Phenotype-genotype correlations in *FBN1* inform genetic diagnosis in patients with unusual clinical presentation

NHS South West Genomic Laboratory Hub

Carolyn Dent[1], Julie Honeychurch[1], Mary Gable[1], Isabelle Nash[1], Adrian Waite[1], Ingrid Scurr[2], Ana Beleza[1] and Rebecca Whittington[1].

[1]North Bristol NHS Trust, [2]University Hospitals Bristol NHS Foundation Trust

NHS University Hospitals Bristol NHS Foundation Trust

Syndromic and non-syndromic thoracic aortic aneurysm and dissection panel testing (R125 TAAD, 32 genes) is available through the cardiology specialist testing service under the National Genomic Test Directory. The genes represented on the R125 panel are listed below along with OMIM disease associations (Table 1). We have tested 343 patients through the R125 panel (since its introduction in November 2019) with a definitive or likely diagnosis in 13% of patients. We have established a diagnosis (pathogenic or likely pathogenic variant detected) in 10 of the 32 genes on the panel, with the majority being in *FBN1* (Figure 1). We have established a diagnosis in a further 5 genes represented on the R125 panel in previous aortopathy panels (*COL3A1, MYH11, SKI, SMAD2*) or through the 100,000 genomes project (*BGN*).

	Relevant OMIM Associations
ABL1	AD Congenital heart defects and skeletal malformations syndrome
ACTA2	AD Familial thoracic aortic aneurysm 6
BGN	XL Meester-Loeys syndrome
CBS	AR homocystinuria
COL3A1	AD vascular Ehlers-Danlos syndrome
COL5A1	AD Classic Ehlers-Danlos syndrome 1
COL5A2	AD Classic Ehlers-Danlos syndrome 2
EFEMP2	AR Cutis laxa 1B
ELN	AD Cutis laxa; AD Supravalvar aortic stenosis
FBLN5	AD Cutis laxa 2; AR Cutis laxa 1A
FBN1	AD Marfan syndrome; AD Ectopia lentis; AD Marfan lipodystrophy syndrome; AD MASS syndrome; AD stiff skin syndrome; AD Weill-Marchesani syndrome 2
FBN2	AD Contractural arachnodactyly
FKBP14	AR Kyphoscoliotic Ehlers-Danlos syndrome 2
FLNA	XL Cardiac valvular dysplasia
FOXE3	AD Susceptibility to familial thoracic aortic aneurysm
LOX	AD Familial thoracic aortic aneurysm 10
MFAP5	AD Familial thoracic aortic aneurysm 9
MYH11	AD Familial thoracic aortic aneurysm 4
MYLK	AD Familial thoracic aortic aneurysm 7
NOTCH1	AD Adams-Oliver syndrome 5; AD Aortic valve disease 1
PLOD1	AR Kyphoscoliotic Ehlers-Danlos syndrome 1
PRKG1	AD Familial thoracic aortic aneurysm 8
SKI	AD Shprintzen-Goldberg syndrome
SLC2A10	AR Arterial tortuosity syndrome
SMAD2	AD Loeys-Dietz syndrome 5
SMAD3	AD Loeys-Dietz syndrome 3
SMAD4	AD Myhre syndrome
SMAD6	AD Aortic valve disease 2
TGFB2	AD Loeys-Dietz syndrome 4
TGFB3	AD Loeys-Dietz syndrome 5
TGFBR1	AD Loeys-Dietz syndrome 1
TGFBR2	AD Loeys-Dietz syndrome 2

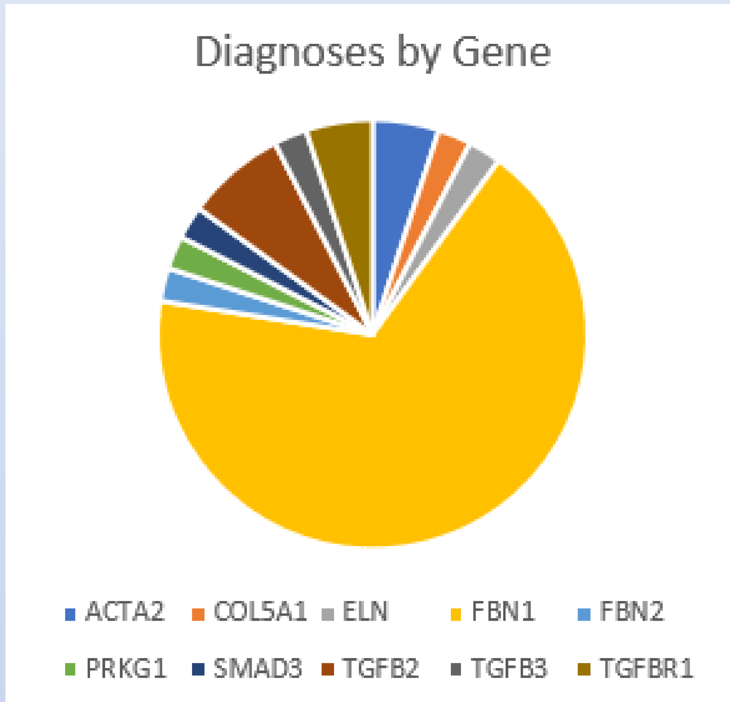


Table 1. List of the 32 genes on the R125 thoracic aortic aneurysm and dissection gene panel.

Figure 1. Genes in which a pathogenic or likely pathogenic variant has been detected through the R125 panel.

Variants in *FBN1* have been detected in 72 index patients using R125, previous aortopathy panels and standalone *FBN1* testing, 92% of which are classified pathogenic or likely pathogenic. The mechanism of pathogenicity for *FBN1*-associated disease is broad with pathogenic missense, truncating and whole exon deletions and duplications being described. *FBN1* variants can be broadly classified as acting through two mechanisms: dominant negative (altered protein folding leading to destabilisation of the extracellular matrix – missense and in-frame deletion and splice variants) and haploinsufficiency (production of less protein - nonsense, frameshift and out-of-frame exon deletions and splice variants). This mechanism of pathogenicity may have implications for the severity of the aortic phenotype and response to Losartan therapy[1,2,3]).

The fibrillin protein structure is modular, comprising multiple EGF-like and TB domains where structural integrity is maintained by disulphide bonding between cysteine residues and, in the case of the Cb-EGF-like domains, binding to a central calcium ion. Missense genetic variation that removes or creates a cysteine residue or disrupts calcium binding is not tolerated and therefore disease-causing.

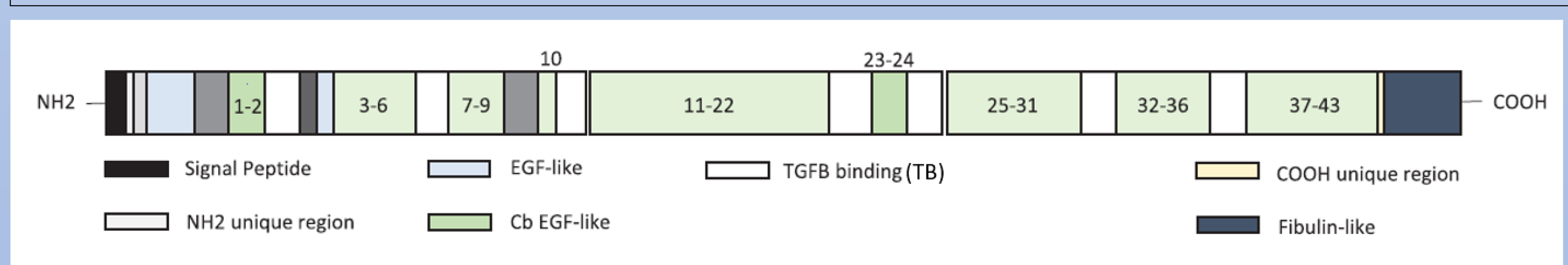
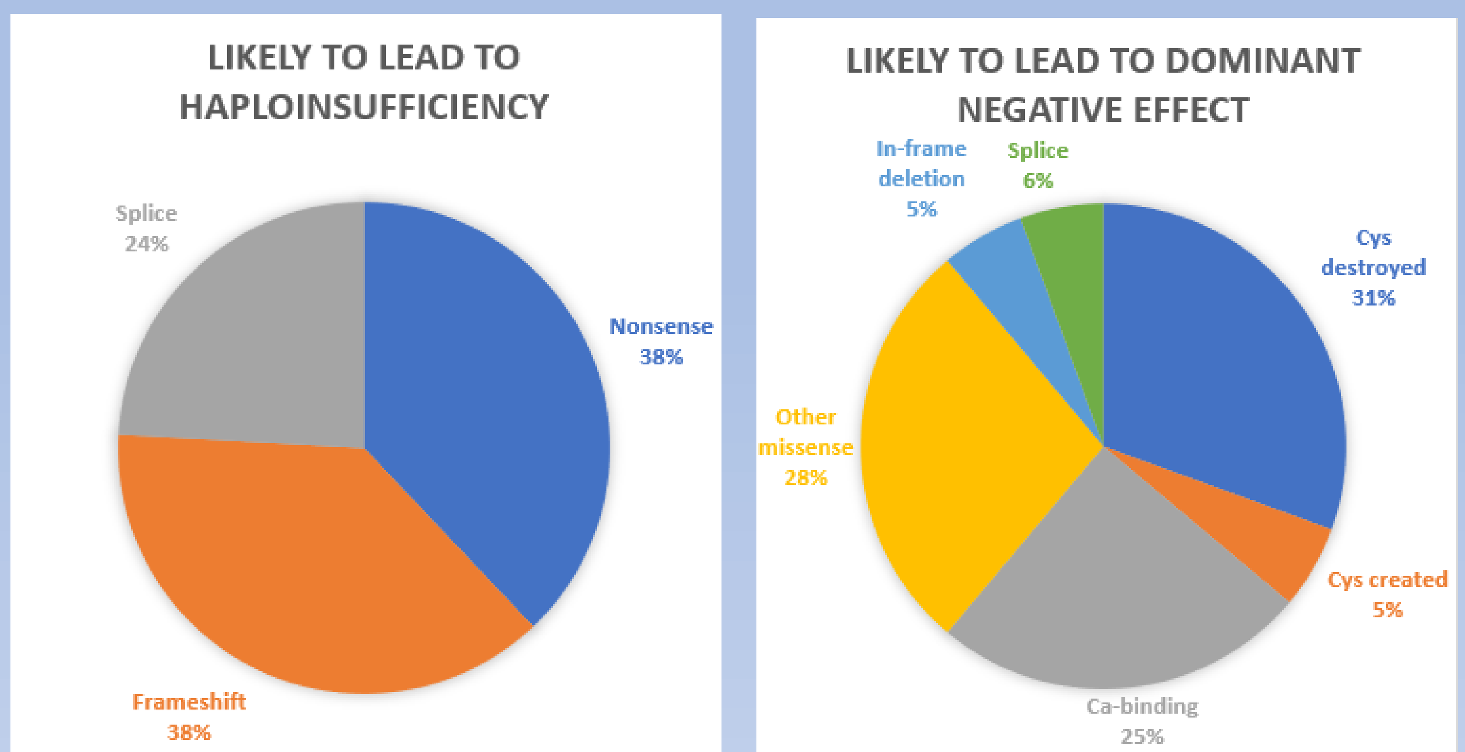


Figure 2: The modular structure of the fibrillin 1 protein (taken from Muino-Mosquera et al (modified)[4].



An audit of *FBN1* variants detected at Bristol genetics laboratory has shown an equal split between variants expected to operate through haploinsufficiency and a dominant negative effect. Figure 3 shows proportions of the different classes of *FBN1* variants detected.

Figure 3: Representation of the proportion of variants detected at Bristol Genetics Laboratory expected to lead to haploinsufficiency (n=29) or exert their effect through a dominant negative mechanism of action (n=36). A caveat is that some cysteine variants have been proven, through functional studies, to lead to haploinsufficiency through degradation of protein (does not include any of the cysteine variants detected at the Bristol Laboratory).

References: 1. Franken *et al* (2015) Circ Cardiovasc Genet 8:383. 2. Franken *et al* (2016) Eur Heart J 37:3285. 3. Franken *et al* (2017) Heart 103:1795. 4. Muino-Mosquera *et al* (2018) Circ Genom Precis Med 11:e002039. 5. McGettrick *et al* (2000) Hum Mol Genet 9:1987. 6. Khau Van Kien *et al* (2010) Hum Mutat 31:E1021

Phenotype-Genotype correlation

FBN1 variants lead to a range of clinical phenotypes (see Table 1). The majority of *FBN1* variants identified through diagnostic gene panel testing in this laboratory are associated with *FBN1*-related Marfan syndrome, but we have also identified variants in patients with isolated ectopia lentis, stiff skin syndrome and isolated thoracic aortic aneurysm/dissection. With the use of case-studies we explore clinical and genetic aspects of patients with non-Marfan clinical presentation.

Case Study 1 – *FBN1*-related Stiff skin syndrome (OMIM 184900)

29 year-old female with clinical features of stiff skin syndrome including bilateral elbow and knee contractures and an unusual eye shape. Clinical Genetics referral. No other family members have been tested.

Case Study 2 – Possible Marfan lipodystrophy syndrome

12 year-old female with a complex Marfan syndrome phenotype. Referred by referred by the orthopaedic team with a longstanding history of pain in the soles of her feet, a diagnosis of plantar fasciitis and features of an underlying connective tissue disorder. She had had a previous admission to Paediatric intensive care with cavitating pneumonia and pneumothoraces following severe influenza type A infection. She also had recurrent episodes of facial swelling and had had extensive investigations for an underlying immunodeficiency. On examination the patient had features consistent with a diagnosis of Marfan syndrome and was also noted to lack subcutaneous fat. These findings led us to query Marfan lipodystrophy syndrome.

Case study1

***FBN1* NM_000138.4 c.4687T>A p.(Cys1563Ser)**

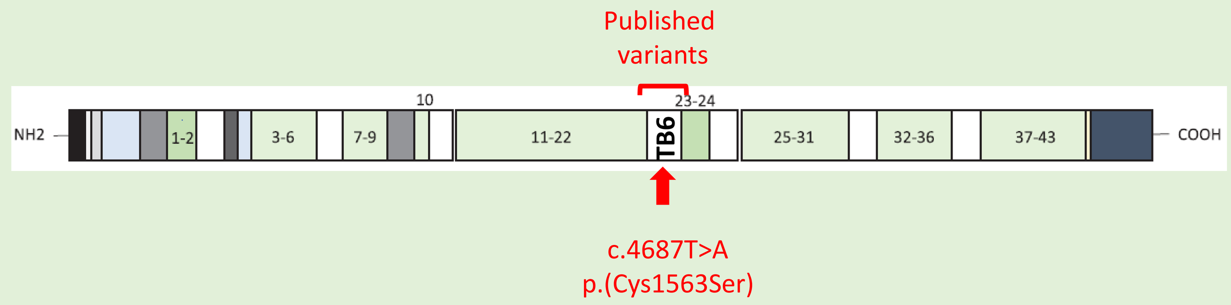
ACMG Classification: Likely Pathogenic

PM1 Strong: Cysteine removed from TB6 domain of fibrillin 1

PM2 Moderate: No reports of variant to population databases

PS1 Supporting: Alternative variant resulting in the same amino acid alteration, *FBN1* c.4688G>C p.(Cys1563Ser), reported to ClinVar as a *de-novo* variant in patient with likely stiff skin syndrome.

All published variants associated with stiff skin syndrome are missense variants removing or creating cysteine residues in TB6.



Case Study 2

***FBN1* NM_000138.4 c.4466A>G p.(Asn1489Ser)**

ACMG Classification: Likely Pathogenic

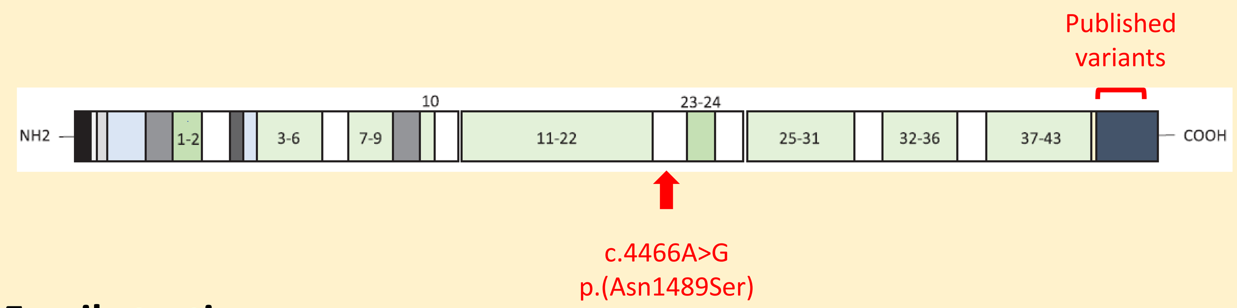
PM1 Moderate: Missense variant affecting the consensus calcium-binding sequence in one of the Ca-EGF-like domains[5].

PM2 Moderate: No reports of variant to population databases

PM5 Moderate: Alternative variant affecting the same codon, c.4467T>A p.(Asn1489Lys) reported in several index cases and other affected family members with relevant phenotypes.

PS4 Supporting: Several ClinVar reports in patients with related phenotypes.

All published variants associated with Marfan-lipodystrophy syndrome are truncating variants in the penultimate exon of *FBN1*. This patient's lipodystrophy is therefore likely coincidental.



Family testing

Variant segregates in this patient's affected father. He has clinical features in keeping with diagnosis of Marfan syndrome and had corrective surgery as a child for severe micrognathia. He was noted to have a bicuspid aortic valve, moderate to severe aortic regurgitation and a notably dilated aortic root.

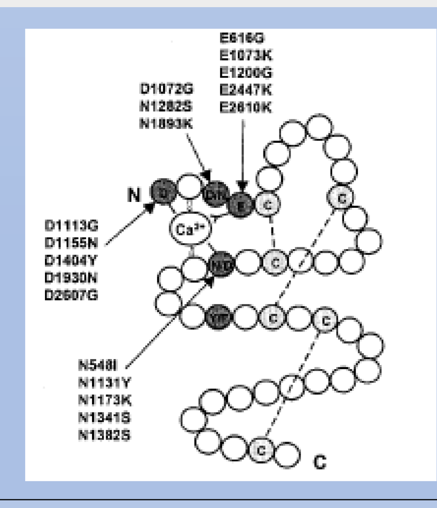
Prediction of functional significance of *FBN1* variants by domain

Variants affecting highly conserved amino acid residues, particularly those likely to disrupt calcium binding or disulphide bonding and affecting conserved structural glycine residues[6], are more likely to be disease causing (see figures 4 and 5 below). Variants detected in less well conserved regions (amino acids shown in light grey below) are less likely to be disease causing and variants remain uncertain pending additional evidence.

Figure 4: Multiple alignment of Ca-EGF-like domains (Alignment performed using COBALT; 10 of the 47 domains shown) highlighting Ca-binding residues (Ca) and variants detected at the Bristol laboratory (red border = Bristol lab likely pathogenic or pathogenic variant; blue border = Bristol lab variant of uncertain significance, red shading = HGMD disease-causing variants).

	Ca		Ca	Ca																		Ca					Ca																			
Ca-EGF-like 4 (246-287)	D	V	D	E	C	Q	A		I	P	G	L	C			Q	G	G	N	C	I	N	T	V	G	S	F	E	C	K	C	P	A	G	H	K	L	N	E	V	S		Q	K	C	E
Ca-EGF-like 5 (288-329)	D	I	D	E	C	S	T		I	P	G	I	C			E	G	G	E	C	T	N	T	V	S	S	Y	F	C	K	C	P	P	G	F	Y	T	S	P	D	G		T	R	C	I
Ca-EGF-like 7 (490-529)	D	V	D	E	C				E	K	N	P	C			A	G	G	E	C	I	N	N	Q	G	S	Y	T	C	Q	C	R	A	G	Y	Q	S	T	L	T	R		T	E	C	R
Ca-EGF-like 8 (530-571)	D	I	D	E	C	L	Q		N	G	R	I	C			N	N	G	R	C	I	N	T	D	G	S	F	H	C	V	C	N	A	G	F	H	V	T	R	D	G		K	N	C	E
Ca-EGF-like 9 (572-612)	D	M	D	E	C	S	I			R	N	M	C		L		N	G	M	C	I	N	E	D	G	S	F	K	C	I	C	K	P	G	F	Q	L	A	S	D	G		R	Y	C	K
Ca-EGF-like 10 (613-653)	D	I	N	E	C	E	T			P	G	I	C			M	N	G	R	C	V	N	T	D	G	S	Y	R	C	E	C	F	P	G	L	A	V	G	L	D	G		R	V	C	V
Ca-EGF-like 11 (723-764)	D	I	N	E	C	A	L		D	P	D	I	C		P		N	G	I	C	E	N	L	R	G	T	Y	K	C	I	C	N	S	G	Y	E	V	D	S	T	G		K	N	C	V
Ca-EGF-like 12 (765-806)	D	I	N	E	C	V	L		N	S	L	L	C			D	N	G	Q	C	R	N	T	P	G	S	F	V	C	T	C	P	K	G	F	I	Y	K	P	D	L		K	T	C	E
Ca-EGF-like 13 (807-846)	D	I	D	E	C	E	S				S	P	C			I	N	G	V	C	K	N	S	P	G	S	F	I	C	E	C	S	S	E	S	T	L	D	P	T	K		T	I	C	I
Ca-EGF-like 14 (910-951)	D	I	D	E	C	E	V		F	P	G	V	C			K	N	G	L	C	V	N	T	R	G	S	F	K	C	Q	C	P	S	G	M	T	L	D	A	T	G		R	I	C	L
Ca-EGF-like 15 (1028-1069)	D	I	N	E	C	K	M		I	P	S	L	C			T	H	G	K	C	R	N	T	I	G	S	F	K	C	R	C	D	S	G	F	A	L	D	S	E	E		R	N	C	T

Figure 5: Illustration showing Calcium-binding domains and disulphide bonding (from McGettrick et al (2000)[5].



Conclusions:

- R125 gene panel testing currently results in a diagnosis for 15% of patients. Further audit is underway to understand factors associated with an improved diagnostic yield.
- 13% of R125 tests result in a confirmed or likely genetic diagnosis through detection of a pathogenic or likely pathogenic variant. 67% of these positive tests resulted in a diagnosis of *FBN1*-related Marfan syndrome, stiff skin syndrome, isolated ectopia lentis or isolated thoracic aortic aneurysm and dissection.
- *FBN1* variants can be subdivided into those leading to fibrillin 1 haploinsufficiency and those working through a dominant negative effect. This mechanism may inform severity of aortic phenotype and response to Losartan therapy.
- Variants associated with Marfan syndrome, isolated ectopia lentis and thoracic aortic aneurysm and dissection may be truncating or missense and occur throughout *FBN1* (data not shown).
- Variants associated with stiff skin syndrome and Marfan lipodystrophy syndrome are more specific in location and mechanism. This can aid genetic diagnosis as illustrated by the two case studies above.