Genotype–phenotype correlations in Marfan syndrome

Benjamin J Landis,[1] Gruschen R Veldtman,[2] Stephanie M Ware[1]

Marfan syndrome (MFS) is an autosomal dominant connective-tissue disorder associated with abnormalities of the cardiovascular, ocular and musculoskeletal systems. Aortopathy, manifest as thoracic aortic aneurysm (TAA) and dissection, is the major cause of morbidity and mortality. Most individuals with MFS carry mutations in the gene *FBN1*. This gene encodes the extracellular matrix (ECM) protein fibrillin-1, which plays essential roles in the formation of microfibrils, organisation of ECM components and sequestration of growth factors such as transforming growth factor beta (TGFβ). Thus, mutations in *FBN1* lead to deleterious biomechanical effects and aberrant signalling pathway activation within the aorta and other affected tissues.

Though autosomal dominant, MFS has wide phenotypic variability. For example, the age of onset, severity and rate of progression of TAA is currently unpredictable. These gaps in knowledge pose important limitations in clinical decision making with respect to timing of elective surgery, frequency of imaging follow-up, physical activity restriction and drug management. In their *Heart* paper, , Franken *et al* have undertaken the important task of investigating whether the specific subtype of *FBN1* mutation could be used to predict the risk of TAA severity.[1] The current study examined 290 patients with MFS and known *FBN1* mutations followed at two specialist units, including Universitat Autonoma de Barcelona in Spain, and the Academic Medical Centre of Amsterdam, The Netherlands. The investigators tracked aortic diameter, aortic dilation rate and clinical endpoints of dissection and death from 2004 to 2015.

THE PATHOGENESIS OF MFS

Mutations in *FBN1* can cause (1) reduction in the fibrillin produced in the cell, that is, quantitative defects, (2) change the structure or stability of the protein, and/or (3) alter the ability of fibrillin to be exported to the ECM (figure 1) . Mutations in *FBN1* that result in haploinsufficiency (HI) lead to reduced amounts of fibrillin, fewer microfibrils and increased activated TGFβ levels due to decreased TGFβ sequestration. In contrast, dominant negative (DN) mutations in *FBN1* result in qualitative defects of the protein that may affect function such as folding or protein–protein interactions leading to disorganisation of the ECM, altered ECM strength and increased TGFβ. The understanding of disease pathogenesis has been facilitated by mouse models of MFS that demonstrate the importance of HI.[2] Conceptually, DN mutations should result in more phenotypic variability because mutations at different region of the protein will have distinct effects, whereas HI mutations should have more consistent phenotypes. Only patients for whom a DN or HI mutation type could be assigned were studied by Franken *et al*. Along with software predictions, the authors also used existing literature to classify the functional effects of several specific variants in *FBN1* that were previously studied in detail in vitro. This interesting work presents evidence that patients with HI mutations have a greater aortic risk as compared with DN mutation carriers. Specifically, those patients with HI mutations had aortic surgery at a younger age (but similar overall rate of surgery), had a larger aortic root at the time of initial referral to their centres and showed more rapid dilation of the aortic root over the course of clinical follow-up.

GENETICS AND MUTATION TYPE IN MFS

Previous studies have also examined mutation type to determine whether genotype–phenotype correlations exist in MFS, dividing mutations into protein truncating or in-frame mutation types.[3 4] These studies fail to completely account for the effect of a mutation on the protein product. For example, a protein truncating mutation may result in HI or DN effects depending on the stability of the protein. Interestingly, there was no difference noted in aortic dilation when classifying mutations in this manner, but comparison within the in-frame group based on whether a cysteine residue was involved did show a significantly higher probability of aortic dilation and mitral valve prolapse.[4] It would be of interest to determine whether the subset of DN mutations that alter cysteine residues show similar results in the current study. The current study's approach to mutation classification is more sophisticated and is supported by prior evidence that the software accurately predicts presence or absence of the mutated transcript.[5] Nevertheless, examples in the literature demonstrate that in order to assign HI versus DN effects with confidence, one must understand the mutation's consequences at the protein level.

A limitation for this retrospective study is that patients with the most severe disease who had aortic dissection or aortic replacement surgery (ARS) prior to referral to the centre were not eligible for longitudinal study. Mutations in exons 24–32 are particularly associated with severe, early aortic disease. The majority of mutations in this region are missense or in-frame insertions/deletions, which manifest a more severe phenotype than the minority of truncating variants within this region,[4] and a DN effect was observed in fibroblasts derived from patients with mutations in this region.[6] The severe nature of these DN mutations seems to contradict the study's major conclusion of HI mutations' having more severe effects. One potential explanation would be that patients carrying in-frame variants in this region died at a young age or underwent ARS and were thus ineligible for longitudinal study. Consistent with prior literature, patients with DN mutations in the study cohort were more likely to have ectopia lentis. This may have introduced an important ascertainment bias if ectopia lentis led to earlier diagnosis of MFS at referring centres and facilitated earlier initiation of medical therapies.

The wide phenotypic variability among patients with MFS includes variability even between relatives who carry the same mutation. Intrafamilial variability is a strong counterargument for classifying patients according to *FBN1* genotype. Related individuals were included in this study for the majority of the analyses between HI and DN groups. There were 169 probands among the 290 patients studied, with the remaining cohort comprised of relatives. It is interesting

[1]Department of Pediatrics and Medical and Molecular Genetics, Indiana University School of Medicine, Ringgold Standard Institution, Indianapolis, Indiana, USA
[2]Adult Congenital Heart Disease Program, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, USA

Correspondence to Prof Gruschen R Veldtman, Cincinnati Children's Hospital Medical Centre, Adult Congenital Heart Disease Program, Cincinnati, Ohio, 45229, USA; Gruschen.veldtman@cchmc.org

Landis, B. J., Veldtman, G. R., & Ware, S. M. (2017). Genotype-phenotype correlations in Marfan syndrome. Heart (British Cardiac Society), 103(22), 1750–1752. https://doi.org/10.1136/heartjnl-2017-311513

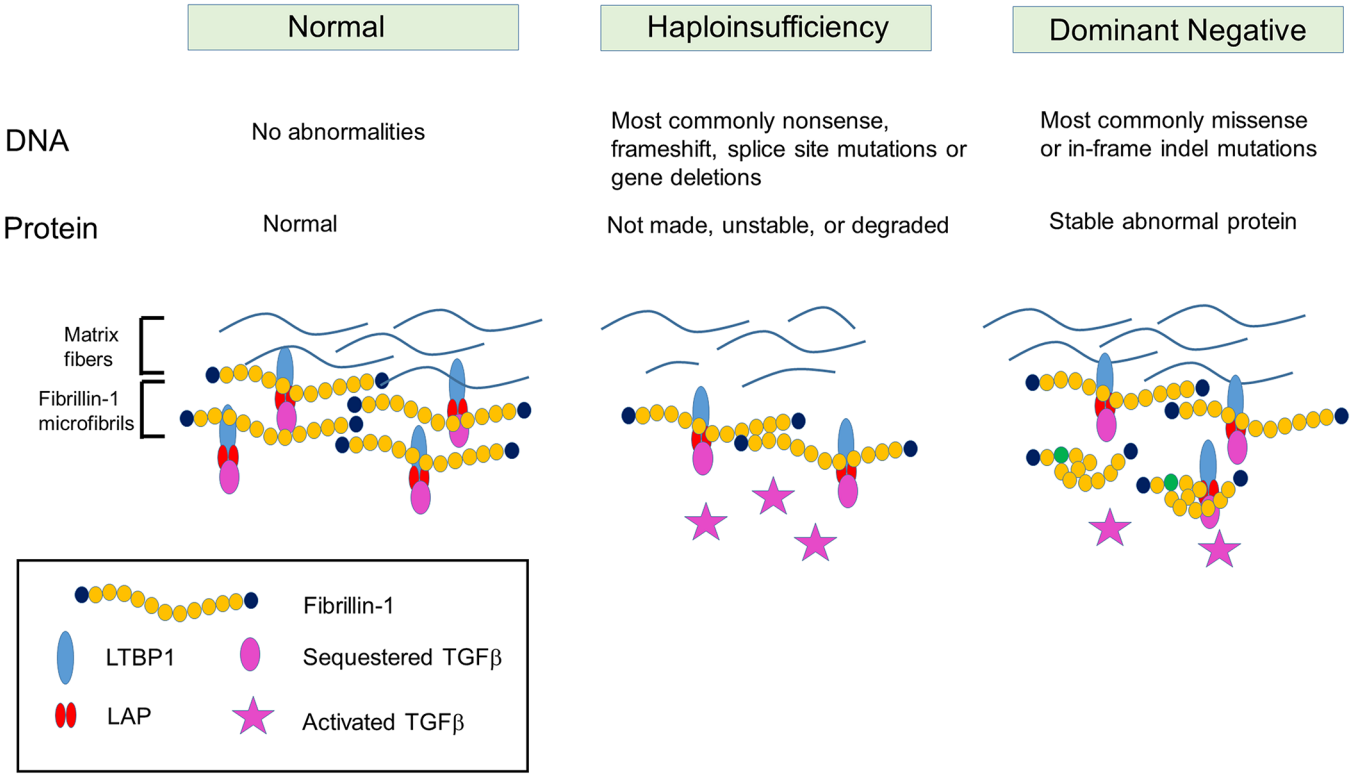

Figure 1 Genetics and pathogenesis in Marfan syndrome. A schematic overview of the effect of haploinsufficiency (HI) or dominant negative (DN) mutations is shown. The most common type of mutations that lead to HI or DN effect are listed. However, these classifications are not absolute, and the precise effect of a specific DNA mutation must be determined at the protein level. Mutations that cause HI result in less protein because only the normal, non-mutated *FBN1* allele generates protein products. In contrast, a combination of normal protein products (fibrillin-1) generated by the non-mutated *FBN1* allele and stable abnormal protein products generated by the DN allele are seen with DN mutations. The green circle in the fibrillin-1 protein represents the altered amino acid residue. Normal microfibrils are associated with latency associated peptides (LAP; red circles), which bind TGFβ in a biologically inactive complex in combination with latent TGFβ binding protein (LTBP1; blue oval). When microfibrils are reduced due to HI, interaction with matrix fibres is disrupted, and increases in activated TGFβ are seen due to a reduced number of microfibrils. DN mutations can have a range of effects depending on the specific mutation. Shown here, a DN mutation results in abnormalities in fibrillin-1 protein folding and increased activated TGFβ. TGFβ, transforming growth factor beta.

that when only one patient from each family was included, the SD of the rate of aortic root dilation decreased, in spite of an overall decrease in number tested. This indirectly suggests that including family members resulted in greater variability in aortic dilation. However, this could have been an artefact of how the patients were selected. Whether certain genotypes are more susceptible to specific genetic or environmental modifiers is an important question for future research.

CARDIAC PHENOTYPING OF PATIENTS WITH MFS

In the longitudinal analyses of the current study, patients with HI mutations were found to have more rapid dilation than DN mutation carriers at the levels of the aortic root and the ascending aorta by approximately 0.3 mm/year. As the authors discuss, despite having a relatively large cohort, this study was still underpowered to conclusively evaluate differential risk of aortic dissection or need for ARS between HI and DN. Requirement for elective ARS is inherently vulnerable to variations in clinical practice, and institutional biases may have amplified the observations documented here. Given these limitations, the authors used absolute aortic size as another marker of aortic disease severity. When using absolute aortic root size or dilation rate as an endpoint, it is important to appraise the specifics of centre practice variations, individual technical limitations, interval duration between measurements and, importantly, differences in overall body size. Though we would advocate adjusting aortic size relative to body surface area, we recognise important limitations to this approach. First, there are numerous published nomograms that substantially differ from one another due to inconsistent factors including different methods of diameter measurement (eg, in systole or diastole), distribution of ages, genders and race/ethnicity within the reference population, formula for body surface area calculation and whether to include age and gender in Z-score calculations.[7 8] Guidelines for timing of elective ARS are mostly based on absolute diameter, but the revised Ghent nosology uses a Z-score threshold as a criterion to diagnose MFS.[9 10] This lack of a consensus approach for how to index aorta to body size hinders consistent clinical care and creates research challenges. Given the important genotype–phenotype correlations identified in the current study, the design and interpretation of future validation studies will need to consider the method for determining aortic size. Based on the data provided for the current study, it appears that longitudinal analyses included children as young as age 5 years and included around 50 who were age 18 or younger at the start of this study. This means that some of the observed growth in aortic size was physiological.[11] Whether using Z-scores would alter the findings of

this study is undetermined, but at baseline there were no differences between age, gender or body surface between mutation type groups. Finally, morphological and haemodynamic factors, such as bicuspid aortic valve and aortic regurgitation, should be considered, if available, when assessing factors that control TAA progression.

PRECISION DIAGNOSTICS

Altogether, the current study makes an important stride towards improving cardiovascular risk classification of patients with MFS and supports the authors' previous findings in the Dutch CONgenital CORvitia registry.[12] The study detects an aortic phenotype difference based on *FBN1* mutation classifications and stands as an example of the increasingly powerful capability to stratify patients based on individualised genetic testing results. Thus, the study shows the potential value of genotyping all patients with MFS and consideration of the role of allelic heterogeneity in management. In addition, the study highlights the value of large volume centres specialised in genetic and cardiac management of aortopathy to analyse robustly phenotyped cohorts and propel clinical practice forward. Whether and how the study's main finding, that is, that HI mutations confer worse prognosis than DN mutations, should be incorporated into routine clinical management practices remains to be determined and will require follow-up in larger cohorts. There is prior evidence that mutation type may alter the patient's response to losartan, providing promise for increasingly tailored patient-specific therapy.[5] Additional mechanistic studies to determine how *FBN1* mutation type alters aortic phenotype severity or response to medical therapies are warranted. Genotype–phenotype correlations promise to improve clinical management decisions to prevent adverse outcomes such as aortic dissection in higher risk patients and also to avoid unnecessary interventions in patients at lower risk.

Contributors BJL, GRV and SMW drafted and critically reviewed the manuscript.

Funding BJL is supported by funding from the National Institutes of Health (K12HD068371). SMW is supported by funding from March of Dimes Research Foundation (grant nos. 6-FY13-167 and 6-FY16-176), the National Institutes of Health (P01 HL 134599-01), an American Heart Association Established Investigator Award (AHA 13EIA13460001) and the Indiana University Health—Indiana University School of Medicine Strategic Research Initiative and PhysicianScientist Initiative.

Competing interests None declared.

Provenance and peer review Commissioned; internally peer reviewed.

To cite Landis BJ, Veldtman GR, Ware SM. *Heart* Published Online First: [*please include* Day Month Year]. doi:10.1136/heartjnl-2017-311513

► http://dx.doi.org/10.1136/heartjnl-2016-310631

Heart 2017;**0**:1–3. doi:10.1136/heartjnl-2017-311513

REFERENCES

1 Franken R, Teixido-Tura G, Brion M, *et al*. Relationship between fibrillin-1 genotype and severity of cardiovascular involvement in Marfan syndrome. *Heart* 2017:Published Online First: 03 May 2017. doi: 10.1136/heartjnl-2016-310631.
2 Judge DP, Biery NJ, Keene DR, *et al*. Evidence for a critical contribution of haploinsufficiency in the complex pathogenesis of marfan syndrome. *J Clin Invest* 2004;114:172–81.
3 Faivre L, Collod-Beroud G, Callewaert B, *et al*. Clinical and mutation-type analysis from an international series of 198 probands with a pathogenic FBN1 exons 24-32 mutation. *Eur J Hum Genet* 2009;17:491–501.
4 Faivre L, Collod-Beroud G, Loeys BL, *et al*. Effect of mutation type and location on clinical outcome in 1,013 probands with marfan syndrome or related phenotypes and FBN1 mutations: an international study. *Am J Hum Genet* 2007;81:454–66.
5 Franken R, den Hartog AW, Radonic T, *et al*. Beneficial outcome of losartan therapy depends on type of FBN1 mutation in marfan syndrome. *Circ Cardiovasc Genet* 2015;8:383–8.
6 Godfrey M, Raghunath M, Cisler J, *et al*. Abnormal morphology of fibrillin microfibrils in fibroblast cultures from patients with Neonatal Marfan syndrome. *Am J Pathol* 1995;146:1414–21.
7 Devereux RB, de Simone G, Arnett DK, *et al*. Normal limits in relation to age, body size and gender of two-dimensional echocardiographic aortic root dimensions in persons ≥15 years of age. *Am J Cardiol* 2012;110:1189–94.
8 Cantinotti M, Scalese M, Molinaro S, *et al*. Limitations of current echocardiographic nomograms for left ventricular, valvular, and arterial dimensions in children: a critical review. *J Am Soc Echocardiogr* 2012;25:142–52.
9 Hiratzka LF, Bakris GL, Beckman JA, *et al*. 2010 ACCF/AHA/AATS/ACR/ASA/SCA/SCAI/SIR/STS/SVM guidelines for the diagnosis and management of patients with thoracic aortic disease: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice guidelines, American Association for thoracic surgery, American College of Radiology, American Stroke Association, Society of Cardiovascular Anesthesiologists, Society for Cardiovascular Angiography and Interventions, Society of Interventional Radiology, Society of Thoracic Surgeons, and Society for vascular medicine. *Circulation* 20102010;121:e266–369;121:e266–369.
10 Loeys BL, Dietz HC, Braverman AC, *et al*. The revised Ghent nosology for the Marfan syndrome. *J Med Genet* 2010;47:476–85.
11 Campens L, Demulier L, De Groote K, *et al*. Reference values for echocardiographic assessment of the diameter of the aortic root and ascending aorta spanning all age categories. *Am J Cardiol* 2014;114:914–20.
12 Franken R, Groenink M, de Waard V, Waard de V, *et al*. Genotype impacts survival in Marfan syndrome. *Eur Heart J* 2016;37:3285–90.