Peer Review File

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Comment 1: It should be mentioned that the term “hypertrabeculation” can be synonymously used for noncompaction.

Reply 1: According to your suggestion, we modified the first sentence in introduction.
Changes in the text: Left ventricular noncompaction (LVNC), also used synonymously as hypertrabeculation, is a cardiomyopathy characterized that is morphologically by two layers of highly thickened myocardium, numerous prominent trabecular processes, and deep intertrabecular recesses that connect to the LV cavity (see Page 5, line 2).

Comment 2. It should be stressed that a causal link between any of the so far reported genetic variants and LVHT remains unproven.

Reply 2: According to your suggestion, we added the following sentence in genetics.
Changes in the text: They identified 189 genes associated with LVNC; 11 were classified as definitive, 21 as moderate, 140 as limited, while 17 were classified as no evidence (sse Page 9, line 9).

Comment 3. Discussion about LGE should be broadened.

Reply 3: According to your suggestion, we added the following sentences.
Changes in the text: Patients with isolated LVNC and no LGE have a better prognosis than patients with LVNC and LGE. In recent years, native T1 mapping has proven to be a useful tool to quantitatively measure myocardial tissue characteristics without the administration of exogenous contrast agents. Myocardial T1 values in patients with diffuse myocardial fibrosis have been reported to correlate well with fibrosis confirmed by myocardial biopsy. Native T1 mapping is superior for characterizing diffuse fibrosis and subtle focal lesions in comparison with LGE. Previous studies that applied T1 mapping to patients with LVNC and without LGE showed that LVNC patients had elevated native myocardial T1 values compared to normal controls, suggesting that native T1 mapping is a more useful indicator of early myocardial fibrosis than LGE imaging in LVNC patients (see Page 16, line 3-13).

Comment 4. The English should be corrected by a native speaker.

Reply 4: The manuscript was checked and modified correctly.

Comment 5: Indeed, LVNC is clinically and genetically heterogeneous as stated correctly by the authors. For that reason, it is important to discuss in a review how we can or cannot distinguish between the phenomenon of LV hypertrabeculation in healthy population and the cardiomyopathy with LV hypertrabeculation in LVNC.

Reply 5: Thank you for providing an important suggestion. According to your suggestion, we added the following sentence in the diagnosis section.
Changes in the text: Another common problem in echocardiographic criteria for LVNC is that interobserver reliability tends to be suboptimal. Saleeb et al. compared 104 LVNC cases with 100 controls and found that interobserver agreement in counts of trabeculations >3 and N/C ratio >2 ranged from 53 to 77% and did not statistically factor in the possibility of chance agreement. Stöllberger et al. recently evaluated the interobserver reliability among three observers evaluating 100 echocardiograms of LVNC patients and reported discordance in 35% of cases. Related to all LVNC diagnostic studies assessing the validity and inter-rater reliability is the lack of a gold standard for diagnosis, as many of these studies rely on the original echocardiographic diagnosis as their overarching criteria. Furthermore, these echocardiographic diagnostic tests may be too sensitive, as suggested by Kohli et al. They studied echocardiograms of patients referred for HF and found that 24% met one or more of the LVNC criteria (see Page 20, line 3-11).

Comment 6: The next hallmark of LVNC is the genetic and clinical overlap with DCM and HCM. And that also need to be presented more clearly early on.

Reply 6: According to your suggestion, we added the following sentence in introduction.
Changes in the text: LVNC has a broad morphologic spectrum and can appear alone or in association with DCM, HCM, early onset of arrhythmias, and congenital heart disease (CHD) (see Page 6, line 4-5).

Comment 7: In contrast to what is stated it should be clear that the majority of genetic causes are autosomal dominantly inherited. It is confusing if this is reported differently throughout the manuscript. All other inheritance patters are rare. In table 1. The genetics are grouped according to function: however, non-sarcomere, non-arrhythmia, or X linked, or genes associated with CHD do not describe a function.

Reply 7: According to your suggestion, we reorganized this part and added the following sentences in genetics section and modified Table 1.
Changes in the text: Rojanasopondist et al. sought to systematically analyze and score the strength of all published evidence linking each gene to LVNC, and then verified which molecular pathways are likely to be involved in the pathogenesis of LVNC. They identified 189 genes associated with LVNC; 11 were classified as definitive, 21 as moderate, 140 as limited, and 17 were classified as no evidence. Of the 32 genes classified as definitive or moderate, the most common gene functions were sarcomere function (34%), transcription/translation regulator (19%), mitochondrial function (9%), and cytoskeletal proteins (9%) (Table 1). In addition, 18 genes (56%) were involved in the presentation of noncardiac syndromes. In addition, there were 20 genes shared between LVNC and DCM, and 18 genes shared between LVNC and HCM. Analysis of pathways and protein-protein interactions suggested that LVNC, more than DCM and HCM, is responsible for abnormalities in cardiomyocyte development and

differentiation during cardiomyogenesis (see Page 9, line 7-15).

Comment 8: The manuscript misses on many occasions' complete references, one of main the purposes of a review, for instance in the epidemiology section.

Reply 8: We apologize our mistake. We added the references in the manuscript appropriately.

Comment 9: Please note that genetic diversity should be rephrased into genetic heterogeneity. Instead of describing very rare genetic defects as provisional, it should be stated that the association with LVNC is weak since these are based on single reports. In contrast to the text, neuromuscular disorder and mitochondrial disorder can also be single gene disorder. One has thus single gene causes and chromosomal defect, the latter may affect multiple genes.
In the genetic section as is, is not an improvement compared to previous reviews of the genetics of LVNC.
In the pathogenesis section one would expect to read something referring to sarcomere genes.: Since sarcomere gene defects are a major cause of LVNC, it is important to discuss how these causes for LVNC correspond to the presented embryologic or acquired presented pathogenesis.
Overall, this section has many redundancies and should be condensed and rewritten.

Reply 9: We rephrased these words and expressions as you suggested correctly.
We condensed and rewrote the genetics section.
Regarding pathogenesis, we focused on sarcomeric gene and its relevance with pathogenicity according to your suggestion.
We condensed and rewrote the pathogenesis section to reduce redundancies.
Changes in the text: Indeed, we reported that sarcomere variants are most commonly identified in fetus-onset patients, and fetus-onset patients with LVNC are more likely to develop HF. Moreover, we found a time-dependent increase in the ratio of noncompacted to compressible layers (N/C) of the LV wall in patients with fetal-onset LVNC, with the N/C ratio being greater than 2 before and after birth. This change is in contrast to patients with DCM and HCM, suggesting that the formation of the trabeculated layer proceeds late in gestation. Thus, studies focusing on fetal LVNC provide new clues to previous hypotheses of mouse models and the development of LVNC in humans (see Page 15, line 8-13).

Comment 10: The echo and MRI section need to refer to the table where all different criteria are listed, and should discuss the differences.

Reply 10: According to your suggestion, we added the following sentences to understand criteria and these differences.
Changes in the text: 6.1. Echocardiography
The first echocardiographic criteria were proposed by Chin et al. This criterion

proposed the ratio of the distance from the epicardium to the trabecular trough (X) to the trabecular peak (Y) is diagnostic of LVNC if X/Y is less than 0.5. Jenni et al. proposed a second criterion. They proposed a maximum ratio of N/C > 2 as a diagnostic criterion for LVNC. Measurements in this study were made on the parasternal short-axis image at end-systole. Currently, this criterion is the most widely accepted and clinically applied echocardiographic criterion for the diagnosis of LVNC. Stöllberger et al. proposed the third criterion. They proposed that the presence of three or more trabeculae at the tip of the papillary muscle and blood flow by color Doppler imaging or evidence of echo-enhancing agents in the interventricular recesses should be required. Joong et al. compared the usefulness and reproducibility of the previously mentioned Chin, Jenni, and Stöllberger echocardiographic criteria and found that Chin's criterion has the highest reproducibility and interrater reliability for counting the parasternal short axis and apical trabeculae using the X/Y ratio in the apical anterolateral region. Jenni and Stöllberger's criteria are probably overdiagnosed in many adult HF cases that meet the diagnostic criteria. Moreover, echocardiography has several limitations. First, in the literature, echocardiographic diagnostic criteria vary widely and are based on a small number of studies using different research methods. Second, since myocardial thickness is greatest in systole and least in diastole, the cardiac cycle (end-systole or end-diastole) during which the uncompressed and compressed layers are measured is also important, which directly affects the N/C ratio. Paterick et al. suggest that the two-layer myocardium be measured at end-diastole because the thickness of each layer can be more accurately measured, and this approach is in line with the end-diastole. They proposed measuring the N/C ratio of the LV myocardium at end-diastole and diagnosing with a ratio > 2. This approach is consistent with the recommendation of the American Society of Echocardiography to measure wall thickness at the end of diastole. (see Page 18, line 1-19, Page 19, line 1-2)

6.2 Cardiac MRI

If uncertainty still exists in the diagnosis of LVNC, additional diagnoses, such as CMR, are warranted. The most widely accepted criteria for CMR diagnosis of LVNC were proposed by Petersen et al. They suggested a larger ratio (N/C >2.3) and measurements in diastole to better understand the bilayer myocardium and to improve accuracy. Jacquier et al. proposed another cross-sectional evaluation method by determining the mass of the trabecular LV. They proposed that trabecular LV mass be greater than 20% of the total hemispheric mass. Stacey et al. subsequently compared the Petersen and Jacquier criteria with the conventional end-systolic ratio N/C > 2 proposed in the Jenni criterion and found the latter to be strongly associated with arrhythmia, HF, and subsequent hospitalization. Grothoff et al. presented a method to measure the LV myocardial mass index of uncompressed myocardium as an absolute value and as a percentage of total LV myocardial mass and proposed cutoffs of 15 g/m2 and 25%, respectively (see Page 20, line 13-19, Page 21, line 1-2).

Comment 11: The section on myocardial biopsy, is confusing, stating that there is thickening of endocardium, elastic fibers and collagen. No reference is provided. And it is very difficult to understand how elastic fibers and collagen are thickened. This part

needs to be explained in more detail.

Reply 11: We apologize our mistake. We modified the sentences and added the references in the manuscript appropriately.
Changes in the text: Endocardial fibrosis or fibroblasts were the most frequently reported abnormalities in adult and pediatric LVNC. It is unclear whether endocardial fibrosis is caused by an immune response, the result of local blood flow abnormalities, or induced by hemodynamic and mechanical factors around the trabecular myocardium. There is marked thickening of the endocardium, elastic fibers, and collagen fibers. Fat cell infiltration and myocyte necrosis may also be seen. Often a mural thrombus is seen on the endocardial surface in the fine-walled column area. The myocardial layer shows various degrees of degeneration, mainly subendocardial, and up to moderate fibrosis. However, there is no extensive myocardial desmoplasia or foci of mottled fibrosis, as is often seen in DCM, and the histological findings are rather similar to those of endocardial fibroelastosis (see Page 17, line 4-11).

Comment 12: Regarding the section on diagnosis. Currently the most widely used are the jenni or stollberger ie the revised jenni) criteria. In this section we need to learn more about the frequent hypertrabeculation in normal population and how to distinguish that form LVNC.
The natural history part it is unclear if the rates confer to CVD in general, and in which country, or to LVNC. Please rephrase also that part.

Reply 12: Thank you for providing an important suggestion. According to your suggestion, we added the following sentence in the diagnosis section. We revised the natural history part more clearly and added references appropriately.
Changes in the text: Moreover, echocardiography has several limitations. First, in the literature, echocardiographic diagnostic criteria vary widely and are based on a small number of studies using different research methods. Second, since myocardial thickness is greatest in systole and least in diastole, the cardiac cycle (end-systole or end-diastole) during which the uncompressed and compressed layers are measured is also important, which directly affects the N/C ratio. Paterick et al. suggest that the two-layer myocardium be measured at end-diastole because the thickness of each layer can be more accurately measured, and this approach is in line with the end-diastole. They proposed measuring the N/C ratio of the LV myocardium at end-diastole and diagnosing with a ratio > 2 This approach is consistent with the recommendation of the American Society of Echocardiography to measure wall thickness at the end of diastole.
Another common problem in echocardiographic criteria for LVNC is that interobserver reliability tends to be suboptimal. Saleeb et al. compared 104 LVNC cases with 100 controls and found that interobserver agreement in counts of trabeculations >3 and N/C ratio >2 ranged from 53 to 77% and did not statistically factor in the possibility of chance agreement. Stöllberger et al. recently evaluated the interobserver reliability among three observers evaluating 100 echocardiograms of LVNC patients and reported discordance in 35% of cases. Related to all LVNC diagnostic studies assessing the

validity and inter-rater reliability is the lack of a gold standard for diagnosis, as many of these studies rely on the original echocardiographic diagnosis as their overarching criteria. Furthermore, these echocardiographic diagnostic tests may be too sensitive, as suggested by Kohli et al. They studied echocardiograms of patients referred for HF and found that 24% met one or more of the LVNC criteria (see Page 18, line 14-20, Page 19, line 1-11).

Comment 13: In the conclusion, lifelong is not a correct description, since we do not have any follow up data to show or refute regression of the cardiac remodeling in LVNC like in people with cardiac overload.
Altogether, the manuscript would benefit greatly from omitting any repeated information, also when stated slightly different, and condensing the presented data retaining only the facts that are really useful. I would have liked to read a novel conclusion, based on the collected information.

Reply 13: Thank you for providing an important suggestion. According to your suggestion, we added the following sentence in conclusion.
Changes in the text: This present review addressed the latest findings on genes reported to be associated with LVNC morphogenesis and possible pathologies in human or non-human model organisms. It reflects the current lack of clarity regarding the pathogenesis and significance of LVNC. This is due to the complexity of imaging diagnostic criteria, interpretation of the role of LVNC as a cause or contributor to cardiomyopathy, and uncertainty regarding the specific genetic basis of LVNC. Hence, the current review highlights both the complexity and heterogeneity of the disease and the continuing need for higher quality evidence. Further extensive genetic and imaging profiling of the patient population would help clarify the risk of LVNC. We hope that this review will not only serve to help with the current status and limitations of LVNC findings but will also help identify areas where current research is lacking and where future efforts may be of interest (see Page 26, line 12-19).

Comment 14: Abstract
Lines 32-50: I suggest to include the study objective in the Abstract.

Reply 14: According to your suggestion, we re-arranged the current abstract into structured abstract.

Comment 15: Introduction
I suggest the authors rewrite the Introduction:
- The authors did not explain clearly why they chose to review LVNC;
- The Introduction only states the purpose of this review and lacks meaning. What is the significance (update or new findings?) and objective of this review (existing research gaps, or points of contention, in this field)?
- Please cite the reference for this sentence: "However, despite first clinical description of LVNC appearing 30 years ago, understanding of LVNC and the its pathogenesis has

not yet fully elucidated, especially when compared to other cardiomyopathies such as hypertrophic cardiomyopathy (HCM) or dilated cardiomyopathy (DCM)" (Lines 73-75). Please check the entire manuscript to address similar concerns.

Reply 15: Thank you for providing an important suggestion. According to your suggestion, we added the following sentences in introduction and added the reference. Changes in the text: Therefore, we addressed the latest findings on genes reported to be associated with LVNC morphogenesis and possible pathologies in human or non-human model organisms to understand the diverse spectrum between genotype and phenotype in LVNC. Moreover, we summarized the latest findings and issues related to the diagnosis of LVNC (see Page 6, line 16-19).

Comment 16: Methods
There is no Methods section provided. Detailed literature search information can help assess whether the search is comprehensive and up-to-date.

Reply 16: According to your suggestion, we added the methods in the manuscript.

Comment 17: Narrative
To make the structure of the article clearer and more concise, we suggest the authors to divide the whole text into several major sections with a serial number. For example:
1. Epidemiology
2. Subtype
3. Genetics
4. Pathogenesis
4.1 Congenital hypothesis
4.2 Acquired hypothesis
5. Imaging
5.1 Echocardiography
5.2 Cardiac MRI

Reply 17: According to your suggestion, we modified the structure of the article.