gene are either very rare in MFS or that the *FBN3* gene is not responsible for MFS (Uyeda et al. 2004). For the presence of *FBN1* mutations, we could not judge whether the mutation in *FBN3* was responsible right now.

All family members above would be followed up regularly to confirm the diagnosis. The identification of the causative gene variant in those uncertain phenotype or complex subjects by WES is of inestimable value for screening, clinical diagnosis and directing a personalized patient care with development of specific small-molecule therapies.

243

244 Conclusions

- 245 In conclusion, these interpreted results could help us further understand the genetic pathology of
- 246 MFS, and these mutations could be taken as genetic markers for pre- and postnatal screening and
- 247 genetic diagnosis for MFS.

248 Additional information and declarations

249 Data Availability

- 250 The following information was supplied regarding data availability:
- 251 The data is included in the manuscript (Table 1, 2 ;Fig. 1,2, and Supplementary data).

252 Web Resources

- 253 QiagenTM, https://www.qiagen.com
- 254 AgilentTM, https://www.agilent.com
- 255 Trimmomatic-0.3.2, http://www.usadellab.org/cms/index.php?page=trimmomatic
- 256 Genome Analysis Toolkit (GATK v3.5), https://software.broadinstitute.org/gatk
- 257 Burrows–Wheeler Aligner, BWA v0.7.12, http://bio-bwa.sourceforge.net
- 258 Picard v1.141, http://picard.sourceforge.net
- 259 Annovar (2016Feb01), http://annovar.openbioinformatics.org
- 260 Mendelian Inheritance in Man (OMIM), http://www.omim.org
- 261 Exome Aggregation Consortium (ExAC) Browser, http://exac.broadinstitute.org
- 262 MutationTaster2, http://www.mutationtaster.org/
- 263 The Combined Annotation Dependent Depletion (CADD), http://cadd.gs.washington.edu
- 264 GeneCards, http://www.genecards.org
- 265 Integrated Genome Viewer (IGV 2.3.80), http://www.broadinstitute.org/igv
- 266 Human Gene Mutation Database (HGMD), http://www.hgmd.cf.ac.uk
- 267 Universal Mutation Database (UMD), http://www.umd

269 **References**

270 Biggin A, Holman K, Brett M, Bennetts B, and Ades L. 2004. Detection of thirty novel FBN1 mutations in patients with 271 Marfan syndrome or a related fibrillinopathy. Hum Mutat 23:99. 10.1002/humu.9207 272 Cain SA, Baldock C, Gallagher J, Morgan A, Bax DV, Weiss AS, Shuttleworth CA, and Kielty CM. 2005. Fibrillin-1 273 interactions with heparin. Implications for microfibril and elastic fiber assembly. J Biol Chem 280:30526-274 30537. 10.1074/jbc.M501390200 275 Chiu HH, Wu MH, Chen HC, Kao FY, and Huang SK. 2014. Epidemiological profile of Marfan syndrome in a general 276 population: a national database study. Mayo Clin Proc 89:34-42. 10.1016/j.mayocp.2013.08.022 277 Collod-Beroud G, Beroud C, Ades L, Black C, Boxer M, Brock DJ, Godfrey M, Hayward C, Karttunen L, Milewicz D, 278 Peltonen L, Richards RI, Wang M, Junien C, and Boileau C. 1997. Marfan Database (second edition): software 279 and database for the analysis of mutations in the human FBN1 gene. *Nucleic Acids Res* 25:147-150. 280 Collod-Beroud G, Beroud C, Ades L, Black C, Boxer M, Brock DJ, Holman KJ, de Paepe A, Francke U, Grau U, Hayward 281 C, Klein HG, Liu W, Nuytinck L, Peltonen L, Alvarez Perez AB, Rantamaki T, Junien C, and Boileau C. 1998. 282 Marfan Database (third edition): new mutations and new routines for the software. Nucleic Acids Res 283 26:229-223. 284 Collod-Beroud G, Le Bourdelles S, Ades L, Ala-Kokko L, Booms P, Boxer M, Child A, Comeglio P, De Paepe A, Hyland 285 JC, Holman K, Kaitila I, Loeys B, Matyas G, Nuytinck L, Peltonen L, Rantamaki T, Robinson P, Steinmann B, Junien C, Beroud C, and Boileau C. 2003. Update of the UMD-FBN1 mutation database and creation of an 286 287 FBN1 polymorphism database. Hum Mutat 22:199-208. 10.1002/humu.10249 288 Collod G, Beroud C, Soussi T, Junien C, and Boileau C. 1996. Software and database for the analysis of mutations in 289 the human FBN1 gene. Nucleic Acids Res 24:137-140. 290 Cotran RS, Kumar V, Collins T, and Robbins SL. 1999. Robbins pathologic basis of disease. Philadelphia: Saunders. 291 DePristo MA, Banks E, Poplin R, Garimella KV, Maguire JR, Hartl C, Philippakis AA, del Angel G, Rivas MA, Hanna M, 292 McKenna A, Fennell TJ, Kernytsky AM, Sivachenko AY, Cibulskis K, Gabriel SB, Altshuler D, and Daly MJ. 293 2011. A framework for variation discovery and genotyping using next-generation DNA sequencing data. Nat 294 Genet 43:491-498. 10.1038/ng.806 295 Dietz H. 1993. Marfan Syndrome. In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LIH, Mefford HC, Stephens 296 K, Amemiya A, and Ledbetter N, eds. *GeneReviews((R))*. Seattle (WA). 297 Faivre L, Collod-Beroud G, Loeys BL, Child A, Binguet C, Gautier E, Callewaert B, Arbustini E, Mayer K, Arslan-Kirchner 298 M, Kiotsekoglou A, Comeglio P, Marziliano N, Dietz HC, Halliday D, Beroud C, Bonithon-Kopp C, Claustres M, 299 Muti C, Plauchu H, Robinson PN, Ades LC, Biggin A, Benetts B, Brett M, Holman KJ, De Backer J, Coucke P, 300 Francke U, De Paepe A, Jondeau G, and Boileau C. 2007. Effect of mutation type and location on clinical 301 outcome in 1,013 probands with Marfan syndrome or related phenotypes and FBN1 mutations: an 302 international study. Am J Hum Genet 81:454-466. 10.1086/520125 303 Habashi JP, Judge DP, Holm TM, Cohn RD, Loeys BL, Cooper TK, Myers L, Klein EC, Liu G, Calvi C, Podowski M, Neptune 304 ER, Halushka MK, Bedja D, Gabrielson K, Rifkin DB, Carta L, Ramirez F, Huso DL, and Dietz HC. 2006. Losartan, 305 an AT1 antagonist, prevents aortic aneurysm in a mouse model of Marfan syndrome. Science 312:117-121. 306 10.1126/science.1124287 307 Hilhorst-Hofstee Y, Hamel BC, Verheij JB, Rijlaarsdam ME, Mancini GM, Cobben JM, Giroth C, Ruivenkamp CA, 308 Hansson KB, Timmermans J, Moll HA, Breuning MH, and Pals G. 2011. The clinical spectrum of complete 309 FBN1 allele deletions. Eur J Hum Genet 19:247-252. 10.1038/ejhg.2010.174 310 Holm TM, Habashi JP, Doyle JJ, Bedja D, Chen Y, van Erp C, Lindsay ME, Kim D, Schoenhoff F, Cohn RD, Loeys BL, 311 Thomas CJ, Patnaik S, Marugan JJ, Judge DP, and Dietz HC. 2011. Noncanonical TGFbeta signaling 312 contributes to aortic aneurysm progression in Marfan syndrome mice. Science 332:358-361. 313 10.1126/science.1192149 314 Judge DP, Biery NJ, Keene DR, Geubtner J, Myers L, Huso DL, Sakai LY, and Dietz HC. 2004. Evidence for a critical 315 contribution of haploinsufficiency in the complex pathogenesis of Marfan syndrome. J Clin Invest 114:172-316 181. 10.1172/JCI20641 317 Judge DP, and Dietz HC. 2005. Marfan's syndrome. Lancet 366:1965-1976. 10.1016/S0140-6736(05)67789-6 318 Li H, and Durbin R. 2010. Fast and accurate long-read alignment with Burrows-Wheeler transform. Bioinformatics 319 26:589-595. 10.1093/bioinformatics/btp698

Li J, Wu W, Lu C, Liu Y, Wang R, Si N, Liu F, Zhou J, Zhang S, and Zhang X. 2017. Gross deletions in FBN1 results in variable phenotypes of Marfan syndrome. *Clin Chim Acta* 474:54-59. 10.1016/j.cca.2017.08.023

- Lin G, Tiedemann K, Vollbrandt T, Peters H, Batge B, Brinckmann J, and Reinhardt DP. 2002. Homo- and heterotypic
 fibrillin-1 and -2 interactions constitute the basis for the assembly of microfibrils. *J Biol Chem* 277:50795 50804. 10.1074/jbc.M210611200
- Loeys BL, Dietz HC, Braverman AC, Callewaert BL, De Backer J, Devereux RB, Hilhorst-Hofstee Y, Jondeau G, Faivre L,
 Milewicz DM, Pyeritz RE, Sponseller PD, Wordsworth P, and De Paepe AM. 2010. The revised Ghent
 nosology for the Marfan syndrome. *J Med Genet* 47:476-485. 10.1136/jmg.2009.072785
- Magyar I, Colman D, Arnold E, Baumgartner D, Bottani A, Fokstuen S, Addor MC, Berger W, Carrel T, Steinmann B,
 and Matyas G. 2009. Quantitative sequence analysis of FBN1 premature termination codons provides
 evidence for incomplete NMD in leukocytes. *Hum Mutat* 30:1355-1364. 10.1002/humu.21058
- Nagase T, Nakayama M, Nakajima D, Kikuno R, and Ohara O. 2001. Prediction of the coding sequences of unidentified
 human genes. XX. The complete sequences of 100 new cDNA clones from brain which code for large
 proteins in vitro. DNA Res 8:85-95.
- Pinard A, Salgado D, Desvignes JP, Rai G, Hanna N, Arnaud P, Guien C, Martinez M, Faivre L, Jondeau G, Boileau C,
 Zaffran S, Beroud C, and Collod-Beroud G. 2016. WES/WGS Reporting of Mutations from Cardiovascular
 "Actionable" Genes in Clinical Practice: A Key Role for UMD Knowledgebases in the Era of Big Databases.
 Hum Mutat 37:1308-1317. 10.1002/humu.23119
- Pyeritz RE, and McKusick VA. 1979. The Marfan syndrome: diagnosis and management. *N Engl J Med* 300:772-777.
 10.1056/NEJM197904053001406
- Reinhardt DP, Ono RN, and Sakai LY. 1997. Calcium stabilizes fibrillin-1 against proteolytic degradation. J Biol Chem
 272:1231-1236.
- Reinhardt DP, Sasaki T, Dzamba BJ, Keene DR, Chu ML, Gohring W, Timpl R, and Sakai LY. 1996. Fibrillin-1 and fibulin-2
 interact and are colocalized in some tissues. *J Biol Chem* 271:19489-19496.
- Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, Voelkerding K,
 Rehm HL, and Committee ALQA. 2015. Standards and guidelines for the interpretation of sequence variants:
 a joint consensus recommendation of the American College of Medical Genetics and Genomics and the
 Association for Molecular Pathology. *Genet Med* 17:405-424. 10.1038/gim.2015.30
- Schrijver I, Liu W, Odom R, Brenn T, Oefner P, Furthmayr H, and Francke U. 2002. Premature termination mutations
 in FBN1: distinct effects on differential allelic expression and on protein and clinical phenotypes. *Am J Hum Genet* 71:223-237. 10.1086/341581
- Sponseller PD, Hobbs W, Riley LH, 3rd, and Pyeritz RE. 1995. The thoracolumbar spine in Marfan syndrome. J Bone
 Joint Surg Am 77:867-876.
- Stheneur C, Collod-Beroud G, Faivre L, Buyck JF, Gouya L, Le Parc JM, Moura B, Muti C, Grandchamp B, Sultan G,
 Claustres M, Aegerter P, Chevallier B, Jondeau G, and Boileau C. 2009. Identification of the minimal
 combination of clinical features in probands for efficient mutation detection in the FBN1 gene. *Eur J Hum Genet* 17:1121-1128. 10.1038/ejhg.2009.36
- 357Thorvaldsdottir H, Robinson JT, and Mesirov JP. 2013. Integrative Genomics Viewer (IGV): high-performance358genomics data visualization and exploration. Brief Bioinform 14:178-192. 10.1093/bib/bbs017
- Tiedemann K, Batge B, Muller PK, and Reinhardt DP. 2001. Interactions of fibrillin-1 with heparin/heparan sulfate,
 implications for microfibrillar assembly. *J Biol Chem* 276:36035-36042. 10.1074/jbc.M104985200
- Tjeldhorn L, Amundsen SS, Baroy T, Rand-Hendriksen S, Geiran O, Frengen E, and Paus B. 2015. Qualitative and
 quantitative analysis of FBN1 mRNA from 16 patients with Marfan Syndrome. *BMC Med Genet* 16:113.
 10.1186/s12881-015-0260-4
- Uyeda T, Takahashi T, Eto S, Sato T, Xu G, Kanezaki R, Toki T, Yonesaka S, and Ito E. 2004. Three novel mutations of
 the fibrillin-1 gene and ten single nucleotide polymorphisms of the fibrillin-3 gene in Marfan syndrome
 patients. J Hum Genet 49:404-407. 10.1007/s10038-004-0168-x
- von Kodolitsch Y, and Robinson PN. 2007. Marfan syndrome: an update of genetics, medical and surgical
 management. *Heart* 93:755-760. 10.1136/hrt.2006.098798
- Wang K, Li M, and Hakonarson H. 2010. ANNOVAR: functional annotation of genetic variants from high-throughput
 sequencing data. *Nucleic Acids Res* 38:e164. 10.1093/nar/gkq603
- Wieczorek P, Riegel MB, Quattro L, and DeMaio K. 1996. Marfan's syndrome and surgical repair of ascending aortic
 aneurysms. AORN J 64:895-913; quiz 916-898, 921-892.

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380381 Abbreviations:

382 Figure 1. Pedigree and mutations in *FBN1* for Marfan syndrome patients

- **1A:** *FBN1* insertion mutation (M1) was identified in two subjects with MFS (I-1 and II-1) from
- Family 1; 1B: FBN1 deletion mutation (M2) was identified in four subjects with MFS (I-5, I-6,
- II-1 and III-1) from Family 2; 1C: *FBN*1 the nonsense mutation (M3) was identified in two subjects
- 386 with MFS (I-2 and II-2) from Family 3. 3 individuals in the pedigrees were not sequenced
- including Family 2: I-2, I-7 and Family 3: I-4. W indicates wildtype allele. Circles represent female
- 388 participants and squares male participants. Black symbols indicate patients with Marfan syndrome.
- A slash through the symbol indicates that the family member is deceased. Arrows indicate the proband.

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392 Figure 2. The locations of mutations in *FBN1* across different species

The positions of three LOF mutations in *FBN1* are shown. The affected amino acid residues are conserved across species.

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396 Supplementary Figure 1. Analytical pipeline for exome sequence filtration and

397 prioritization

398 Abbreviations: MAF, minor allele frequency; UTR, untranslated region.

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Table 1(on next page)

Clinical symptoms of all 19 members in three Marfan families

Facial features (3/5) = 1 (dolichocephaly, enophthalmos, downslanting palpebral fissures,

malar hypoplasia, retrognathia) ND = not detected NA = not available *Suspected case

1 Table 1. Clinical symptoms of all 19 members in three Marfan families

| Family ID | Member ID | Age of onset | Age | Wrist AND thumb sign | Pectus carinatum deformity(pectus excavatum or chest asymmetry) | Hindfoot deformity (plain pes planus) | Dural ectasia | Protrusio acetabuli | Pneumothorax | Reduced upper segment/lower segment ratio AND increased arm/height AND no severe scoliosis | Scoliosis or thoracolumbar kyphosis | Reduced elbow extension | Facial features | Skin striae (stretch marks) | Myopia > 3 diopters | Mitral valve prolapse | Systemic score | Aortic root Z- score | Case |
|--------------|--------------|-----------------|-----|-------------------------------|--|--|------------------|------------------------|--------------|--|---|-------------------------------|--------------------|--------------------------------------|---------------------------|-----------------------------|-------------------|----------------------------|------|
| Score | | | | 3 | 2(1) | 2(1) | 2 | 2 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | | |
| F1 | I-1 | 25 | 46 | \checkmark | \checkmark | × | × | × | \checkmark | × | × | × | × | × | × | \checkmark | 8 | ND | 1 |
| F1 | I-2 | | 43 | × | × | × | × | × | × | × | × | × | × | × | × | × | 0 | ND | 0 |
| F1 | II-1 | 10 | 21 | \checkmark | \checkmark | × | × | × | \checkmark | × | × | × | × | × | × | \checkmark | 8 | ≥ 2 | 1 |
| F2 | I-1 | | 66 | × | × | × | × | × | × | × | × | × | × | × | × | × | 0 | ND | 0 |
| F2 | I-3 | | 60 | × | × | × | × | × | × | × | × | × | × | × | × | × | 0 | ND | 0 |
| F2 | I-4 | | 63 | × | × | × | × | × | × | × | × | × | × | × | × | × | 0 | ND | 0 |
| F2 | 1-5 | 30 | 65 | × | × | \checkmark | × | × | × | \checkmark | × | \checkmark | | × | × | \checkmark | 6 | ND | 1 |
| F2 | I-6 | 20 | 67 | × | × | \checkmark | × | × | × | \checkmark | × | \checkmark | \checkmark | × | × | \checkmark | 6 | ND | 1 |

Manuscript to be reviewed

| F2 | II-1 | 30 | 36 | × | × | \checkmark | × | × | × | \checkmark | × | \checkmark | \checkmark | × | × | \checkmark | 6 | ≥ 2 | 1 |
|----|--------|----|----|--------------|--------------|--------------|---|---|---|--------------|---|--------------|--------------|---|---|--------------|---|----------|----|
| F2 | II-2 | | 30 | × | × | × | × | × | × | × | × | × | × | × | × | × | 0 | ND | 0 |
| F2 | II-3 | | 50 | × | х | × | × | × | × | × | × | × | × | × | × | × | 0 | ND | 0 |
| F2 | III-1* | 2 | 5 | × | \checkmark | × | × | × | × | × | × | × | × | × | × | × | 1 | ND | 0* |
| F3 | I-1 | | 52 | × | × | × | × | × | × | × | × | × | × | × | × | × | 0 | ND | 0 |
| F3 | I-2 | 30 | 49 | × | \checkmark | \checkmark | × | × | × | × | × | × | × | × | × | \checkmark | 5 | ND | 1 |
| F3 | I-3 | | 46 | \checkmark | \checkmark | × | × | × | × | × | × | × | × | × | × | × | 4 | <2 | 0 |
| F3 | II-2 | 27 | 29 | × | \checkmark | \checkmark | × | × | × | × | × | × | × | × | × | \checkmark | 5 | ≥ 2 | 1 |
| F3 | II-3 | | 28 | × | × | × | × | × | × | × | × | × | × | × | × | × | 0 | ND | 0 |
| F3 | II-4 | | 22 | × | × | × | × | × | × | × | × | × | × | × | × | × | 0 | ND | 0 |
| F3 | III-1 | | 6 | × | × | × | × | × | × | × | × | × | × | × | × | × | 0 | ND | 0 |

2 Facial features (3/5) = 1 (dolichocephaly, enophthalmos, downslanting palpebral fissures, malar hypoplasia, retrognathia)

3 ND = not detected

4 NA = not available

5 *Suspected case



Table 2(on next page)

FBN1 variants identified for affected individuals in three Marfan families

| Family ID | F1 | F2 | F3 |
|---------------------------------|-----------------------|--------------------|------------|
| Chr. | chr15 | chr15 | chr15 |
| Position | 48,756,133 | 48,737,634 | 48,707,750 |
| Ref allele | - | G | С |
| Alt allele | TGTCCTCC | - | А |
| Gene | FBN1 | FBN1 | FBNI |
| Mutation type | insertion | deletion | nonsense |
| Exon | 41/66 | 48/66 | 64/66 |
| Codon change | c.5027_5028insTGTCCTC | C c.5856delG | c.8034C>A |
| Amino acid change | p.D1677Vfs*8 | p.S1953Lfs*27 | p.Y2678* |
| Affected individuals | I-1/II-1 | I-5/I-6/II-1/III-1 | I-2/II-2 |
| CADD Raw Score | 9.18 | 7 | 16.63 |
| PHRED scaled score [†] | 35 | 33 | 56 |

1 Table 2. FBN1 variants identified for affected individuals in three Marfan families

2 † PHRED-like scaled C-scores = - 10 * $\log_{10}(\frac{\operatorname{rank}}{\operatorname{total}})$, the recommended deleterious threshold was > 15 for scaled C-scores

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Figure 1

Pedigree and mutations in FBN1 for Marfan syndrome patients

1A: *FBN1* insertion mutation (M1) was identified in two subjects with MFS (I-1 and II-1) from Family 1; **1B:** *FBN1* deletion mutation (M2) was identified in four subjects with MFS (I-5, I-6, II-1 and III-1) from Family 2; **1C:** *FBN*1 the nonsense mutation (M3) was identified in two subjects with MFS (I-2 and II-2) from Family 3. 3 individuals in the pedigrees were not sequenced including Family 2: I-2, I-7 and Family 3: I-4. W indicates wildtype allele. Circles represent female participants and squares male participants. Black symbols indicate patients with Marfan syndrome. A slash through the symbol indicates that the family member is deceased. Arrows indicate the proband.



Figure 2

The locations of mutations in FBN1 across different species

The positions of three LOF mutations in *FBN1* are shown. The affected amino acid residues are conserved across species.

