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Molecular characterization and investigation of the role of genetic variation in phenotypic variability and response to treatment in a large pediatric Marfan syndrome cohort



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ABSTRACT

Purpose: In a large cohort of 373 pediatric patients with Marfan syndrome (MFS) with a severe cardiovascular phenotype, we explored the proportion of patients with MFS with a pathogenic *FBNI* variant and analyzed whether the type/location of *FBNI* variants was associated with specific clinical characteristics and response to treatment. Patients were recruited on the basis of the following criteria: aortic root z-score > 3, age 6 months to 25 years, no prior or planned surgery, and aortic root diameter < 5 cm.

Methods: Targeted resequencing and deletion/duplication testing of *FBNI* and related genes were performed.

Results: We identified (likely) pathogenic *FBNI* variants in 91% of patients. Ectopia lentis was more frequent in patients with dominant-negative (DN) variants (61%) than in those with haploinsufficient variants (27%). For DN *FBNI* variants, the prevalence of ectopia lentis was highest in the N-terminal region (84%) and lowest in the C-terminal region (17%). The association with a more severe cardiovascular phenotype was not restricted to DN variants in the neonatal *FBNI* region (exon 25–33) but was also seen in the variants in exons 26 to 49. No difference in the therapeutic response was detected between genotypes.

Conclusion: Important novel genotype–phenotype associations involving both cardiovascular and extra-cardiovascular manifestations were identified, and existing ones were confirmed. These findings have implications for prognostic counseling of families with MFS.

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Introduction

Marfan syndrome (MFS, OMIM 154700) is an autosomal dominant connective tissue disorder with multisystemic manifestations, and the disorder mainly affects the skeletal (eg, long bone overgrowth, joint hypermobility, scoliosis, pectus deformity), ocular (eg, myopia, ectopia lentis [EL]), and cardiovascular systems. This disorder has an estimated prevalence between 1 in 3000 and 1 in 5000.¹ The vast majority of patients with MFS ultimately develop an aortic aneurysm, typically at the level of the sinuses of Valsalva, with a propensity to aortic dissection.

Although MFS was initially described in 1898, it took approximately 100 years to discover its genetic cause. Pathogenic variants in the *FBNI* gene, which codes for the extracellular matrix protein FBN1, are the cause of MFS.² Over the past 25 years, >3000 pathogenic *FBNI* variants have been described (*FBNI*-Universal Mutation Database). Today, variants curated as pathogenic or likely pathogenic (P/LP) are identified in over 90% to 95% of patients with MFS, and molecular testing has become an integral part of the diagnostic decision-making process (revised Ghent nosology).³

Although genetic analysis of *FBNI* now contributes significantly to the diagnostic process, its predictive value to phenotypic outcomes is still limited. Nevertheless, uncovering genotype–phenotype associations facilitates genetic counseling and potentially allows the development of more personalized surgical and/or pharmacological patient-management strategies. *FBNI* variants causing MFS are distributed across the entire gene, encompass all variant types, and can be categorized largely into 2 groups, namely haploinsufficiency (HI) and dominant-negative (DN) variants. HI variants lead to a decreased amount of FBN1 protein in the extracellular matrix. DN variants result in the production of a mutant protein that can interfere with the function of wild-type FBN1 derived from the normal allele of the gene. The effects of both HI and DN variants can contribute to the pathogenesis of MFS.^{4,5}

In this study, we performed a molecular screening and sought genotype–phenotype associations in a cohort of well-characterized patients with MFS participating in the Pediatric Heart Network (PHN) Marfan trial (atenolol vs losartan).^{6–8} This is the largest pediatric and adolescent MFS genotype–phenotype study reported to date.

Materials and Methods

Patient cohort

The PHN Marfan Trial (atenolol vs losartan; [ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT00429364) Identifier: NCT00429364) randomized 608 patients; of these patients, only a subset had previously undergone *FBNI* genetic testing.⁶ Patients were recruited on the basis of the following criteria: aortic root z-score > 3, age 6 months to 25 years, no prior or planned surgery, and aortic

root diameter < 5 cm. All patients were offered the opportunity to participate in this genetics ancillary cohort study by donating an additional blood sample after signing informed consent. In total, 304 patients (50%) donated a blood sample for genetic analysis, which resulted in bad quality DNA of 2 patients (patient identifier (ID) 372 and 373). For the genotype–phenotype analyses, we also incorporated the *FBNI* analysis results from 69 patients who did not provide a blood sample but agreed to share their genetic test results through their treating physician. Overall, detailed genetic information and clinical phenotyping for 373 patients with MFS,⁶ as well as aortic measurements over 3 years of treatment with losartan or atenolol, were available at the start of this genetics ancillary study.⁷ At least 27 patients had 1 or 2 family members included in the genetics ancillary study (as indicated in [Supplemental Tables 1A and 1B](#)).

Molecular analysis

After standard DNA extraction methods and HaloPlex enrichment of target regions (Agilent Technologies) of 14 core thoracic aortic aneurysm and dissection (TAAD) genes ([Supplemental Table 2](#), genes in bold), sequencing was performed on MiSeq System (Illumina).⁹ Data analysis was performed using an in-house developed pipeline (for details see Proost et al⁹). When no pathogenic variant in *FBNI* could be identified, multiplex ligation-dependent probe amplification for the detection of deletions/duplications of *FBNI* and *TGFBR2* was performed (MRC Holland).⁹ If both sequencing and multiplex ligation-dependent probe amplification were negative, an analysis using an extended TAAD panel comprising 34 genes was carried out ([Supplemental Table 2](#), underlined genes). Variants were classified as pathogenic, likely pathogenic, or variant of uncertain significance (VUS) according to the adjusted American College of Medical Genetics and Genomics /Association for Molecular Pathology criteria ([Supplemental Tables 1A, 1B, and 3](#)).¹⁰ P/LP *FBNI* variants were grouped into 2 classes on the basis of molecular criteria only: DN and HI ([Supplemental Table 4](#)). All variants were submitted to ClinVar.

Statistical analysis

Statistical analyses were performed using SPSS Statistics (IBM SPSS Statistics for Windows version 27.0, IBM Corporation), and all hypothesis tests were 2-sided. Comparisons of the categorical clinical characteristics between different patient groups were performed using the χ^2 or Fisher exact tests. Continuous clinical variables were compared using the unpaired *t* test. When there were >2 groups, differences in aortic root z-score were tested using 1-way analysis of variance. The effect of variant type on response to treatment (change in aortic root z-score/year) was modeled using analysis of covariance, including age (the only factor found significant in the main PHN clinical trial) as a covariate. Assumptions of the used tests were verified, including the use

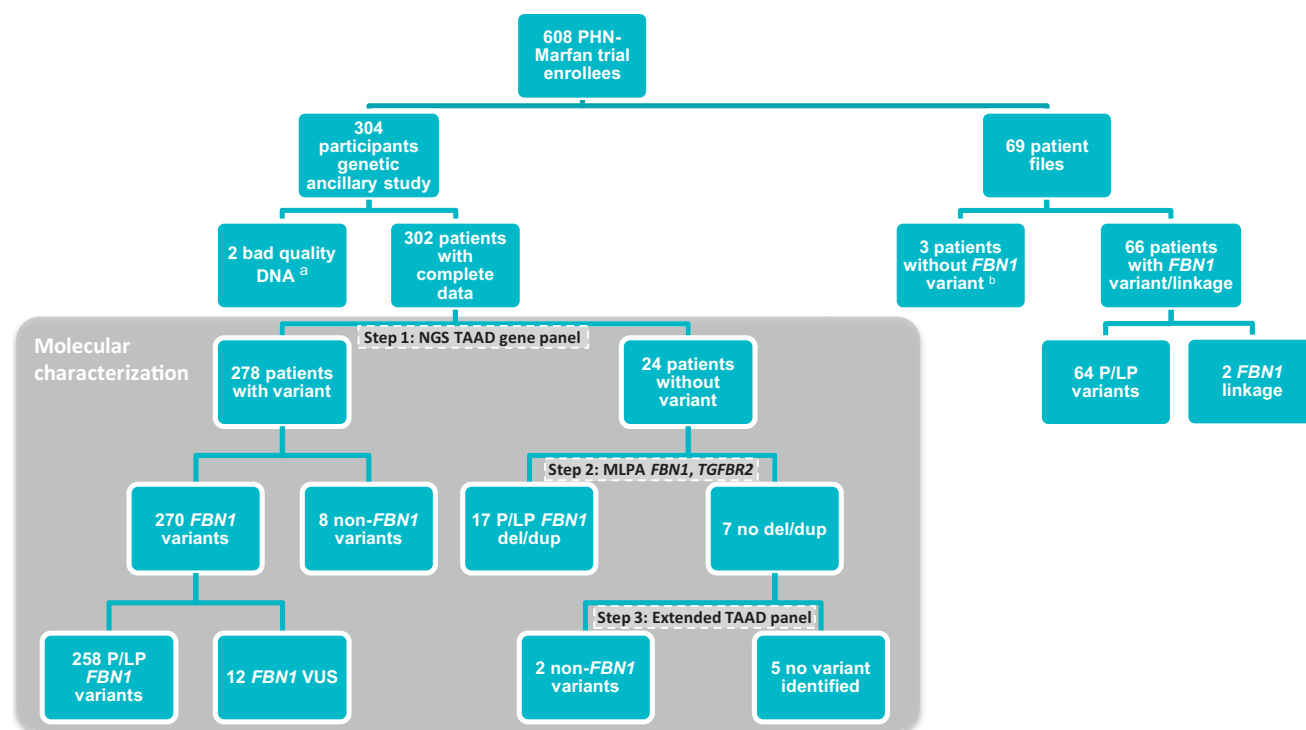


Figure 1 Patient cohort and workflow of molecular characterization. Overview of the participants included in this study and the results of their molecular screening. ^aIdentifiers (IDs) of patients with bad quality DNA: 372 and 373. ^bIDs of patients who did not have an *FBNI* variant described in their patient report and were not sequenced in our genetic ancillary study: 369, 370, and 371. del, deletion; dup, duplication; MLPA, multiplex ligation-dependent probe amplification; NGS, next-generation sequencing; P/LP, pathogenic or likely pathogenic; PHN, Pediatric Heart Network; TAAD, Thoracic Aortic Aneurysm and Dissection; VUS, variant of uncertain significance

of Levene's test to assess the equality of variances. Patients who were lost for follow-up were excluded from the response to treatment analysis. Missing data were left as missing, without imputation. We carried out a false discovery analysis to account for the multiple hypotheses testing in R (R version 4.0.3, R Foundation for Statistical Computing) and R-package *Q*-value (package version 2.18.0). $Q \leq 0.05$ was considered significant.

Results

Molecular screening of 302 patients with MFS and *FBNI* variant spectrum

Figure 1 illustrates the workflow and results of the molecular screening. In total, we identified 270 variants in *FBNI* (Supplemental Table 1A) and 10 patients with variants in other TAAD genes (Supplemental Table 1B). Furthermore, 17 deletions/duplications of (part of) the *FBNI* gene were identified. Overall, 275 (91%) patients had a P/LP *FBNI* variant, 12 (4%) patients had a *FBNI*-VUS, 10 (3%) patients had a P/LP variant or VUS in another gene associated with syndromic aortic aneurysms, and 5 (2%) patients had no causal variant identified. The clinical features of these TAAD panel-negative patients ($n = 5$) are listed in Supplemental Table 5. In 4 patients, 2 *FBNI* variants were identified (always 1 P/LP *FBNI* variant and 1 VUS).

To study the *FBNI* variant spectrum, we combined the results from our molecular screening with 64 patient reports that contained a *FBNI* P/LP variant. In total, 339 P/LP *FBNI* variants were identified in this extended cohort. These variants included 46% missense (of which 68% were cysteine-involving), 19% frameshift, 16% nonsense, and 13% splice site variants. In addition, 5% of subjects harbored a large deletion/duplication and 1% a small in-frame deletion/duplication (Figure 1). The overall distribution of the variant subtypes is comparable between the largest MFS cohort reported in the literature and our cohort (Supplemental eFigure 1).¹¹

Genotype-phenotype associations

Overall cohort

No significant differences regarding major clinical characteristics, except positive family history (56% vs 71%, $P = 2.4 \times 10^{-4}$, $Q = 0.003$), were found between the genetics ancillary cohort ($n = 373$) and the trial patients not enrolled in this ancillary study ($n = 235$) (Supplemental Table 6).

Variant type: HI vs DN

We compared the phenotypes of individuals in the predicted DN ($n = 174$) and HI ($n = 125$) *FBNI* groups (Figure 2, Table 1). In total, 40 P/LP *FBNI* variants could not be classified in either of these groups because of uncertainty

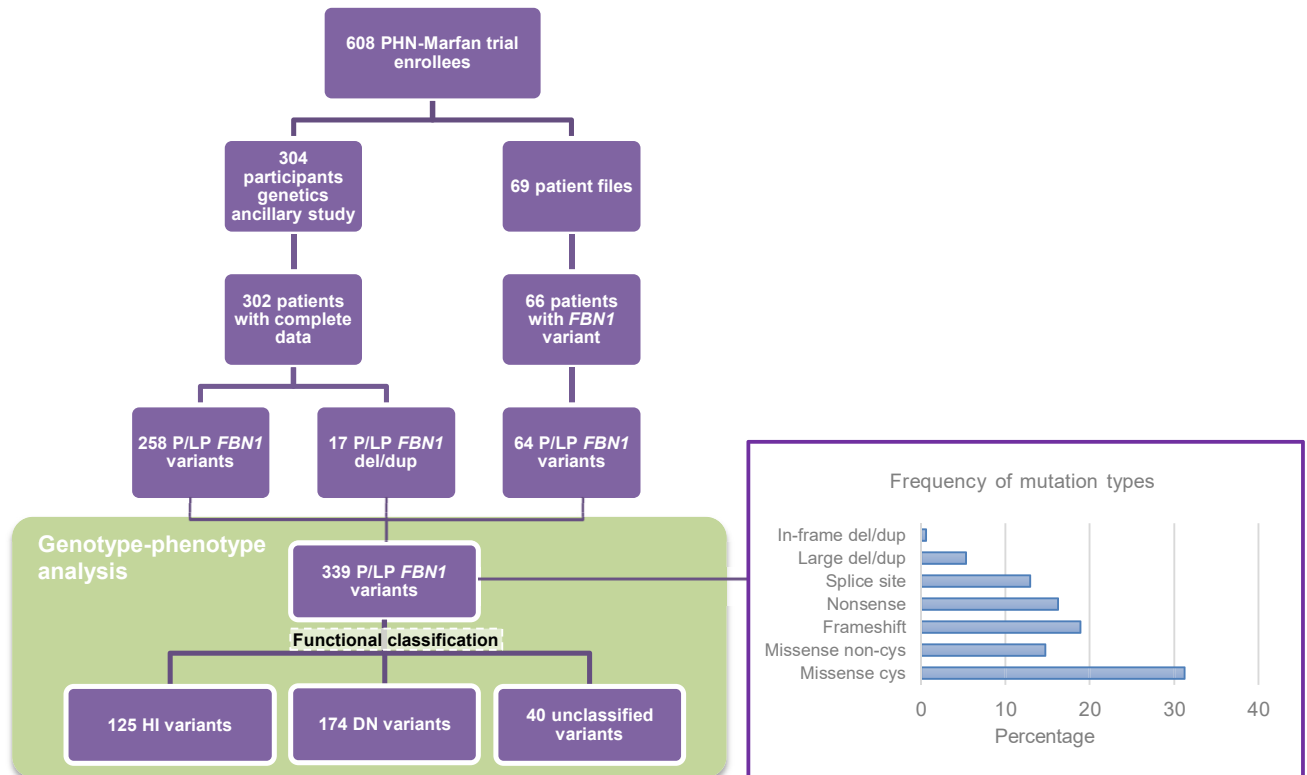


Figure 2 Workflow of genotype-phenotype analysis. Overview of the variant workflow, functional classification, and variant types. cys, cysteine; del, deletion; DN, dominant negative; dup, duplication; HI, haploinsufficient; P/LP, pathogenic or likely pathogenic; PHN, Pediatric Heart Network.

about their effect and were excluded from this comparative analysis. We observed no difference in aortic root diameters (absolute or z-score) or aortic root growth progression between the 2 groups. DN *FBNI* variants in the entire gene were significantly associated with EL (61% [99/161]) when compared with HI variants (27% [29/109]) ($P = 1.8 \times 10^{-8}$, $Q = 5.4 \times 10^{-7}$), especially when only the cysteine-involving variants (71% [72/102]) were compared with HI variants (27% [29/109]) ($P = 1.6 \times 10^{-10}$, $Q = 1.0 \times 10^{-8}$). Skeletal features were more pronounced in patients harboring HI variants; pectus excavatum was more frequent in patients with HI variants (36% [45/125]) than in patients harboring DN variants (21% [36/174]) ($P = .003$, $Q = 0.03$), and the patients harboring HI variants had a taller stature (height z-score = 2.4 ± 1.1) than those heterozygous for DN variants (1.9 ± 1.2) ($P = .001$, $Q = 0.01$). A similar trend was observed for joint hypermobility (HI: 89% [109/122] vs DN: 80% [134/168]) ($P = .03$, $Q = 0.18$), but this P -value did not survive multiple hypotheses analysis. The P -values for these skeletal features did not change significantly when only the subset of patients with cysteine-involving or other missense variants were compared with patients with HI variants.

Variant location

Exon 25 to 33 vs other

This neonatal *FBNI* region is formerly described to include exons 24 to 32. However, in the most recent reference transcript of *FBNI* (NM_000138.4), the noncoding exon 1

is added, which causes a shift in the numbering. Therefore, the neonatal region in this manuscript is now referred to as exon 25 to 33. Although we observed a higher aortic root z-score for patients with P/LP DN *FBNI* variants in the so-called neonatal or middle region of the *FBNI* gene (exon 25-33, 4.9 ± 1.5 , $n = 33$) than for patients with HI and other P/LP DN *FBNI* variants (4.2 ± 1.1 , $n = 265$, $P = .02$, $Q = 0.11$, Supplemental Table 7), this did not survive multiple hypotheses analysis. We also observed a lower prevalence of striae (21% [7/33] vs 40% [105/264]) ($P = .04$, $Q = 0.21$) and higher prevalence of arachnodactyly (84% [26/31] vs 66% [173/261]) ($P = .05$, $Q = 0.22$) in the P/LP DN *FBNI* neonatal group than in the HI and other P/LP DN *FBNI* variant group. However, none of these 3 P -values survived multiple hypotheses analysis.

Exon 26 to 49 vs other

When visualizing the average aortic root z-score per exon for DN variants, the region with a more severe aortic phenotype appeared to be bigger than just the exon 25 to 33 region (Supplemental Table 8, Supplemental eFigure 2). We observed a significantly higher aortic root z-score for patients with P/LP DN variants in exons 26 to 49 (4.8 ± 1.4 , $n = 76$) than for patients with other P/LP DN or HI *FBNI* variants (4.1 ± 1.0 , $n = 223$, $P = 5.0 \times 10^{-5}$, $Q = 7.6 \times 10^{-4}$).

5'-End vs middle vs 3'-end

On visualizing the proportion of EL in patients with DN P/LP variants on the basis of their location, a novel

Table 1 Comparison of clinical phenotype in DN and HI variants patient groups

Clinical Features	DN (<i>n</i> = 174)	HI (<i>n</i> = 125)	<i>P</i>	<i>Q</i>
Age ± SD (y)	11.1 ± 6.1	10.9 ± 6.1	.81	0.75
Height ± SD (z-score)	1.9 ± 1.2	2.4 ± 1.1	.001	0.01
BMI ± SD (kg/m ²)	16.7 ± 3.5	16.5 ± 4.1	.80	0.75
Ectopia lentis, <i>n</i> (%)	99 (62)	29 (27)	1.78 × 10 ⁻⁸	5.43 × 10 ⁻⁷
Highly arched palate, <i>n</i> (%)	147 (87)	109 (88)	.81	0.75
Pulmonary artery diameter z-score > 2.0, <i>n</i> (%)	20 (24)	19 (33)	.22	0.44
Dural ectasia, <i>n</i> (%)	13 (42)	5 (26)	.26	0.46
Striae, <i>n</i> (%)	64 (37)	48 (39)	.69	0.75
Arachnodactyly, <i>n</i> (%)	114 (68)	85 (69)	.77	0.75
Pes plani, <i>n</i> (%)	115 (68)	95 (78)	.07	0.23
Pectus excavatum, <i>n</i> (%)	36 (21)	45 (36)	.003	0.03
Pectus carinatum, <i>n</i> (%)	78 (45)	49 (39)	.33	0.53
Scoliosis, <i>n</i> (%)	49 (29)	34 (28)	.85	0.75
Joint hypermobility, <i>n</i> (%)	134 (80)	109 (89)	.03	0.18
Aortic root diameter ± SD (z-score ± SD)	3.4 ± 0.7 cm (4.4 ± 1.3)	3.4 ± 0.7 cm (4.2 ± 1.1)	.89 (.27)	0.75 (0.46)
Change in aortic root diameter (z-score/y) ± SD	-0.15 ± 0.25	-0.15 ± 0.24	.95	0.77

BMI, body mass index; DN, dominant negative; HI, haploinsufficient.

genotype–phenotype association emerged. A clear gradient was observed when comparing the proportion of EL between individuals with DN P/LP pathogenic variants across the *FBNI* gene. On the basis of this gradient, 3 statistically different regions were defined with the following frequencies of EL: exon 2 to 24: 84% (31/37); exon 25 to 57: 64% (63/99); and exon 58 to 66: 17% (4/24) (Figure 3) ($P = 7.3 \times 10^{-7}$, $Q = 1.5 \times 10^{-5}$). Within this gradient, 2 specific subregions with 100% penetrance for EL were identified, which included all patients with cysteine-involving variants between residues 611 to 921 ($n = 23$) and 1348 to 1429 ($n = 13$).

Association of genotype with response to treatment

The patients included in the genetics ancillary study cohort showed comparable phenotypic characteristics in both treatment groups (losartan: $n = 189$, atenolol: $n = 184$), including aortic z-scores at the start of the treatment (Supplemental Table 9). Within the treatment groups, no difference in baseline aortic z-scores between DN and HI genotypes was observed. When analyzing the effect of variant type (ie, DN and HI) and treatment (ie, losartan and atenolol) on treatment outcome, we found no significant difference after accounting for age (change in aortic root z-score/year; HI + atenolol: -0.16 ± 0.04 ; HI + losartan: -0.15 ± 0.03 ; DN + atenolol: -0.17 ± 0.03 ; DN + losartan: -0.12 ± 0.03 , $P = .18$, $Q = 0.39$, Figure 4).

No association between the nature or location (cysteine missense variants vs other missense variants, DN variants in the middle region [exon 26–49] vs other variants [including HI and DN in other exons], and DN variants in neonatal vs non-neonatal region) of the P/LP *FBNI* variants and the treatment outcome was detected when accounting for age, but subgroups were smaller in size (data not shown). Throughout analysis of covariance analyses, we never

observed any significant interaction between the independent variables.

Discussion

In the current study, our molecular analyses identified a P/LP *FBNI* variant and VUS in the overwhelming majority (91% and 4%, respectively) of 302 clinically diagnosed pediatric patients with MFS. Only 3% presented with non-*FBNI* variants and, as such, confirmed the solid association between MFS and the *FBNI* gene.¹² In total, 4 patients presented with 2 *FBNI* variants, of which always 1 was a P/LP variant and 1 was a VUS. Owing to the use of a short read next-generation sequencing technology, the fact that the identified variants were located in different exons, and the fact that no complementary DNA was available for testing, we were not able to determine whether the *FBNI* variants were located in *cis* or in *trans*. Furthermore, owing to the low number of patients (ie, 4) with 2 variants in *FBNI*, we were not able to confirm the association with a more severe phenotype, as has been suggested in literature.¹³

Despite significant progress in the understanding of the molecular defects underlying MFS, only a limited number of convincing genotype–phenotype associations have emerged. Several studies have attempted to define possible associations, but these were often refuted by subsequent analyses.^{2–17}

Previous studies have suggested an association between DN *FBNI* variants and EL.^{14,18,19} Our study convincingly confirmed that patients with EL are significantly enriched for DN variants ($Q = 5.4 \times 10^{-7}$), a finding even more pronounced when only cysteine-involving DN variants were considered ($Q = 1.0 \times 10^{-8}$). Overall, a cysteine-involving missense variant gives a 2.7-fold higher risk for developing EL than HI variants. For the first time, when considering DN variants, we found that EL was more

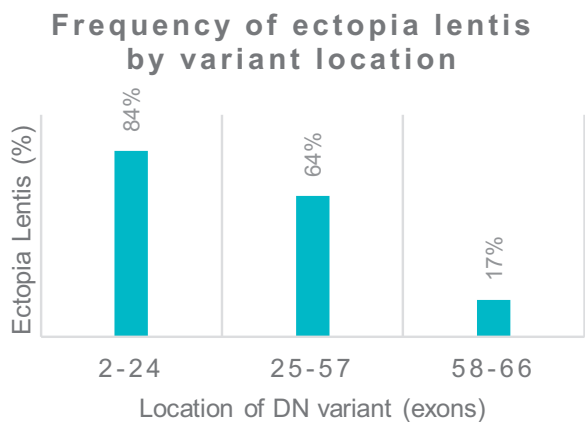


Figure 3 Effect of location of DN variants on the proportion of ectopia lentis. Groups were made on the basis of the proportion of ectopia lentis. DN, dominant negative.

frequent in the 5'-end than in the 3'-end. In their study, Pees et al¹⁴ described that EL occurs in 80% of cases with a pathogenic variant in exon 1 to 21, but we now describe a clear gradient that implies that DN variants that are located more toward the 5'-end of the gene are more likely to lead to EL. Furthermore, the following 2 subregions showed 100% penetrance of EL: cysteine-involving variants between residues 611 to 921 and 1348 to 1429. To confirm our observations in an independent cohort, we used the clinically classified patients by Groth et al.¹⁵ Our observations of 100% EL penetrance in patients with cysteine-involving variants in these subregions were confirmed in 49 and 14 patients. These regions overlap with LTBP2- and ADAMTS10-binding sites in *FBN1*.^{16,17} Pathogenic variants in those genes are linked to nonsyndromic autosomal recessive EL¹⁸⁻²¹ and Weill-Marchesani syndrome, respectively, in which EL is frequently observed.²² These observations have important clinical implications and aid patient-tailored management.

Another historical observation is that the vast majority of pathogenic variants in the central region of the *FBN1* gene (exons 25-33) is linked to more severe MFS presentations,²³ although exceptions have been reported.²⁴ While analyzing this neonatal region of the *FBN1* gene in our cohort, we did not observe a significant result for severity of cardiovascular involvement (aortic root z-score) for DN neonatal variants. However, it should also be noted that children at the most severe end of the MFS spectrum are underrepresented in this cohort on the basis of the exclusion criteria including aortic root diameters >5 cm and the presence of prior or planned aortic surgery. When we extended this region to exons 26 to 49, on the basis of the average aortic z-score per exon, we did observe a highly significant result, which also survived multiple hypotheses analysis ($Q = 7.6 \times 10^{-4}$). This indicates that on average patients with MFS with P/LP DN variant in exons 26 to 49 have a more severe aortic phenotype than patients with either HI variants or DN variants in other exons. This finding is in contrast with previous literature findings that suggest that patients with HI variants

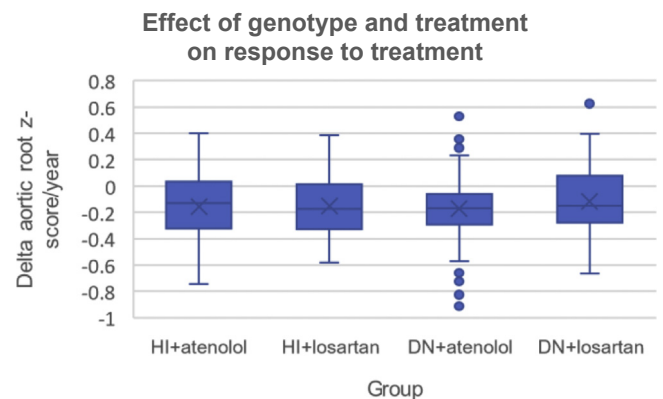


Figure 4 Effect of variant type (DN vs HI) and treatment (atenolol vs losartan) on response to treatment. Boxplot of the change of aortic root z-score per year for the different groups. The thick line in the middle is the median. The green box shows the first and third quartiles. The whiskers show the maximum and minimum values, with the exceptions of outliers (circles). DN, dominant negative; HI, haploinsufficient.

may have higher aortic risk.²⁵⁻²⁸ A recent study ($n = 207$) suggested that truncating and splice site variants more frequently correlate with aortic events at young age.²⁶ The study by Franken et al²⁷ also looked into the effect of the *FBN1* variant subtype (HI vs DN) on prospective cardiovascular outcome, including survival and time free of dissection in patients with MFS. Overall, 357 adult patients with MFS were included. The adjusted hazard ratios (for age, sex, and prior events) showed a 2.5-fold increased risk of cardiovascular death ($P = .05$) and a 2.4-fold increased risk of the combined end point, comprising cardiovascular death and dissection ($P < .001$), in patients with MFS with HI *FBN1* variants compared with those with DN variants. In the same cohort, the effect of variant type on treatment outcome was analyzed. As indicated by aortic root dilatation rate, patients with MFS with HI *FBN1* variants seemed to be more responsive to losartan therapy than patients with DN variants.²⁸ In our study cohort we were not able to replicate these findings because all patients received treatment (either losartan or atenolol) and could not be compared with patients without treatment.

Possible explanations for the different observations between our study and the study by Franken et al²⁷ are the different ages of the patients with MFS (6 months-25 years vs >18 years), the greater cardiovascular severity at baseline in our study ($z\text{-score} \geq 3$ as inclusion criterion), and the larger contribution of multiple individuals from the same families in the study by Franken et al.²⁷ Our study classified *FBN1* variants as DN and HI on the basis of sequence information alone, when in fact experimental evidence has shown that an accurate prediction of the ultimate effect of a *FBN1* variant at the protein level is difficult to achieve. For example, it was shown that upregulation of the wild-type *FBN1* allele can (partially) compensate for the loss of protein derived from a mutant *FBN1* nonsense allele.²⁹ In addition, *FBN1* premature termination codon alleles can escape decay of the mutant

messenger RNA through alternative splicing or other mechanisms and hence produce a truncated protein with DN potential. Alternatively, predicted DN variants, including cysteine-replacing missense variants, can encode mutant proteins that stall in the secretion process, limiting their ability to interfere with normal *FBNI* in the extracellular matrix.³⁰ Therefore, without experimental protein work, the effect of a specific *FBNI* variant remains speculative.

Taken together, the presented and published data suggest that the nature and location of the implicated *FBNI* defect can only partially explain phenotypic variability. Validation of observed genotype–phenotype associations is further hampered by the observation that related individuals harboring an identical pathogenic *FBNI* variant vary widely with respect to disease severity. It has been speculated that differences in expression levels between the variant *FBNI* allele and the wild-type *FBNI* allele contribute to intrafamilial clinical variability.^{31–33} However, residual *FBNI* expression in cultured cells has not proven to be a reliable predictor of phenotypic severity in small studies.^{5,29,31} In the largest systematic investigation to date ($n = 80$), low levels of wild-type *FBNI* messenger RNA have been shown to associate with increased risk of EL and pectus abnormalities and a trend toward an increased risk of aortic dilatation.³² An alternative explanation for the intrafamilial variability is the involvement of modifier genes.³⁴

Limitations

The uniqueness of this cohort lies in the fact that all patients with MFS are younger than 25 years and have an aortic root z-score > 3 , which makes them rather severely affected. However, patients with an aortic dissection, a prior or planned aortic surgery, or an aortic root diameter > 5 cm were excluded from the study.⁶ As such, the most severe end of the spectrum was excluded, and consequently cardiovascular events were rare. Overall, these selection criteria could have created a bias regarding genotype–phenotype associations. Owing to the fact that this MFS cohort is very young, some phenotypic features could still develop later in life as reported in literature that most aortic events occur above the age 20 years.³⁵ Therefore, it is possible that some genotype–phenotype associations are missed in this cohort. Furthermore, classification into DN and HI variants is based on predictions. No functional analyses were performed to confirm these predictions, and therefore, some variants could have been misclassified. Finally, no complementary DNA was available for analysis, and therefore, deep intronic variants could have been missed.

Conclusion

In conclusion, we present novel genotype–phenotype data in the largest pediatric and adolescent MFS cohort reported to date. A more severe aortic phenotype was not limited to the neonatal region because patients with (likely) pathogenic

DN variants in a more extended central region of the gene (exons 26–49) had a significantly worse aortic phenotype than other patients. Furthermore, we observed a clear difference in the proportion of EL on the basis of the location of the DN variant. Patients with MFS with cysteine-involving variants have 2.6-fold higher risk of EL than those carrying HI variant. Furthermore, patients with cysteine-involving variants between residues 611 to 921 and 1348 to 1429 probably have a near 100% chance of developing EL. These findings could guide frequency of ocular follow-up. According to our data, both the location and type of the *FBNI* variant influence the phenotype of patients with MFS. No effect of variant type on treatment outcome was detected in this cohort.

Data Availability

All data relevant to the study are included in the article or uploaded as [Supplemental Tables 1–9](#) and [Supplemental Figures 1 and 2](#). Additional data are available from the corresponding author upon request. All variants observed in the present cohort were submitted to ClinVar (<https://www.ncbi.nlm.nih.gov/clinvar/>): accession numbers SCV002025277 - SCV002025581.

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Ethics Declaration

All patients from the Pediatric Heart Network Marfan Trial were offered the opportunity to participate in this genetics ancillary cohort study by donating an additional blood sample after signing informed consent. Institutional Review Board approval was received from Ethical Committee University Hospital Ghent (Belgian Registry number B67020084735).

Conflict of Interest

The authors declare no conflicts of interest.

Additional Information

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