SUPPLEMENTAL MATERIAL

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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SUPPLEMENTAL CONTENT

Supplemental Tables

Supplemental Figures

Supplemental Tables

Supplemental Table 1A. Identified *FBN1* variants.

See separate excel file.

Supplemental Table 1B. Overview of non-*FBN1* genetic variants.

Sample ID	Gene	cDNA change[a]	Protein change	Classification criteria	ACMG/AMP classification [1]	Previous *FBN1* testing
354	*ACTA2; TGFBR2*	c.247G>A; deletion exon 7	p.(Asp83Asn); deletion exon 7	PM2, PP3; PM2, PM4	VUS; VUS	Negative
355[b,e]	*BGN*	c.5G>A	p.(Trp2*)	PM2, PVS1	Likely pathogenic (I)	Unkown
356[b,e]	*BGN*	c.908A>C	p.(Gln303Pro)	PM2, PP5	VUS	Negative
357	*FLNA; FLNA*	c.3106C>T; c.3127G>T	p.(Arg1036Cys); p.(Val1043Leu)	PM2, PP3; PP3	VUS; VUS	Negative
358	*FLNA*	c.1041G>C	p.(Val347Ile)	PM2	VUS	Unkown
359	*SMAD3*	c.1211T>C	p.(Leu404Ser)	PM2, PP3, PM1	VUS	Unkown
360[c,d]	*TGFBR2*	c.1411G>A	p.(Asp471Asn)	PM2, PS1, PM1, PP1	Likely pathogenic (II)	Unkown
361[c,d]	*TGFBR2*	c.1411G>A	p.(Asp471Asn)	PM2, PS1, PM1, PP1	Likely pathogenic (II)	Unkown
362	*TGFBR2*	c.1684C>T	p.(Arg562Cys)	PM2, PS1	Likely pathogenic (II)	Unkown
363	*TGFBR2*	c.1091G>C	p.(Arg364Pro)	PM2, PP3, PM1	VUS	Unkown

[a] GenBank reference sequence and version number for *ACTA2*: NM_001141945.2; *BGN*: NM_001711.6; *FLNA*: NM_001110556.2; *SMAD3*: NM_005902.4; *TGFBR2*: NM_001024847.2; numbering is from +1 as A of the ATG initiation codon.
[c], this sample has already been described in Baetens *et al*., 2011.[2]
[b], this sample has already been described in Proost *et al*., 2014.[3]
[d], this sample is related to one or two other individuals (with the same variant) in this cohort.
[e], this sample has already been described in Meester *et al*., 2014.[4]
Abbreviations: DN, dominant-negative; HI, haploinsufficient.

Supplemental Table 2. Genes included in (extended) TAAD panel.

Gene	Reference
ABL1	NM_007313
ACTA2	**NM_001141945**
ARIH1	NM_005744
BGN	NM_001711
COL3A1	**NM_000090**
EFEMP2/FBLN4	**NM_016938**
ELN	NM_001278939
EMILIN1	NM_007046
FBN1	**NM_000138**
FBN2	NM_001999
FLNA	**NM_001110556**
FOXE3	NM_012186
HCN4	NM_005477
LMOD1	NM_012134
LOX	NM_001178102
LTBP3	NM_001130144
MAT2A	NM_005911
MFAP5	NM_003480
MYH11	**NM_001040113** **NM_001040114**
MYLK	**NM_053025**
NOTCH1	**NM_017617**
PLOD1	NM_000302
PMEPA1/TMEPAI	NM_020182
PRKG1	NM_001098512
SKI	**NM_003036**
SLC2A10	**NM_030777**
SMAD2	NM_001003652
SMAD3	**NM_005902** **NM_001145103**
SMAD4	NM_005359
SMAD6	NM_005585
TGFB2	**NM_001135599**
TGFB3	NM_003239
TGFBR1	**NM_004612**
TGFBR2	**NM_001024847**

Bold: genes included in the standard TAAD panel. Underlined: genes included in the extended TAAD panel.

Supplemental Table 3. Modified ACMG/AMP criteria for variant classification.

See separate excel file.

Supplemental Table 4. Criteria used for HI and DN variant classification.

Dominant-negative variants	Haploinsufficient variants
Missense variants	Complete gene deletion
In-frame (exon) deletions/duplications	Deletion of first or last exon
Premature terminaton codon (PTC) escaping nonsense mediated decay (NMD) based on standard predictive rules (e.g. PTC in last exon)	PTC with NMD
Splice variants with proven in-frame exon skipping	Frameshift
	Splice variants with proven frameshift

Abbreviations: DN, dominant-negative; HI, haploinsufficient.

Supplemental Table 5. Clinical characteristics of TAAD panel-negative patients.

Patient	Gender	Age (years)	Ectopia lentis	Aortic root diameter z-score	Number of major Ghent criteria	*FBN1* variant previously identified	Family history (first degree relative)
364	Male	12.3	No	3.46	2	No	No
365	Female	20.0	No	3.22	3	Unknown	Yes
366	Male	18.1	No	2.52	2	No	No
367	Female	2.2	No	3.14	2	Unknown	Yes
368	Male	11.5	Yes	3	3	Unknown	No

Abbreviations: TAAD, Thoracic aortic aneurysm and dissection.

Supplemental Table 6. Clinical characteristics of the PHN trial cohort, stratified by inclusion in the genetics ancillary study cohort.

Characteristic	Included in genetics ancillary study cohort (N=373)	Not included in genetics ancillary study cohort (N=235)	*P*	*Q*
Age ± SD (years)	11.1±6.1	11.4±6.7	.49	.64
No. (%) Male	226 (61%)	140 (60%)	.80	.75
No. (%) with Family history	199 (56%)	161 (71%)	<.001	.003
No. (%) with Mitral valve prolapse	228 (62%)	140 (61%)	.85	.75
No. (%) with Ectopia lentis	162 (48%)	93 (49%)	.89	.75
No. (%) with Dural ectasia	22 (38%)	10 (27%)	.27	.46
No. (%) with Striae	146 (39%)	104 (45%)	.14	.35
No. (%) with Pneumothorax	7 (2%)	10 (4%)	.08	.25
Aortic root diameter ± SD (z-score ± SD)	3.4±.7 cm (4.3±1.2)	3.4±.8 cm (4.3±1.6)	.74 (.72)	.75 (.75)

Abbreviations: PHN, Pediatric Heart Network.

Supplemental Table 7. Comparison of clinical characteristics of dominant-negative neonatal and non-neonatal cohort.

Characteristic	Dominant-negative neonatal cohort[a] (N=33)	Non-neonatal[a] (N=265)	*P*	*Q*
Age ± SD (years)	9.4±6.2	11.2±6.1	.10	.28
No. (%) with Ectopia lentis	17 (57%)	111 (46%)	.28	.46
No. (%) with Highly arched palate	30 (97%)	226 (86%)	.15	.35
No. (%) with Pulmonary artery diameter z-score > 2.0	3 (23%)	36 (28%)	>.99	.79
No. (%) with Dural ectasia	4 (50%)	14 (33%)	.44	.62
No. (%) with Striae	7 (21%)	105 (40%)	.04	.21
No. (%) with Arachnodactyly	26 (84%)	173 (66%)	.05	.22
No. (%) with Pes plani	22 (73%)	188 (72%)	.88	.75
No. (%) with Pectus excavatum	5 (15%)	76 (29%)	.10	.28
No. (%) with Pectus carinatum	12 (36%)	115 (43%)	.45	.62
No. (%) with Scoliosis	10 (32%)	73 (29%)	.66	.75
No. (%) with Joint hypermobility	30 (94%)	213 (83%)	.11	.28
Aortic root diameter ± SD (z-score ± SD)	3.3±.7 cm (4.9±1.5)	3.4±.7 cm (4.2±1.1)	.46 (.02)	.62 (.11)
Change in aortic root diameter z-score/year ± SD	-.14±.28	-.15±.24	.61	.74

[a] The “neonatal” classification is purely based on the presence of a P/LP variant in exons 25-33 and is not based on any specific phenotypic features.

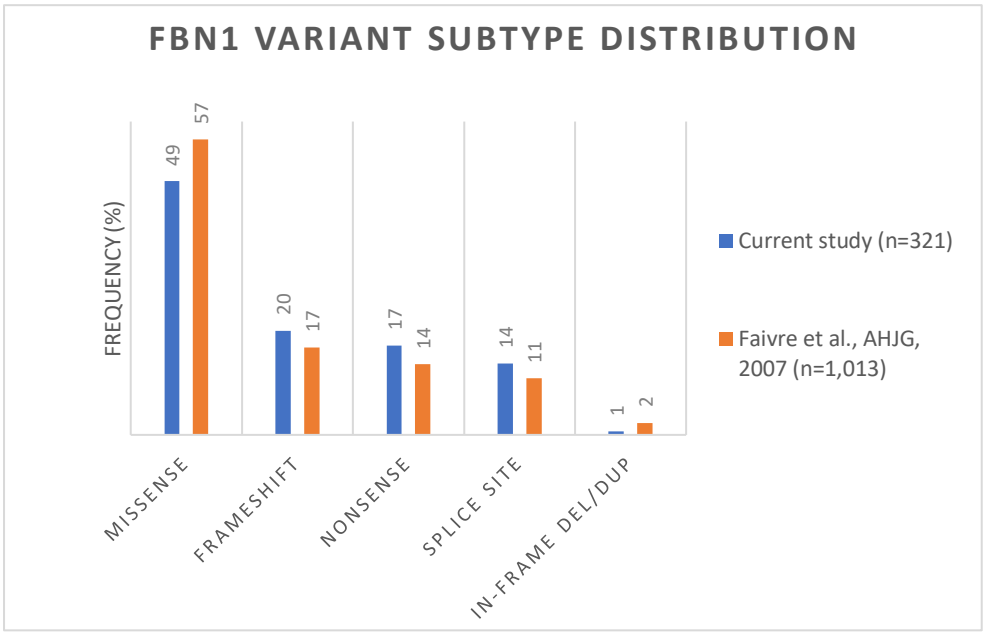
Supplemental Table 8. Number of patients with a dominant-negative variant per exon.

See separate excel file.

Supplemental Table 9. Comparison of clinical characteristics of atenolol and losartan cohort.

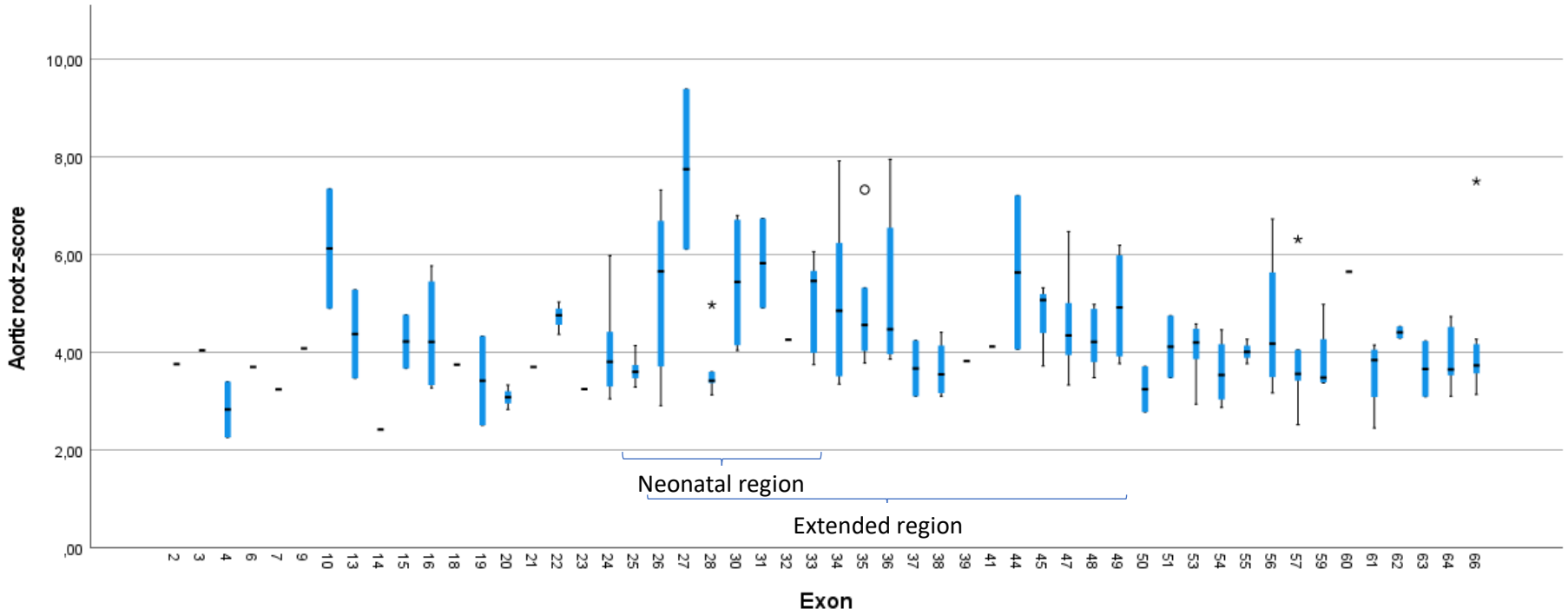
Characteristic	Atenolol cohort (N=184)		Losartan cohort (N=189)		*P*	*Q*
Age ± SD (years)	11.2±6.2		11.0±6.0		.78	.75
No. (%) Male	109 (59%)		117 (62%)		.60	.74
No. (%) with Family history	79 (49%)		83 (51%)		.74	.75
No. (%) with Highly arched palate	150 (83%)		166 (90%)		.06	.22
No. (%) with Mitral valve prolapse	110 (60%)		116 (62%)		.70	.75
No. (%) with Pulmonary artery diameter z-score >2.0	29 (32%)		22 (24%)		.27	.46
No. (%) with Ectopia lentis	81 (49%)		80 (47%)		.79	.75
No. (%) with Striae	71 (39%)		75 (40%)		.89	.75
No. (%) with Arachnodactyly	122 (68%)		121 (65%)		.63	.74
No. (%) with Pes plani	125 (70%)		138 (74%)		.35	.54
No. (%) with Pectus excavatum	46 (25%)		54 (29%)		.44	.62
No. (%) with Pectus carinatum	84 (46%)		77 (41%)		.34	.53
No. (%) with Scoliosis	59 (34%)		46 (25%)		.09	.28
No. (%) with Joint hypermobility	155 (87%)		151 (82%)		.19	.39
Aortic root diameter ± SD (z-score ± SD)	3.4±0.7cm (4.3±1.0)		3.4±0.7 cm (4.4±1.3)		.90 (.44)	.75 (.62)
	DN: 3.3±.7 cm (4.2±1.0)	HI: 3.4±.7 cm (4.2±1.0)	DN: 3.4±.7 cm (4.5±1.4)	HI: 3.3±.7 cm (4.2±1.1)	.95 (.24)	.77 (.46)
Change in aortic root diameter z-score/year ± SD	-.19±.25		-.14±.24		.06	.22

Supplemental Figures



Supplemental eFigure 1. Comparison of variant subtype distribution to literature.

A comparison of the variant subtype distribution between our MFS cohort (n=321) and the study by Faivre *et al.*, AJHG, 2007.[5] del, deletion; dup, duplication.



Supplemental eFigure 2. Boxplots of aortic root z-score of patients with DN (likely) pathogenic variants according to exonic location.

The thick line in the middle is the median. The blue boxes show the first and third quartiles. The whiskers show the maximum and minimum values, with the exceptions of outliers (circles) and extremes (asterisks).

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