Genet. Res., Camb. (2016), vol. 98, e13. © Cambridge University Press 2016
doi:10.1017/S0016672316000112

Identification of *FBN1* gene mutations in Ukrainian Marfan syndrome patients

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(*Received 2 February 2016; revised 21 June 2016; accepted 29 June 2016*)

Summary

Marfan syndrome is an autosomal dominant connective tissue disorder, predominantly affecting the ocular, skeletal and cardiovascular systems. Here, we present the results of the first genetic testing in 40 Ukrainian Marfan (-like) patients and 10 relatives. We applied a targeted next generation sequencing panel comprising *FBN1* and 13 thoracic aortic aneurysm genes. We identified 27 causal mutations in *FBN1*, obtaining a mutation yield of 67·5%. A significant difference in age at aortic surgery between mutation positive and negative patients was observed. Thus, we conclude that genetic testing is important to identify patients at higher risk for developing life-threatening cardiovascular complications.

1. Introduction

Marfan syndrome (MFS) (OMIM#154700) is an autosomal dominant connective tissue disorder with a prevalence of 0·075 to 0·86 per 5000 individuals (von Kodolitsch *et al.*, 2015). MFS is a multisystemic disorder involving the ocular, skeletal and cardiovascular systems. Myopia and lens dislocation are the most common ocular features, while skeletal involvement is characterized by long bone overgrowth, pectus deformity and arachnodactyly (Fig. 1(*a*) and (*b*)). However, the most life-threatening complications in MFS are related to the cardiovascular system (Fig. 1(*c*)). These include aortic root dilatation primarily at the level of the sinuses of Valsalva, aortic dissection and rupture, mitral valve prolapse, mitral regurgitation and arrhythmias (Van Laer *et al.*, 2013; Cherkas *et al.*, 2016). In 1991, the fibrillin-1 gene (*FBN1*), which encodes a 350 kDa glycoprotein, was identified as the gene responsible for MFS (Dietz *et al.*, 1991). Fibrillin-1, as a part of the extracellular matrix (ECM), provides elasticity and structural support to tissues and plays an important role in TGF-β signalling. Thus, mutations in *FBN1* lead to a loss of ECM integrity and to a dysregulation of the downstream TGF-β signalling pathway (Neptune *et al.*, 2003; Ramachandra *et al.*, 2015).

In 80 to 100% of MFS patients, a *FBN1* mutation can be identified (Loeys *et al.*, 2004; Faivre *et al.*, 2011; Radonic *et al.*, 2011; Sheikhzadeh *et al.*, 2011; Aalberts *et al.*, 2012; Yang *et al.*, 2012; Proost *et al.*, 2015; von Kodolitsch *et al.*, 2015). Despite the presence of identical mutations, a large inter- and intrafamilial phenotypic variability is observed, suggesting that modifiers may be involved (Van Laer *et al.*, 2013). Genetic testing is important, as on the one hand, the identification of a pathogenic *FBN1* mutation can be very helpful to establish an adequate treatment and management scheme for the proband and affected family members. On the other hand, unaffected family members can be reassured and be released from further clinical follow-up. Here, we present the first genetic testing in a Ukrainian cohort of 40 MFS probands and 10 family members.

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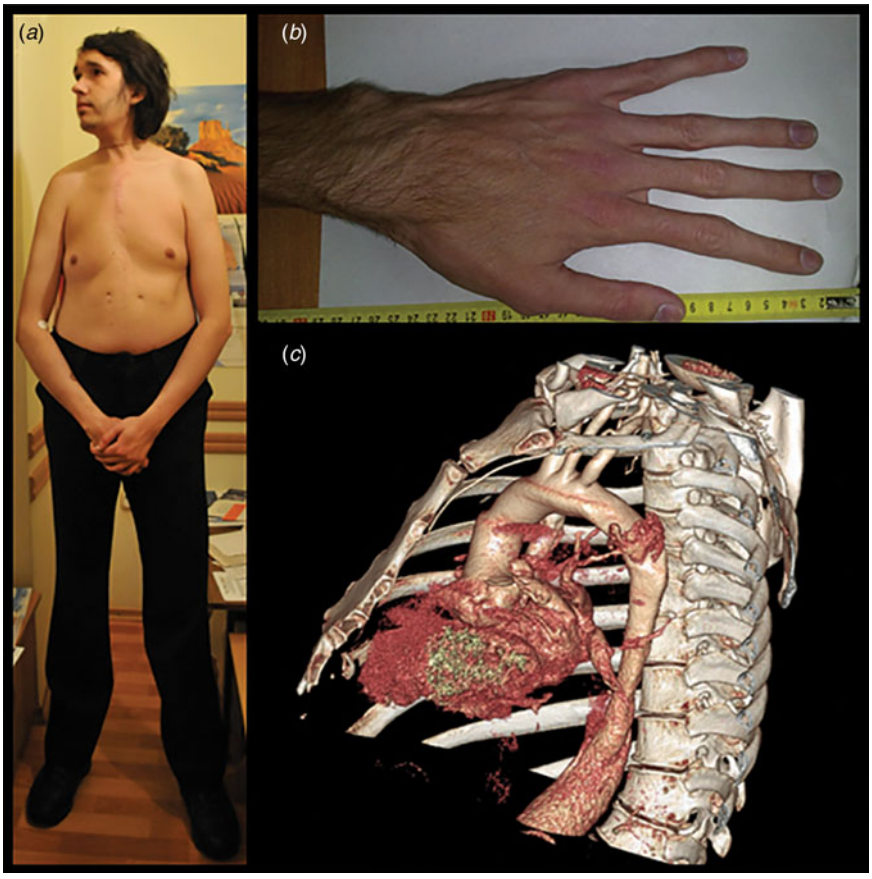

Fig. 1. Clinical features of MFS. Typical MFS patients with (*a*) disproportionate long bone overgrowth, (*b*) arachnodactyly and (*c*) thoracic aortic aneurysm.

2. Materials and methods

Probands in this study were consecutive patients derived from the two largest cardiovascular centres of Ukraine, accessible to all Ukranians. The probands were referred for evaluation of aortic root aneurysm or aortic root surgery. As such, this group is most probably biased towards more extreme cardiovascular phenotypes, but can be considered as representative for the Ukranian population (Zhuraev *et al.*, 2014). All probands and their family members underwent a thorough clinical examination, including a slitlamp exam and physical exam. Based on the clinical findings, the systemic score was calculated according to www.marfan.org/dx/score. Twenty-eight patients met the diagnostic criteria for MFS, based on the original and revised Ghent nosology (www.marfan.org/dx/rules) (De Paepe *et al.*, 1996; Loeys *et al.*, 2010), while 12 were suspected of a MFS-related syndrome. None of the individuals refused inclusion in the study, but three probands were excluded because their DNA was of insufficient quality. The local ethical committee approved the clinical and genetic program for MFS.

Probands were screened with a next generation sequencing (NGS) panel, comprising 14 genes involved in thoracic aortic aneurysms (TAA) (Proost *et al.*, 2015). Enrichment of the regions of interest was performed with a custom Haloplex target enrichment kit according to the supplier's protocol (Agilent Technologies, Santa Clara, CA), followed by NGS on MiSeq (Illumina, San Diego, CA) using 150 bp paired-end sequencing reads. Next, data analysis was performed with a tailored pipeline and our in-house developed VariantDB was used to annotate and interpret the variants (Vandeweyer *et al.*, 2014; Proost *et al.*, 2015). Decisions on the pathogenicity of variants were based on their presence in specific mutation databases, including Human Gene Mutation Database (HGMD) (www.hgmd.cf.ac.uk) and Universal Mutation Database (UMD) FBN1 (www.umd.be/FBN1/), which also contain the relevant links to the literature and/or on the functional importance of specific residues and their conservation across the TGF-β binding (TB) and the (calcium-binding) epidermal growth factor (EGF)-like domains as demonstrated in Supplementary Tables S1(*a*)–(*c*) (e.g., the conserved cysteine residues in EGF-like domains and the first four amino acids of the EGF-like domain, the so-called DIDE motif).

The variants found with NGS were confirmed by Sanger sequencing using the BigDye® Terminator Cycle Sequencing kit (Applied Biosystems, Life Technologies, Carlsbad, CA), followed by capillary electrophoresis on an ABI3130XL (Applied Biosystems).

https://doi.org/10.1017/S0016672316000112 Published online by Cambridge University Press

Table 1. *Mutation or VUS positive probands.*

#	Exon	Gene	cDNA base change	Predicted amino acid change	Type of mutation; domain[a]	Prediction[b] MutationTaster, PolyPhen-2 and Sift	Previously described[c]	ExAC Frequency
MFS patients								
2	14	*FBN1*	c.1709G > A	p.Cys570Tyr	Missense mutation; conserved cys in calcium-binding EGF-like#8	P (0·999), P (0·997), P (0·002)	CM013918; UMD	/
3	44	*FBN1*	c.5368C > T	p.Arg1790*	Nonsense mutation	P (1·000), NA, NA	CM054694; UMD	/
	25	*FBN1*	c.2956G > A	p.Ala986Thr	VUS	P (0·999), P (0·458), B (0·100)	UMD	0·001508
4	Intron 51	*FBN1*	c.6313 + 3insT	/	Splice site mutation	NA	CS022105	/
5	Intron 37	*FBN1*	c.4582 + 1G > T	/	Splice site mutation	NA	Novel	/
6	64	*FBN1*	c.7828G > A	p.Glu2610Lys	Missense mutation, DIDE consensus sequence[d]	P (0·999), P (0·999), P (0·003)	CM972822; UMD	/
7	Intron 13	*FBN1*	c.1589–1G > A	/	Splice site mutation	NA	Novel	/
8	10	*FBN1*	c.1090C > T	p.Arg364*	Nonsense mutation	P (1·000), NA, NA	CM032224; UMD	/
9	55	*FBN1*	c.6629G > A	p.Cys2210Tyr	Missense mutation; conserved cys in calcium-binding EGF-like#38	P (0·999), P (0·997), P (0·000)	Novel	/
10	34	*FBN1*	c.4096G > A	p.Glu1366Lys	Missense mutation, DIDE consensus sequence[d]	P (0·999), P (0·995), P (0·980)	CM040037; UMD	/
	25	*FBN1*	c.2956G > A	p.Ala986Thr	VUS	P (0·999), P (0·458), B (0·100)	UMD	0·001508
12	49	*FBN1*	c.5947A > T	p.Lys1983*	Nonsense mutation	P (1·000), NA, NA	Novel	/
13	38	*FBN1*	c.4621C > T	p.Arg1541*	Nonsense mutation	P (1·000), NA, NA	CM993159; UMD	/
14	4	*FBN1*	c.254G > T	p.Cys85Phe	Missense mutation; conserved cys in EGF-like#1	P (1·000), P (0·999), P (1·000)	Novel	/
16	25	*FBN1*	c.2963G > A	p.Trp988*	Nonsense mutation	P (1·000), NA, NA	UMD	/
17	32	*FBN1*	c.3845A > G	p.Asn1282Ser	Missense mutation, DIDE consensus sequence[d]	P (0·757), P (0·803), P (0·970)	CM972807; UMD	0·0000742
	9	*SMAD3*	c.1269T > G	p.Ser423Arg	VUS	P (0·999), P (0·980), P (0·002)	Novel	/
19	32	*FBN1*	c.3960T > A	p.Cys1320*	Nonsense mutation	P (1·000), NA, NA	CM054723; UMD	/
20	63	*FBN1*	c.7712G > A	p.Cys2571Tyr	Missense mutation; conserved cys in calcium-binding EGF-like#45	P (1·000), P (0·999), P (1·000)	Novel	/
23	58	*FBN1*	c.7180C > T	p.Arg2394*	Nonsense mutation	P (1·000), NA, NA	CM993162; UMD	/
24	22	*FBN1*	c.2639G > A	p.Gly880Asp	Missense mutation	P (1·000, P (1·000), P (1·000)	UMD	/
25	35	*FBN1*	c.4222T > C	p.Cys1408Arg	Missense mutation; conserved cys in calcium-binding EGF-like#24	P (1·000), P (0·999), P (1·000)	CM098517; UMD	/
26	66	*FBN1*	c.8352_8353insT	p.Thr2785Tyrfs*16	Frameshift mutation	NA	Novel	/
27	Intron 16	*FBN1*	c.1960 + 1G > A	/	Splice site mutation	NA	UMD	/
	34	*NOTCH1*	c.6413C > T	p.Pro2138Leu	VUS	P (0·999), P (0·494), B (0·599)	Novel	0·000008763
	2	*FLNA*	c.182G > A (A/–)	p.Ser61Asn	VUS	P (0·824), B (0·000), B (1·000)	Novel	/
28	64	*FBN1*	c.7831T > C	p.Cys2611Arg	Missense mutation; conserved cys in calcium-binding EGF-like#45	P (1·000), P (0·998), P (1·000)	Novel	/
29	4	*FBN1*	c.254G > A	p.Cys85Tyr	Missense mutation; conserved cys in EGF-like#1	P (1·000), P (0·999), P (1·000)	Novel	/

Table 1. (*Cont.*)

#	Exon	Gene	cDNA base change	Predicted amino acid change	Type of mutation; domain[a]	Prediction[b] MutationTaster, PolyPhen-2 and Sift	Previously described[c]	ExAC Frequency
MFS-like patients								
1	4	*TGFBR1*	c.709A > G	p.Arg237Gly	VUS	P (0·999); P (0·997); P (0·002)	Novel	/
11	63	*FBN1*	c.7754T > C	p.Ile2585Thr	Missense mutation	P (0·999), P (0·642), B (0·543)	CM972820; UMD	/
15	61	*FBN1*	c.7549C > T	p.Gln2517*	Nonsense mutation	P (1·000), NA, NA	Novel	/
18	58	*FBN1*	c.7039_7040delAT	p.Met2347 fs*19	Frameshift mutation	NA	CD020234; UMD	/
21	14	*FBN1*	c.1693C > T	p.Arg565*	Nonsense mutation	P (1·000), NA, NA	CM950438; UMD	/
22	22	*FLNA*	c.3421G > A (A/–)	p.Ala1141Thr	VUS	P (0·999), P (0·939), B (0·051)	Novel	0·0003147

Nucleotide numbering uses +1 as the A of the ATG translation initiation codon in the reference sequence, with the initiation codon as codon 1. Patient numbers are in accordance with the patient numbers in Table 2.

/: not present in ExAC B: benign; NA: not available; P: pathogenic.

[a] Numbering of domains is based on Uniprot entry: P35555 (see Supplementary Table S1(*a*)–(*c*) for the relevant alignments).

[b] dbSNFP, integrated into the VariantDB annotation tool (Vandeweyer *et al.*, 2014), was used to automatically generate the prediction scores of MutationTaster, Polyphen-2 and SIFT respectively, as described by Liu *et al.*, 2011.

[c] In the UMD FBN1 (www.umd.be/FBN1/) or in the HGMD (public part: www.hgmd.cf.ac.uk/ac).

[d] DIDE consensus sequence: Asp–Ile–Asp–Glu (see Supplementary Table S1(*a*)).

Multiplex ligation-dependent probe amplification (MLPA) was performed on samples that remained negative after TAA NGS panel testing.

We performed statistical analysis for possible genotype/phenotype correlations in the *FBN1* mutation positive and mutation negative groups using the Mann–Whitney U-test for continuous variables and the Fisher's exact test for categorical variables. The patients carrying a variant of unknown significance (VUS) in *TGFBR1* and *FLNA* (patient 1 and 22, respectively) were excluded from this analysis. The patients carrying both a *FBN1* mutation and one or more VUS were placed in the mutation positive group.

3. Results and discussion

Of the 40 probands, 27 had causal mutations in *FBN1*, one patient had a VUS in *TGFBR1*, and one patient had a VUS in *FLNA*. In addition to the *FBN1* mutation, four patients had additional variants in either *FBN1, SMAD3, FLNA* or *NOTCH1* (Table 1). At this moment, we cannot exclude that these VUS may modify the phenotype caused by the *FBN1* mutation. Of the 27 mutations in *FBN1*, 12 were missense, 11 predicted a premature termination codon (nine nonsense and two frameshifts) and four affected splice sites. Ten of these *FBN1* variants were novel. Except one (c.3845A > G; p.Asn1282Ser in patient 17), the *FBN1* mutations were not present in the ExAC database (Table 1). No large deletions/duplications could be detected by MLPA.

As our TAA NGS assay has a validated high sensitivity, and as MLPA excluded the presence of large deletions/insertions, we can largely rule out the possibility of false negatives in the coding regions. Only deep intronic mutations and mutations in the 5′- and 3′-untranslated regions will remain undetected with the applied methodology, but we expect that these account for only a minor fraction of all MFS patients. Thus, a possible explanation for the relatively low yield may be the fact that several mutation negative probands did not fulfill the diagnostic criteria for MFS. Indeed, of the 27 *FBN1*-positive probands, only 15% (four out of 27) had a systemic score below seven, while this was 67% (six out of nine) for the nine *FBN1*-negative probands. Furthermore, only one of the 15 *FBN1* negative patients did present ectopia lentis. As such, more Marfan-like than true Marfan (fulfilling clinical diagnostic criteria) presentations were present in the *FBN1*-negative group. Moreover, Marfan-like patients that remained negative with the gene panel, may carry mutations in more recently identified TAA genes or yet to be identified TAA genes.

For six of the probands, DNA of family members was available and segregation analysis was performed. The p.Cys570Tyr mutation (patient 2) was found in

https://doi.org/10.1017/S0016672316000112 Published online by Cambridge University Press

Table 2. *Clinical data of 40 MFS probands and their family members.*

Patient	Gender	Diagnosis	Mutation	Systemic score	Surgery	Surgery aortic root (mm)	Age at surgery	Ectopia lentis
MFS patients and their relatives								
2·1	F	MFS	*FBN1*: p.Cys570Tyr	10	Yes	63	20	Yes
2·2	M	MFS	*FBN1*: p.Cys570Tyr	11	Yes	73	33	Yes
2·3	M	MFS	*FBN1*: p.Cys570Tyr	8	No	/	/	Yes
2·4	F	MFS	*FBN1*: p.Cys570Tyr	7	No	/	/	Yes
3	F	MFS	*FBN1*: p.Arg1790*, *FBN1*: p.Ala986Thr	8	Yes	53	41	No
4	M	MFS	*FBN1*: c.6313 + 3insT	11	Yes	79	28	No
5	M	MFS	*FBN1*: c.4582 + 1G > T	11	Yes	59	32	Yes
6	F	MFS	*FBN1*: p.Glu2610Lys	7	Yes	67	25	No
7	M	MFS	*FBN1*: c.1589–1G > A	13	Yes	64	33	No
8	F	MFS	*FBN1*: p.Arg364*	8	Yes	66	31	No
9	F	MFS	*FBN1*: p.Cys2210Tyr	9	Yes	62	42	No
10	M	MFS	*FBN1*: p.Glu1366Lys, *FBN1*: p.Ala986Thr	12	Yes	72	40	No
12·1	M	MFS	*FBN1*: p.Lys1983*	11	Yes	57	22	No
12·2	F	MFS	*FBN1*: p.Lys1983*	6	No	/	/	No
13	M	MFS	*FBN1*: p.Arg1541*	9	Yes	70	52	No
14	M	MFS	*FBN1*: p.Cys85Phe	12	Yes	73	22	Yes
16	F	MFS	*FBN1*: p.Trp988*	9	Yes	72	25	No
17	M	MFS	*FBN1*: p.Asn1282Ser, *SMAD3*: p.Ser423Arg	9	Yes	78	29	No
19	M	MFS	*FBN1*: p.Cys1320*	11	Yes	65	32	No
20	M	MFS	*FBN1*: p.Cys2571Tyr	9	Yes	71	19	Yes
23·1	F	MFS	*FBN1*: p.Arg2394*	8	Yes	60	32	Yes
23·2	F	MFS	*FBN1*: p.Arg2394*	7	No	/	/	Yes
24	M	MFS	*FBN1*: p.Gly880Asp	9	No	/	/	Yes
25	M	MFS	*FBN1*: p.Cys1408Arg	7	Yes	110	24	Yes
26·1	F	MFS	*FBN1*: p.Thr2785Tyr fs*16	14	Yes	79	24	No
26·2	F	Unaffected	No	3	No	/	/	No
26·3	M	Unaffected	No	2	No	/	/	No
27	M	MFS	*FBN1*: c.1960 + 1G > A, *NOTCH1*: p.Pro2138Leu, *FLNA*: p.Ser61Asn	13	Yes	64	35	Yes
28	M	MFS	*FBN1*: p.Cys2611Arg	9	Yes	90	23	No
29·1	F	MFS	*FBN1*: p.Cys85Tyr	6	Yes	61	39	Yes
29·2	F	MFS	*FBN1*: p.Cys85Tyr	11	No	/	/	Yes
29·3	M	MFS	*FBN1*: p.Cys85Tyr	5	No	/	/	Yes
31	M	MFS	No	10	Yes	65	49	No
32	M	MFS	No	11	Yes	78	31	No
33	M	MFS	No	11	Yes	68	37	No
34	M	MFS	No	10	No	/	/	No
40	M	MFS	No	11	Yes	120	31	Yes
MFS-like and their relatives								
1	M	MFS-like	*TGFBR1*: p.Arg237Gly	6	Yes	67	42	No
11·1	F	MFS-like	*FBN1*: p.Ile2585Thr	3	Yes	70	49	No
11·2	M	MFS-like	*FBN1*: p.Ile2585Thr	5	No	/	/	No
15	M	MFS-like	*FBN1*: p.Gln2517*	5	Yes	57	43	No
18	F	MFS-like	*FBN1*: p.Met2347 fs*19	5	Yes	64	48	No
21	M	MFS-like	*FBN1*: p.Arg565*	6	Yes	74	30	No
22	M	MFS-like	*FLNA*: p.Ala1141Thr	3	Yes	70	57	No
30	F	MFS-like	No	2	Yes	78	43	No
35	M	MFS-like	No	2	Yes	73	45	No
36	M	MFS-like	No	4	Yes	50	46	No
37	M	MFS-like	No	6	No	/	/	No
38	M	MFS-like	No	4	Yes	57	64	No
39	M	MFS-like	No	4	Yes	67	64	No

Patient numbers are in accordance with the patient numbers in Table 1 and suffixes indicate family members.
/ : no surgery.

https://doi.org/10.1017/S0016672316000112 Published online by Cambridge University Press

Table 3. *Clinical data of 38 MFS probands and their family members.*

	Mutation		
	Negative	Positive	*p*-value
Male	11	20	0·099
Female	2	15	0·099
Surgery	9	27	0·710
Aortic diameter at surgery (mm)	73	69	0·693
Age at surgery	46	32	0·006
Ectopia lentis	1	15	0·036

three additional affected family members (Table 2). Although all family members presented with ectopia lentis, only two have undergone aortic surgery (ages 20 years and 33 years). Also the p.Ile2585Thr (patient 11), the p.Arg2394* (patient 23) and the p.Cys85Tyr (patient 29) mutations segregated with the MFS phenotype in four additional family members. The truncating mutation (p.Lys1983*) identified in patient 12 was also found in his mother. She had no aortic root dilatation, but presented with mitral valve prolapse and skeletal features including wrist sign, pectus carinatum and tall stature. As a direct result of our genetic testing, she is now in regular cardiovascular follow-up and has been started on losartan in order to delay future aortic surgery.

Next, statistical analysis for possible genotype/phenotype correlations was performed (Table 3). We found a significant difference between the *FBN1* mutation positive and mutation negative patients for the age at surgery (Mann–Whitney U, $p = 0{\cdot}006$) and the presence of ectopia lentis (Fisher's exact test, $p = 0{\cdot}036$). Patients with a mutation in *FBN1* had aortic surgery at an earlier age (32 years on average) than mutation negative patients (46 years on average). This emphasizes the importance of genetic screening for the identification of patients that are at higher risk for developing aortic aneurysms and dissection. According to the literature, a higher frequency of truncating and splicing variants in *FBN1* can be observed in patients with an aortic event (Baudhuin *et al.*, 2015). In our cohort, no significant difference could be observed between truncating or splice variants and missense variants in patients with an aortic event (Fisher's exact test, $p = 0{\cdot}282$). Of course, our study was not sufficiently powered to detect such differences.

4. Conclusion

In conclusion, we identified *FBN1* gene mutations in Ukrainian MFS patients for the first time. Since the clinical picture of these patients is not always clear, genetic screening can help to establish a diagnosis and to identify patients at high risk for developing life-threatening complications such as aortic aneurysm and dissection.

We are grateful to all patients who participated in this study. B. L. Loeys is senior clinical investigator and J. A. N. Meester is a predoctoral researcher of the Fund for Scientific Research, Flanders (FWO, Belgium). This research was supported by funding from the University of Antwerp (Lanceringsproject), the Fund for Scientific Research, Flanders (FWO, Belgium) (G.0221·12), an ERC starting grant to B. L. Loeys and the Hercules Foundation.

Declaration of interest

None.

Supplementary material

The online supplementary material can be found available at http://dx.doi.org/10.1017/S0016672316000112

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https://doi.org/10.1017/S0016672316000112 Published online by Cambridge University Press