Title

Formation and malformation of cardiac trabeculae-Biological basis, clinical significance and special yield of magnetic resonance imaging in assessment

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ABSTRACT

Adult and pediatric cardiologists are familiar with variation in cardiac trabeculation. Abnormal trabeculation is a key feature of left ventricular noncompaction (LVNC), but it is also common in congenital heart diseases and in cardiomyopathies (dilated and hypertrophied). Trabeculae may be a measurable phenotypic marker that will allow insights into how cardiomyopathy and congenital heart disease arise and develop. This will require the linking together of clinical and preclinical information (such as embryology, genetics), with new analysis methods for trabecular quantitation. In adult cardiology several promising quantitative methods have been developed for echocardiography, computed tomography and cardiovascular magnetic resonance (CMR) and earlier cross-sectional caliper approaches have now been refined to permit more advanced assessment. Adapting these methods for use in developmental biology may inform on better ways to measure and track trabecular morphology in model organisms.

This review outlines the biological significance of myocardial trabeculae and their impact on normal cardiac development. It emphasises the heterogeneous spectrum of trabecular complexity in the general population and suggests a clinically useful algorithm to guide the management of patients discovered to have abnormal trabeculation at the time of cardiac imaging.

KEYWORDS

Cardiovascular MRI; Trabeculation; Noncompaction; Genetics; Notch

BRIEF SUMMARY

This review outlines the biological significance of myocardial trabeculae and their impact on the normal cardiac developmental trajectory. It emphasises the heterogeneous spectrum of trabecular complexity in the general population and the coexistence of trabecular abnormalities with other well-known cardiac diseases. A clinically useful management algorithm for patients with a new imaging diagnosis of abnormal trabeculation is suggested.

BIOLOGY AND CLINICAL RELEVANCE OF TRABECULAE

Myocardial trabeculae make their first embryonic appearance in the developing mouse at the end of cardiac looping, specifically embryonic days (E) 9.0–9.5 (Carnegie stage 12 in the human). An early role is believed to be that of optimising efficient nutrient and gas exchanges before the development of the coronary arteries[1] (the mouse heart is devoid of any intramural vessels till E12.5[2]). By E14.5 (Carnegie stage 22 in the human) ventricular septation is complete and a dense trabecular meshwork is established within the ventricular cavities.[3] This reduces by birth with the formation of papillary muscles, the moderator band and effective arterial valves in a functioning adult patterned heart. Morphologically, two sequential phases of trabecular development can be identified in mouse (**Figure 1**): (i) an early phase (E9.0–13.5) dominated by the formation of a thick trabeculated meshwork, mediated through cardiomyocyte differentiation and terminal proliferation, and maintained by intact ligand/receptor interactions between endocardial cells and between endocardial and myocardial cells[4] (**Figure 2**); and (ii) a late phase (beyond E13.5) where the trabecular zone is less dense and a thick compacted layer is observed. The commonly held view that trabeculae in this late phase of cardiac development "condense" somehow to give the compact layer, leaving only a little behind (as seen in the adult human heart) is still lacking compelling evidence. Further research is needed to definitively show how the two events of trabecular remodelling and compact layer formation in the developing heart are linked rather than distinct.

Using small animal models, genetic disruptions affecting the normal endo/myocardial crosstalk in the developing heart have already been shown to manifest as either decreased or increased trabeculation (**Table 1** lists specific examples). Notably,

hypotrabeculated mutants exhibit severe heart failure and embryonic lethality indicating that trabeculae have a 'pro-survival' role in cardiogenesis. Trabecular development also impacts other aspects of normal cardiac structure and function such as the development of the conduction system.[3]

Their biological importance is also reflected in their phylogenetic context: trabeculae make up the ancestral spongy myocardium predominating in fish, reptiles and amphibians that then permitted the evolution of sophisticated circulatory systems in terrestrial birds, mammals and crocodilians.[5] In making the switch from trabeculated to compact myocardium, mammals became obligatorily dependent on a coronary circulation and vulnerable therefore, to the effects of atherosclerosis – in fact, coronary ligation may kill a mammal but not a rattlesnake[6] where the well developed trabecular myocardium rescues the reptile's cardiac oxygen supply. Likewise, the trabecular myocardium in adult salmon allows it to migrate upstream in high velocity rivers even in the presence of critical coronary atherosclerosis.[7]

While hypotrabeculation (examples in **Table 1**) prevents the developing heart from functioning as an effective cardiovascular organ, an excessive number of abnormal trabeculae has also been shown to link with cardiac disease and it is the principal anatomic characteristic of isolated left ventricular noncompaction (LVNC), a condition for which there is a current lack of consensus on terminology and diagnostic criteria[8] (reviewed elsewhere[9,10]) that rely heavily on imaging and measuring the complex 'fractal' biology of the trabecular meshwork. 'Fractals' are natural forms or mathematical sets with complicated, repeating patterns that defy robust quantification using simple cross-sectional caliper measurements. The intricate, three-dimensional meshwork that is formed from myocardial trabeculae has fractal properties. Careful

quantification of trabecular complexity and its cautious interpretation using ethnically-appropriate reference ranges[11] is needed to diagnose LVNC and prevent misapplied diagnoses of noncompaction in otherwise healthy adult and paediatric subjects. Mathematical methods like fractal analysis (**Figure 3**) have confirmed that a heterogeneous spectrum of trabeculation exists across healthy hearts in the population[11,12] and the sole presence of excessive trabeculae is quite benign in asymptomatic individuals.[13] Excessive trabeculation discovered at the time of cardiac imaging may therefore represent one of three things: a benign phenotype not uncommon in the general population,[13] a distinguishing feature of isolated LVNC, or a shared morphological trait[10,14,15] common to many cardiac diseases clinically and clustering into distinct phenotypes. Phenotyping in small animal models of LVNC has already shown how excessive trabeculation is commonly associated with either dilated cardiomyopathy[16] (DCM) or congenital heart disease (CHD)[17–19] and the same is also true in adult and paediatric cardiology given that 8 of the 12 LVNC phenotypes reported in the Online Mendelian Inheritance in Man database[20] (OMIM) have similar associations (**Table 2**). Human genetic studies have shown how, like many other heart muscle disorders, LVNC exhibits significant genetic heterogeneity[10] (e.g. taffazin, mindbomb homolog 1 (*Mib1*),[21] sarcomere[22] and cytoskeletal protein gene mutations) and diverse inheritance patterns. The number of reported mutations is likely to grow with whole-exome/genome sequencing and more detailed familial investigations with improved phenotyping in LVNC[23,24] but confirmation that these new mutations are causative will require the use of experimental disease models.[25]

The phenotypic and genetic heterogeneity described for LVNC, raises the question of whether use of the term “isolated” should be enforced as a non-optional prefix when

referring to ‘pure’ LVNC, to distinguish it from the more common, cardiomyopathy-associated variant. A proposed nosology could be: ‘isolated LVNC’ and then ‘DCM with LVNC’ and ‘CHD with LVNC’ as recently proposed from the MOGE(S) classification.[26]

ANALYSIS METHODS FOR TRABECULATION

The trabecular abnormalities observed in some animal models (studied principally by histology) resemble those described in a proportion of LVNC patients studied by echocardiography,[8] computed tomography[27] or CMR[28] but other features like the compact wall hypoplasia, may not necessarily match the clinical phenotype. Cardiac morphologists have noted the similarity between trabecular phenotypes in LVNC and the developing heart.[29] The major difference is that in the clinical domain, formal quantitation of trabecular complexity is the norm – visual assessment is simply too subjective and insensitive, and cannot be relied upon for accurate diagnosis. In fact, at least 14 different methods for formal trabecular quantitation across 3 modalities[30] have been proposed clinically. Of these, at least 8 are specific to CMR (**Table 3**) with some even including population-based reference ranges for describing the normal spectrum of trabeculation in health.[11,13,31,32]

The first diagnostic criteria for LVNC were developed for echocardiography (Chin et al.,[33] Jenni et al,[34] Stöllberger et al.[35,36], discussed elsewhere[30]), and to date this modality remains the most accessible, first-line tool for evaluating LV structure and trabecular complexity. Using Doppler, it is possible to demonstrate inter-trabecular blood flow confirming the connection between the ventricular cavity and the deep inter-trabecular recesses, considered to be an important feature for LVNC diagnosis.[34–36] The

most commonly used echocardiographic criterion for LVNC is the noncompacted-to-compacted wall thickness ratio of >2 at end-systole that has been validated against other cardiomyopathies.[37] End-diastolic counts of LV trabeculae (>3 trabeculations protruding from the LV wall, located apically to the papillary muscles and visible in one image plane) were a key feature of the Stöllberger criterion.[35] The criterion that focused on a two-layered structure was added later.[36] LVNC criteria have also been developed for computed tomography[27,38] (CT) and these are especially valuable in patients with limited echocardiographic windows or contra-indications for CMR. CT permits relative ease of acquisition, temporal and spatial resolutions not too dissimilar from those obtained with MR and low radiation doses thanks to recent advances. It also potentially overcomes the partial voluming CMR effects that hamper accurate trabecular quantification particularly in the apico-lateral ventricular segments.

To date all the CMR approaches have been based on measurements derived from balanced steady-state free precession cines that typically provide a spatial resolution of 1.5 mm and 6–8 mm in the *x*/*y* and *z* directions respectively, and a temporal resolution of 40 ms. In the adult heart, the width of the trabecular meshwork is not usually > 3 mm, with individual trabeculae typically of the order 0.05–0.5 mm in diameter.[39] Using transthoracic echocardiography the best possible axial resolution cannot exceed two wavelengths, that is approximately 0.3–2 mm,[40,41] while lateral resolution (based on beam width, tissue depth, line density) tends to be lower–in a good scan, a maximum could be 2 mm at a depth of 10 cm (with a complex slice profile). Because of its ability to provide consistent, high-resolution cardiac cines with good blood-myocardial contrast particularly in the apical half of the ventricle and without the need for ionising

radiation, CMR has become one of the imaging modalities of choice for trabecular quantification in patients.

The nature of trabeculae makes simple cross-sectional measurements sub-optimal. More advanced mathematical solutions like fractal analysis[42] and the Grothoff method[43] (that estimates the mass of the trabeculated myocardium using a combination of automated segmentation and manual correction) have become possible thanks to the high blood-myocardial contrast afforded by CMR. These approaches provide a degree of semi-automation that is of value when attempting to quantify complex biology. A further advantage is that they can also be potentially repurposed for use with other imaging modalities. We tend to favour the use of semi-automated, mathematical approaches for trabecular quantification. The open-source release of such analysis tools and provision of training datasets is key to rendering these approaches more accessible to the imaging community. Like Petersen et al.[28] we recommend (**Figure 4**) that irrespective of the method used, any meter of endocardial complexity be interpreted within its clinical context, searching first for coexisting cardiac disease that is well-known (like DCM, CHD) and taking into account the pre-test probability of LVNC, that is higher in those with impaired LV function, positive family history, neuromuscular disease, arrhythmia, bundle branch block on electrocardiography, syncope or thromboembolism.

The fractal analysis approach using CMR datasets is sensitive to subtle changes in trabecular morphology that cannot be detected by caliper approaches – subjects carrying a disease causing mutation in sarcomeric proteins that cause hypertrophic cardiomyopathy (HCM) have been shown to have at least 4 differences compared to healthy volunteers (crypts, increased length of the anterior mitral valve leaflet, increased systolic function and abnormal trabeculation, possibly through the effect of

hypertrophy. The analysis is robust at both single[42] and multicentre scale, and accurate – the use of a scoring system that includes the fractal dimension of trabeculae is capable of predicting subclinical gene carriage in HCM.[44] It has been used to better define LVNC[12] and it can distinguish differences in trabecular complexity between Afro-Caribbean, Chinese and Caucasian subjects in the Multi-Ethnic Study of Atherosclerosis [MESA]).[11] A fractal dimension greater than 1.385 may be considered to be highly abnormal. We have previously shown how this points either to a pathological diagnosis of LVNC[12] or to the presence of grossly altered trabeculae resulting from the interplay of ethnicity/racial background, LV hypetrophy and abnormal cardiac loading conditions.[11] Just like in the clinical domain, the imaging technologies to permit detailed 3D analysis of trabecular morphology in model organisms already exist. In addition to the traditional 2D techniques (electron-microscopic, histological, and macroscopic sections etc.) developmental biologists can now avail themselves of episcopic 3D imaging methods,[45] where typically a microtome physically sections a tissue block, captures serial images of the freshly-cut block surface and finally generates high-resolution volumetric cardiac data. Amongst the currently available episcopic 3D methods are "fast 3D serial reconstruction",[46] "Epi-3D",[47] "episcopic fluorescence image capturing",[48] "surface imaging microscopy",[49] "high-resolution episcopic microscopy"[50] (HREM), and "serial block face scanning electron microscopy".[51] All episcopic methods utilize sacrificed embryos and tissue samples.[45] HREM first described in 2006 by Mohun et al., permits the detailed morphological study as well as visualization of gene expression of whole mount stained specimens. Specimen may be sacrificed embryos, adult material or even tissue biopsies. More recently high-speed selective plane illumination microscopy (SPIM)[52] has shown

promise for its ability to capture high-resolution 3D images of the beating zebrafish heart *in vivo*.

As more attention is focused on studying trabecular abnormalities and their etiology, it is likely that some of the quantitative solutions developed at the bedside for CMR, may prove useful at the bench.

CLINICAL UTILITY OF CMR IMAGING IN LVNC

The difficulties of quantification of trabeculae may have resulted in the wide-scale over-diagnosis of LVNC - as newer imaging modalities reveal more biological detail, there is a risk of overinterpretation.[30] LVNC has been equated to mitral valve prolapse which was systematically over-diagnosed for at least a decade because diagnostic criteria were no longer concordant with the image quality.[30] The improved images of CMR (when combined with appropriate normal ranges and an appreciation of normal variability) provides added value in the diagnosis, phenotypic characterization and management of LVNC patients. This is based on high and consistent blood-myocardial contrast and spatial resolution in the apical half of the ventricle. CMR can better diagnose LVNC (8 different approaches are available and/or a combination thereof, **Table 3**) and differentiate isolated LVNC from other cardiomyopathies. Echocardiography alone with traditional diagnostic criteria tends to be too sensitive, particularly in black individuals resulting in the over-diagnosis of the condition.[53]

"Usually, the heart is not the only affected organ in patients with noncompaction"[54] so comprehensive anatomical cardiac/extracardiac scrutiny (whether by echocardiography, computed tomography or CMR) can identify common and rare LVNC-associated abnormalities. These may include: CHD[55] and related phenotypes like left isomerism[14]

and coronary artery malformations,[56] aortopathy, pericardial absence,[57] myocardial bridging,[58] vertebral hemangiomas,[59] polycystic kidneys,[60] and bronchiectasis.[61] CMR also has the capacity to reveal (or infer) the presence of LVNC-associated complications like the presence of ventricular thrombi, pulmonary vascular changes due to previous thromboembolism, atrial appendage thrombi in patients with atrial fibrillation and signs of cardiac decompensation in the form of pericardial and pleural effusions. It permits detailed quantitative assessment of biventricular size and function bearing in mind that the right ventricle may also be involved in LVNC.[62] CMR can point to the presence of myocardial hypoperfusion and myocardial or trabecular late gadolinium enhancement (LGE), providing a benchmark for follow-up scans.

Pooled data from some of the more recent case series suggest an mean prevalence of LGE in LVNC of 62% [prevalence as %]: Ivan et al,[63] *n* = 3 [67%]; Dodd et al,[64] *n* = 9 [data not provided]; Yun et al,[65] *n* = 9 [67%]; Dursun et al,[66] *n* = 10 [80%]; Akhbour et al,[67] *n* = 24 [54%]; Wan et al,[68] *n* = 47 [40%]; Nucifora et al, *n* = 56 [52%][69] and *n* = 42 [55%][70]). A heterogeneous pattern of LGE has been reported in LVNC, involving both compacted and noncompacted myocardial segments and also the trabeculae themselves. Subendocardial and less frequently, transmural LGE has been described. Near-transmural LGE in compacted myocardium has been shown to correlate with extensive mid-myocardial wall fibrosis on histology[71] whereas LGE in the noncompacted segments has been associated with endocardial mucoid degeneration and trabecular fibrosis. Endocardial fibroelastosis has also been described in LVNC.[72]

Based on 11 studies the following imaging features may be prognostic in LVNC: signs of cardiac decompensation (e.g. pleural effusions, dilatation of pulmonary veins), systolic dysfunction or elevated LV filling pressure;[73–77] increased LV end-diastolic

diameter;[73] bundle-branch block pattern[73,74] (inferred through dyscoordinate septal motion). LGE of basal trabeculae has been shown to correlate with the clinical severity of LVNC and the amount of trabecular LGE has been shown to independently predict ejection fraction.[64]

CONCLUSION

Myocardial trabeculae are an important property of the adult heart, developmentally, phylogenetically and clinically. Disruptions of normal trabecular development have deleterious effects on the normal cardiogenetic process and may be embryonic lethal. The isolated finding of excessive trabeculae in the adult human is not uncommon in the general population, particularly in certain ethnicities. Clinical management decisions in those with a morphological diagnosis of LVNC need to take into account LV systolic function, pre-test probability for LVNC, presence of co-existent cardiac disease, and emerging evidence in this field. Results from imaging and using reliable criteria need to be interpreted with the help of normative reference ranges and in the appropriate clinical context, taking into account pre-test probability for disease. Driven by this clinical need, multiple approaches for trabecular quantification have already been developed, and it is hoped that biologists will harness these solutions to further improve our understanding of trabecular development during cardiac morphogenesis.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

All authors have contributed significantly to the submitted work: J.C.M., G.L., P.S., C.O. and G.C. wrote the article. J.L.P., L.G. and P.S. provided expert review of the manuscript.

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Table 1 Mouse models showing aberrant trabecular morphology. This list focuses only on abnormalities in Notch signaling or a related pathway. In the majority of cases trabecular phenotypes were ascertained by visual assessment only; formal trabecular quantification was less commonly performed.

Mutant	Function	Mutation effect	Visual assessment of trabeculae (method used)	Quantitative assessment of trabeculae (method used)	Ref.
Bmp10	*Bmp10* is a signaling molecule transiently expressed in the trabeculated myocardium (between E9.0–13.5) and required for cardiomyocyte proliferation.	*Bmp10*-deficient mutants show marked reduction in proliferation of *Bmp10*-deficient cardiomyocytes leading to the formation of a hypoplastic ventricular wall and failure of trabeculation.	Y (assessed visually by histology)	N	78
Notch1	One of four heterodimeric transmembrane Notch receptor proteins.	Notch1 mutants are deficient of trabeculae.	Y (qualitative analysis of histological sections by scanning electron microscopy)	N	4
RBPJk	Transcription factor.	*RBPJk* mutants exhibit defective myocardial differentiation and severe hypotrabeculation compared to wildtype.	Y (qualitative analysis of histological sections by scanning electron microscopy)	N	4
FKBP12	This protein is a member of the immunophilin protein family, which play a role in immunoregulation and basic cellular processes involving protein folding and trafficking. *FKBP12* interacts with several intracellular signal transduction proteins.	Mutant embryos deficient in *FKBP12* exhibit an up-regulation of *Bmp10* activity resulting in enormous overproduction of ventricular trabeculae. The mutation is embryonic lethal with hearts exhibiting impaired cardiac function.	Y (assessed visually by histology)	N	78
Nrg1 and ErbB2/B4	Endocardial-derived *Nrg1* appears to be crucial for cardiac trabeculation by signaling to cardiomyocytes through its receptor complex *ErbB2*/*B4*[79] to drive trabecular initiation. *Nrg1* expressed in the endocardium, modulates trabecular differentiation by activating *ErbB2/B4* receptors in the subjacent myocardium.	Targeted deletion of *Nrg1* or cognate receptors *ErbB2**/*B4* results in the absence of trabecular formation in mice.	Y	Y (manual count of trabecular projections per field by scanning electron microscopy)	80,81
EphB2/EphB4	The *EphB2/EphB4*-signaling system required for trabecular development, operates in both myocardium and	Notch mutants with hypotrabecular phenotypes express reduced activity of *EphB2* ligand in the endocardium and *EphB4* receptor in the	Y	N	[82]

	endocardium.	myocardium.			
Sgk1	A serine/threonine kinase lying downstream of the *PI3* kinase pathway involved in cardiovascular development and in maintaining normal expression of Notch genes.	Disruption of *Sgk1* in the mouse is embryonic lethal by E10.5–11.5 with severe heart failure and hypotrabeculation.	Y (visual assessment of histological section images)	N	83
hesr1;hesr2	Notch target genes.	Double knockout *hesr1;hesr2* mutant mice was used to confirm the presence of hypotrabeculation at E10.5.	Y (visual assessment of histological section images)	N	[84]
Numb/Numbl	*Numb* found in both *Drosophila* and vertebrates, and its mammalian homolog *Numblike* (*Numbl*) found in vertebrates, regulate trabeculation and compaction by acting as endocytic adaptor proteins, supressing *Notch2* and *Bmp10* signalling during normal trabecular development.	Double knockout *Numb* and *Numbl* mutant phenocopies *Notch2* overexpression (increased trabeculation) through increased *Bmp10* expression.	Y	Y (histological measurement of trabeculated/compacted wall thickness)	85
Notch2	*Notch2* activity is specifically down-regulated in the compact layer during normal cardiac development (by E13.5).	Hypomorphic *Notch2* mutants exhibit a defective trabecular phenotype and myocardial hypoplasia while expression of constitutively active *Notch2* in the myocardium of mutants, results in increased trabeculation and reduced compaction of the ventricular wall.	Y	Y (measured trabeculated and compacted wall thicknesses on histological section images)	86
Mib1	*Mib1* is an E3 ubiquitin ligase which mediates ubiquitination of *Jag1* in the myocardium resulting in endocardial *Notch1* activation at the base of trabeculae.	*Mib1* deficient mutants are hypertrabeculated. The trabeculated myocardium remains abnormally proliferative, evidenced by the expansion of compact myocardial markers (like *hesr2*) to the trabeculae, late into embryonic development.	Y	Y (measured noncompacted-to-compacted diastolic wall thickness ratio by echocardiography)	21

*Targeted deletion of *ErbB2* in zebrafish has also been shown to result in defective trabecular development.[87]

Bmp10, bone morphogenetic protein-10; *EphB2/EphB4*, ephrin-B2/B4; *ErbB2/B4*, erythroblastic leukaemia viral oncogene homologues 2 and 4 receptors; *hes*, hairy and enhancer of split family genes; hesr, *hes-related* family genes**;** *FKBP12*, FK506 binding protein-12; *Mib1*, mindbomb homolog-1; N, no; *Nrg1*, neuregulin-1; *PI3*, phosphoinositide 3 kinase pathway; Ref, reference; *Sgk1*, Serum and glucocorticoid-inducible kinase 1; Y, yes.

Table 2 Genetics of left ventricular noncompaction.

Gene symbol	Encoded protein	Inheritance	Gene MIM number	Ref.
ACTC1	Alpha cardiac actin	AD	*102540	88
DTNA	Alpha dystrobrevin	AD	*601239	89
LDB3	LIM domain binding 3 (Z-line protein Cypher/ZASP)	AD	*605906	90
LMNA	Lamin A/C	AD	*150330	91
MIB1	Mindbomb E3 ubiquitin protein ligase 1 (Notch pathway)	AD	*608677	21
MYBPC3	Myosin binding protein C	AD	*600958	22
MYH7	Beta myosin heavy chain 7	AD	*160760	88,92
PRDM16	PR domain-containing protein 16	AD	*605557	93
TAZ (G4.5)	Tafazzin	XL recessive	*300394	94
TNNT2	Cardiac troponin T type 2	AD	*191045	88
TPM1	Alpha tropomyosin 1	AD	*191010	22
mtDNA	Mitochondrial DNA dehydrogenase subunit 1; ATPase subunit 6; ATPase subunit 8	Maternal	*516000 *516060 *516070	95–97

* Derived in part from the Online Mendelian Inheritance in Man database[20] (OMIM) accessed 05-2015.

AD, autosomal dominant; MIM, Mendelian Inheritance in Man; mt, mitochondrial inheritance; mtDNA, mitochondrial DNA; Ref., reference; XL X-linked.

Table 3 Summary of published CMR methods for quantifying LV trabeculae*.

Year	Author	Sequence	Timing	Imaging plane/s	Methodology	LVNC (or other disease~) cut-off	Normal reference range	Refined procedure over time	Applied to RV	Strength	Limitation
2005	Petersen[28]	Cine bSSFP	ED	HLA VLA LVOT	A. NC/C ratio per segment using HLA and VLA; apex excluded.	A. NC/C > 2.3	A. 1.1±0.1	Kawel[98] & Zemrak et al.[13]	N	Quick and easy to implement	Confounded by wall thickness in LV hypertrophy
2010	Jacquier[99]	Cine bSSFP	ED	SAX	A. Compacted LV mass subtracted from global LV mass after semi-automated contouring and indexing to BSA. B. Ratio of TLVM to global LV mass expressed as %.	A. Mean TLVM 43±19 g/m^2 B. %TLVM > 20%	A. 9.0±4.0 g/m^2 B. 12.5±5.0 %	N	N	Evaluates global trabecular load	Time consuming
2011	Dawson[32]	Cine bSSFP	ED ES	SAX	A. 17-segment model excluding apex, evaluated for NC & C wall thickness in ED & ES, taking the peak value for NC per segment.	A. Not available	A. NC/C ratio ranges: ED, 0-0.9; ES, 0-0.5	N	N	Includes ES data	Performance in LVNC unknown
2012	Grothoff[43]	Cine bSSFP	ED ED	HLA VLA SAX SAX	A. Software for contouring NC & C; epicardial border manually traced in ES/ED in HLA & registration marks applied. B. Maximal NC/C from measurements in ED on SAX in 16 of 17 segments.	Ai. LV-$MMI_{noncompacted}$ > 15 g/m^2 Aii. %LV-$MMI_{noncompacted}$ > 25% B. NC/C ≥ 3.1	Ai. 5.3±2.4 g/m^2 Aii. 9.9±4.4 % B. Not available	N N	N N	Semi-automated evaluation of global trabecular load	Dedicated software required – not open source
2013	Stacey[100]	Cine bSSFP	ES	SAX	A. Apical SAX 16 to 24 mm from the true apical slice for measurements; paps. excluded; ES NC/C wall thickness ratio measured.	A. ESNCCR ≥ 2	A. Not available	N	N	Quick (single slice evaluation)	User-dependent exclusion of paps.; no ED data incorporated into criterion
2013	Captur[12]	Cine bSSFP	ED	SAX	A. Java-based box-counting fractal analysis to extract the maximal apical FD after analysing all SAX cines (excluding apex)	A. $FD_{MaxApical}$ ≥ 1.30	A. 1.203±0.06	Captur et al.[42]	N	Quick, reproducible, open source OsiriX version to be released soon	Does not consider wall thickness; some training in mathematical fractals required
2013	Marchal[101]	Cine bSSFP	ED ED	HLA VLA SAX HLA VLA	A. STI measured as NC/C ratio per segment 1-15 in SAX; apical values for segment 16 from HLA and VLA. B. GTI as ratio of the sum	~A. STI range in DCM, 0.1-2.2 (apex included) or 0.1-1.5 (apex excluded) ~B. Mean GTI in DCM	A. Not available B. Not available	N	N	Attempts to circumvent the problem of apical partial voluming effects	Normal reference ranges unavailable; performance not tested beyond DCM

				SAX	of total NC to the sum of total C.	0.68±0.32					
2015	André[102]	Cine bSSFP	ED	SAX	A. Epi & endocardial contours, paps. & trab.-free LV/RV volumes manually outlined.	A. LVTV/BSA not defined in LVNC	A. M 43.1±8.7 ml/m^2; F 38.1±5.9 ml/m^2	N	Y	Only method to have been applied to the RV	Laborious; manual outlines may lack reproducibility

* Values are reported as mean ± standard deviation unless otherwise stated.

BSA, body surface area; bSSFP, balanced steady-state free precession; C, compacted epicardial layer; CMR, cardiovascular magnetic resonance; DCM, dilated cardiomyopathy; D&S, diastole and systole; ED, end-diastole; epi., epicardial; ESNCCR, end-systolic noncompacted-to-compacted ratio; F, female; GTI, global trabeculation index; HLA, horizontal long axis; LV-MMI$_{noncompacted}$, noncompacted LV myocardial mass index; LVNC, left ventricular noncompaction; LVOT, left ventricular outflow tract view; LVTV, left ventricular trabecular volume; M, male; N, no; NC, noncompacted endocardial layer; paps., papillary muscles; RV, right ventricle; SAX, short axis stack; STI, segmental trabeculation index; TLVM, trabeculated left ventricular mass; VLA, vertical long axis; NA, data not published; Y, yes.

Figures

Figure 1A Sequential morphological phases of trabecular development in the mouse are governed by a complex number of signaling pathways and trophic factors. As an example, this figure focuses on the Notch signaling pathway (bottom) for its role in causing aberrant trabecular phenotypes in mutants. Key developmental stages in the embryonic mouse heart (black horizontal timeline arrow) are shown above.

Figure 1B Notch is one of several signaling pathways that influence trabecular development. Notch converts information about the concentration of extracellular ligands into specific transcriptional responses in the nucleus. In mammals, Notch comprises two groups of transmembrane proteins: (i) four receptors (*Notch1, 2, 3, 4*); and (ii) five ligands (that contain a *Delta/Serrate/Lag2* [*DSL*] motif in their extracellular domain). *Notch1* receptor-ligand interaction results in a series of cleavage events (S1, S2, S3) affecting the receptor holoprotein. Cleavage of the Notch extracellular domain (*N1ECD*) by ADAM metallopetidase domain 17 (ADAM17) is followed by cleavage of the intracellular portion by a γ-secretase (γ-S) that releases the Notch intracellular domain (*N1ICD*) into the cytoplasm. From there *N1ICD* translocates to the nucleus where it forms a complex with the recombination signal binding protein for immunoglobulin kappa J region (*RBPJk*, a transcription factor) and other co-activators to transactivate transcription of downstream target genes like the *hairy and enhancer of split* (*hes*) family genes and the *hes-related* (*hesr*) family genes (also known as the *hrt/hey/herp* family genes).[103,104] *Notch1* activation in the developing trabeculae has recently been shown to depend on ubiquitination and endocytosis of ligands *Delta* and *Jagged* by the E3 ubiquitin ligase mindbomb homolog 1 (*Mib1*), shown here in red.

Figure 2 Morphological changes in the ventricular wall and development of trabeculae in mouse. During trabecular development there is spatial heterogeneity of expression of Notch components: for example, *Notch1*, *Notch4*, *Delta4*, *neuregulin-1* (*Nrg1*), *ephrin-B2* (*EphB2*) ligand and *hesr1* activity is mainly restricted to the endocardium, while *Notch2*, *Jagged1*, *bone morphogenetic protein-10* (*Bmp10*), *EphB4* receptor, erythroblastic leukaemia viral oncogene homologues 2 and 4 receptors (*ErbB2/B4* receptor), *Mib1* and *hesr2* activity predominates in the myocardium.[3] A cue (red arrow) from the myocardium promotes *N1ICD* expression (dark green) in endocardial cells. Between E9.0-9.5, *Bmp10* mediates trabecular muscle proliferation while *Nrg1* through *ErbB2/B4*, and *EphB2* through *EphB4*, mediate differentiation of primitive myocardial epithelium to trabecular and compact myocardium. As trabecular projections mature, the endocardium delaminates from the myocardium distancing itself from the *N1ICD*-inducing cue, resulting in *N1ICD* down-regulation on the luminal aspect of trabeculae (dark green > white endocardial cells) compared to the base. By E10.5 a compact (dark blue) and trabecular layer (pale blue) can be distinguished.[4]

Figure 3 The concepts of complexity, self-similarity and scale invariance in fractal maths (**left**) and the proposed method for myocardial trabecular fractal analysis (**right**). In mathematics, a self-similar object (like this mathematically-derived fractal branching set) is exactly similar to a smaller part of itself i.e. A has the same shape as B and C making it scale invariant. Many naturally occurring biological objects (like myocardial trabeculae in adult human hearts or model organisms) have complicated patterns but are only 'statistically' self-similar: only parts of them show the same statistical properties

and only over a limited range of scales. Fractal geometry can still be used to quantify their complexity and we have used a box-counting algorithm applied to cardiovascular magnetic resonance datasets of the adult human heart to measure trabecular complexity as a fractal dimension. FD, fractal dimension; Ln, natural logarithm.

Figure 4 Identification of abnormal trabecular patterning in the LV is not enough to apply a diagnosis of LVNC. Due to the high prevalence of associated cardiac disease (like DCM, CHD), these should be sought as a first step in the workup of patients found to have excessive trabeculation. If LV systolic function is normal and the subject has a low pre-test probability for LVNC, reassurance is reasonable.
This figure was adapted from Petersen SE and Zemrak F. Spot the difference: LV trabeculation vs. LV noncompaction. Cardiology Today, February 2015. Thorofare, NJ: SLACK Incorporated; 2015.
* Where possible/available ethnically-appropriate reference ranges should be used.
BBB, bundle branch block pattern on electrocardiography; CHD, congenital heart disease; CMR, cardiovascular magnetic resonance; CT, computed tomography; DCM, dilated cardiomyopathy; Echo, echocardiography; FH, family history; FU, follow up; LVNC, left ventricular noncompaction.