# Noncompaction Cardiomyopathy: Genotype-Phenotype Associations

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# Noncompaction Cardiomyopathy: Genotype-Phenotype Associations

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# Introduction

## Introduction

Cardiomyopathies are diseases of the heart muscle, classified according to distinct structural and functional features (1, 2). Noncompaction cardiomyopathy (NCCM) is characterized by excessive trabeculation of the endocardial layer of the myocardium of the left ventricle. It is also referred to as left ventricular non-compaction (LVNC) (3). The left ventricle shows a spectrum of hypertrabeculation ranging from physiologic remodeling in adults to perinatal cardiomyopathy in children requiring heart transplantation (4, 5). The diagnosis is established by imaging noncompaction of the left ventricle, usually by transthoracic echocardiography or cardiac magnetic resonance imaging. NCCM is classified as a genetic cardiomyopathy according to the America Heart Association (AHA), while the European society of cardiology (ESC) has classified NCCM as an unspecified cardiomyopathy (1, 2). The reason the AHA classified NCCM as a genetic cardiomyopathy was that mutations in cardiomyopathy genes are equally frequent causes for NCCM as for the more prevalent inherited hypertrophic – and dilated cardiomyopathies. The first report of a genetic cause for NCCM dates back to 1997 describing a family with NCCM linked to a mutation in the X-linked tafazzin (*TAZ*) gene (6). In 2008 the first sarcomere mutations were identified in NCCM patients (7). Since then more and more of the cardiomyopathy genes (genes associated with hypertrophic (HCM) - and dilated (DCM) cardiomyopathy have been associated with NCCM, leading to the current recommendations for DNA testing of panels of more than 50 cardiomyopathy genes in NCCM. A disease causing (likely) pathogenic variant, is observed in 32% to 38% of all NCCM patients (8-10). However, in approximately half of familial NCCM no (likely) pathogenic variant is found, indicating additional genetic causes have to be identified, resulting in an even higher prevalence of genetic NCCM. The motive of the ESC to classify NCCM as an unspecified cardiomyopathy was based on the ongoing debate whether NCCM is a distinct clinical entity or is an epiphenomenon of other cardiomyopathies (11-14), giving the overlapping phenotypes and genotypes with HCM and DCM and because cardiac features complying with the current NCCM imaging criteria may occur in the healthy population (15-17). The aim of this thesis is to establish the genetic spectrum of NCCM and the correlations between genetics and the phenotypic manifestations.

### Diagnostic criteria

The first description of a noncompacted myocardium can be traced back to a pathology report by Grant et al. in 1926 describing a spongy myocardium (18). It was until 1990 that Chin et al. proposed the first diagnostic criteria for NCCM (3). With

the progress in diagnostic imaging techniques, recognition of NCCM increased and with it the number of diagnostic criteria. At first mainly echocardiography criteria were used, later criteria for magnetic resonance imaging (MRI) were introduced (19). All the different composed criteria on various imaging modalities for the diagnosis of NCCM require some kind of measurement of trabeculations, although different thresholds for the area or thickness of the noncompaction are used. In practice only echocardiographic and MRI criteria are used. Currently, the most frequent used criteria on echocardiography are the Jenni criteria (20).

The Jenni criteria for NCCM are composed of four echocardiographic features: 1. an excessively thickened left ventricular myocardial wall with a two-layered structure consisting of a compact epicardial layer (C) and a noncompacted endocardial layer (NC) of prominent trabeculations and deep intertrabecular recesses; 2. an end-systolic NC/C ratio > 2, measured at the parasternal short axis; 3. Color-Doppler evidence of ventricular perfused intertrabecular recesses; 4. absence of coexisting cardiac anomalies (21). The fourth feature however is under debate since congenital heart defects occur frequently in NCCM patients and there is no convincing evidence for this exclusion (22). In fact, some specific defect in sarcomere genes have been associated with distinct congenital heart defects (i.e. and Ebstein anomaly) in NCCM patients (23). Also defects in genes associated with heart development in NCCM patients with a congenital heart defect indicate otherwise (24).

For MRI diagnostics the Petersen criteria are most widely used (20). The Petersen criteria were based on only seven patients with NCCM and require a noncompacted/compacted ratio (NC/C) of >2.3, measured in end-diastole to diagnose NCCM (25). So far the diagnosis of NCCM is solely based on imaging of trabeculation of left ventricle, without taking into account clinical, genetic or functional parameters.

The sensitivity of the echocardiographic and CMR imaging criteria is under debate since it is apparently not uncommon that healthy people (without a cardiomyopathy) meet the current diagnostic criteria for NCCM. Overdiagnosis may occur, as some studies suggested with up to 43% of the general population meeting the current diagnostic criteria as discussed below (16, 17, 26-29). Images of papillary muscles and false tendons can easily be mistaken for trabeculation, therefor these features may be diagnosed erroneously as NCCM. Further issues complicating the diagnosis of NCCM are poor agreement between different imaging criteria (30, 31), and poor inter-observer agreement between NCCM specialist. One study showed that observers agreed in 65% of the cases. After reviewing discordant

cases, still no consensus was achieved resulting in an ambiguous/ questionable diagnosis in 11% (32).

**Epidemiology**

*Prevalence of NCCM*

The exact prevalence of NCCM in the general population has not been established. However, estimates of prevalence of NCCM have been presented for specific groups of cardiologic patients. Estimates of the prevalence among patients undergoing echocardiography ranges from 0.014% to 1.3% (33-37). The prevalence is much higher (3.7%) when selecting patients with left ventricular systolic dysfunction and further increases when patients with left ventricular dilatation (6.8%) were selected (35, 37). The estimated prevalence of NCCM in patients with left ventricular systolic dysfunction on echocardiography ranged from 3% to 24% (31, 38). The difference in estimated prevalence may be explained the selection of the study population; one study excluded the diagnosis NCCM when patients had another cardiac diagnosis, i.e. HCM, DCM, coronary artery disease, hypertension. Whereas the study with the high prevalence included all patients suffering from left ventricular systolic dysfunction and presented the measured NC/C ratios in this group of patients. In patients referred for cardiac MRI the prevalence of NCCM ranged from 3 to 39 percent depending on the applied diagnostic criteria (30). In this population 39% of the patient met the Petersen criteria, the most widely used diagnostic criteria on MRI. Prevalence according to the other criteria was: Jacquier 25%, Stacey 23% and Captur only 3%. The large discrepancy in diagnostic yield between the different diagnostic criteria, each with their own specificity, shows that there is a need for more reliable, improved diagnostic criteria. In large cohorts of pediatric cardiologic patients, estimated prevalence of NCCM was 9% and NCCM was the third most frequent cardiomyopathy, after DCM and HCM (39). Since some patients with NCCM may be asymptomatic, and in some patients signs of NCCM are only detected by MRI without sufficient echo graphic features, the prevalence estimations relying on echocardiography, underestimate the true prevalence in the general population. On the other hand, patients referred for cardiac MRI may be more likely to have cardiac pathology with left ventricular dysfunction, which may lead to an overestimation of the prevalence of NCCM.

*Prevalence of hypertrabeculation in healthy population*

Four large population-based studies were designed to establish the occurrence of hypertrabeculations in the general population (16, 17, 28, 29). These studies showed that 14.8% to 43% of the study populations were meeting diagnostic

criteria for NCCM on MRI. The large differences between these studies and the fact that prevalence was similar to that in cardiac patient referred for cardiac MRI, remains difficult to explain. As discussed above, this may be the result of the lack of specificity of the current diagnostic MRI criteria for NCCM. Endorsing that more accurate diagnostic criteria for NCCM are needed, preferably including besides the imaging, also functional and genetic parameters. There may be an effect of ethnicity on prevalence of hypertrabeculation, which may have influenced the results (17, 29, 31, 40). Based on these observations one may conclude that high prevalence of hypertrabeculation in the population indicates that hypertrabeculation could be an anatomical phenomenon. In our point of view, the validity of this interpretation is questionable, given the broad heterogeneity of the phenotype, as will be discussed below (17, 29). Interestingly, in addition, two of the population-based studies suggested an effect of hypertrabeculation on heart function or vice versa, since decreased left ventricular function was associated with the extent of hypertrabeculation (16, 28). In addition, the other two studies suggested that signs of hypertrabeculation might be associated with LV functioning. In the study of Weir-McCall et al. (17) patients meeting more imaging criteria for NCCM had also a significantly lower left ventricular ejection fraction, indicating the presence of cardiomyopathy in some of these seemingly healthy individuals (20). In the study of Zemrak et al. (29) the cohort was divided by level of trabeculation into quintiles. Similar risk of major adverse cardiac events was observed in the cases with high (>2.46) noncompacted/compacted ratios (quintile 5) and cases with lower levels of trabeculations (≤2.0, quintile 1-3). The conclusion was that hypertrabeculation had no impact on cardiac outcome. In this study however, the group with high (>2.46) noncompacted/compacted ratios had significantly lower incidence of cardiovascular risk factors like obesity, hypertension and diabetes. These factors are predictors for adverse cardiovascular events. Correcting outcome for these risk factors could have led to an association between major adverse cardiac events and hypertrabeculation (>2.46), concluding the opposite of the paper (41). In line, a more recent study conducted with cases from the same MESA cohort, higher rates of trabeculations were associated with worse myocardial strain and outcome (42).

Other studies focused on the occurrence of hypertrabeculation in athletes with excellent cardiac function. Two studies estimated that the prevalence of hypertrabeculation in athletes ranged from 1.4% to 21% (26, 27). Caselli et al. (26) used the Jenni criteria on echocardiography and detected hypertrabeculation in 1.4% of athletes, whereas Luijkx et al. (27) used the Petersen criteria on MRI and detected hypertrabeculation in 21% of the athletes. These observations may lead

again to the conclusion again that better diagnostic criteria are needed to diagnose NCCM accurately.

**Pathology**

Reports on macro – and microscopic characteristics of NCCM are scarce and have been summarized comprehensively by Stöllberger and Finsterer (43). The macroscopic characteristic of NCCM is a two-layered myocardium, with an endocardial layer containing excessive trabeculations and an epicardial layer comprised of compact myocardium (figure 1). This hypertrabeculation affects primordially the apical and the midventricular inferior and lateral wall of the left ventricle (43). The two-layered structure is better visible on short axis cuts (43). The ratio of noncompacted/compacted myocardium in different reports may vary from 0.6 to 5.0 in short axis (44, 45). However, also on pathologic examination it remains difficult to distinguish papillary muscles from hypertrabeculation. Also left ventricular aberrant bands and false tendons can easily be confused with trabeculations (46, 47). Pathologic examination of long axis cuts may help to differentiate between trabeculations and papillary muscles and between trabeculations and muscle bands. Indicating that additional information like genetic and functional data may help interpreting the macroscopic pathology examination.

Microscopic reports of NCCM showed that the trabeculae in NCCM were covered with endocardium and were not communicating with the coronary arteries (43). Interstitial fibrosis (81%) and endocardial or sub-endocardial fibrosis or fibro-elastosis (63%) were the most frequent histopathological findings (43). In eight pathology studies in which fibrosis was described cardiac MRI was performed and 6 showed late gadolinium enhancement (43). Interstitial fibrosis may be the result of dysregulation of the myocardium in ischemia, degeneration, inflammation or as a reaction on mechanical stress in chronic pressure overload (48). The interstitial fibrosis in HCM consists of predominantly of collagen I fibers and is probably resulting from mechanical stress induced upregulation of TGF-beta signaling and related pathways (49). Since inflammation and atherosclerosis are rare in NCCM (43), mechanical stress either by failing sarcomere function or overload may be the main mechanism underlying fibrosis. Since the examined heart tissues are usually from patients with the most advanced stages of heart failure, it is difficult to draw conclusions about the general pathological mechanisms in NCCM based on pathological features. Hypertrophy (47%) and disarray of cardiomyocytes (15%) was also frequently described in NCCM, these are the hallmark s of HCM (43).

These findings highlight overlapping phenotypes in cardiomyopathies.

To date pathologic exams of only five NCCM patients with a known genetic cause have been presented. One study reported on an autosomal dominant inherited mutation carrier of the *ACTC* gene and on a patient with an autosomal recessively inherited mutation in *FKTN* (50, 51). The patient with the *ACTC* mutation had besides NCCM also an atrial septal defect. The cardiac coupes of this patient showed trabeculations, fibrosis and disarray (50). The patient with the *FKTN* mutation had elevated creatine kinase levels, but did not have muscle weakness. The patient had left ventricular dilatation with prominent trabeculations. Microscopically the patient had prominent fibrous band separating the noncompacted from the compact myocardium. The compact myocardium showed myocyte disarray and anisonucleosis with mild interstitial fibrosis (51). Another study reported on a heart of a patient with an autosomal dominant inherited mutation in the *MBL* gene. However the association of NCCM with the *MBL* gene, which has an role in innate immunity, is questionable as this gene is hardly expressed in cardiac tissue (52). One third report concerned a Duchenne muscular dystrophy patient with NCCM who had a mutation in X-linked *DMD* gene (46). In this report the compacted layer consisted of fibrous tissue and some normal and dystrophic cardiomyocytes, while the noncompacted layer consisted of little fibrous tissue in which no dystrophic cardiomyocytes were detected (46). Finally, one NCCM patient with a rare variant in the mitochondrial gene *MT-CO3* was reported (53). The patient with the variant in *MT-CO3* gene was identified in a study of six NCCM patients who underwent cardiac transplantation. These hearts of these 6 transplantation cases were compared to 20 control hearts without a history of heart disease. The NCCM myocardium specimens had significant lower mitochondrial DNA content in myocardium and morphological abnormalities of mitochondria (53). Additional evidence is needed to establish whether these mitochondrial features may help to distinguish histologically NCCM and may shed novel insights on the etiology of NCCM. Regarding hereditary NCCM no specific pathologic findings can be observed.

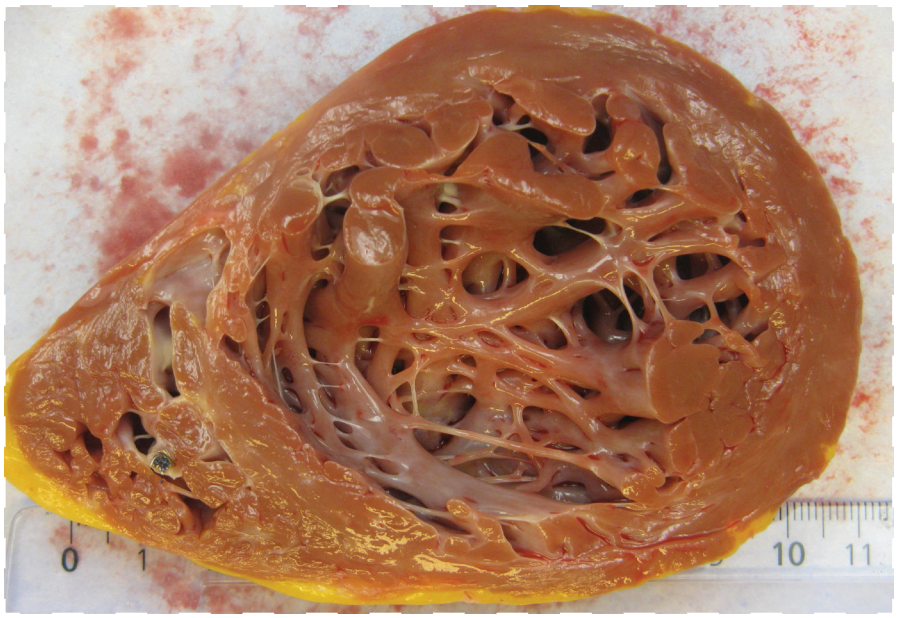


**Figure 1 : Short axis fresh autopsy specimen showing hypertrabeculation.**
NCCM Patient from the Erasmus Medical Center with two mutations in MYBPC3 (c.932C>A, p.Ser311* and c.442G>A, p.Gly148Arg) who had a cardiac transplant at the age of 23 years. The explanted heart was 400g, with dilatation of the left ventricle, marked hypertrabeculation and interventricular septum width of 9 mm. Microscopie showed hypertrophied and architectural disarray of cardiomyocytes with anisonucleosis and polychromasia of the cell nuclei. At the site of the macroscopic hypertrabeculation, round oval clusters of cardiomyocytes encircled with sub-endocardial fibrosis. Interstitial low to moderate levels of fibrosis was observed. Mainly the left ventricle showed degenerative changes with ischemic changes including hyper-eosinophilia and contraction band necrosis. Mild atherosclerosis was present.

## Genetics

Since DNA diagnostics for cardiomyopathies were introduced around 20 years ago, there have been major changes in the assessment in the effect of genetic variants. Before the start of this thesis and before the introduction of the latest stringent variant classification, sarcomere mutations were reported in 17% to 41% of NCCM patients (7, 54). These variants however were classified using different classification for pathogenicity than the classification system used nowadays. The currently widely applied and accepted sequence variant classification system is that from the American College of Medical Genetics and Genomics (55). This classification system classifies variants into classes 1 to 5. Class 5 includes variants with the highest likelihood of being pathogenic, while class 1 includes most likely common innocent genetic variants. This classification is based on population frequency, in silico prediction tools and functional evidence. With the advancing knowledge of

genetic variants and the wider application of DNA testing, refinement of variant classification will continue. As an example, the effect of variant reclassification was presented in a recent comprehensive study, reclassifying missense variants reported in NCCM studies, showing that 46% of the previously reported mutations could be regarded as benign or as variants of unknown clinical significance (56). Current practice is that large cardiomyopathy gene panels consisting on more than 50 genes are analyzed. However, as a review of prevalence of genetic defects in cardiomyopathy population showed, for some genes there is little evidence for association based on population frequency and may lead to more selective DNA testing (57). Overall, improved variant classification, identification of novel genetic causes will help to improve diagnosis of genetic NCCM.

**Aim and outline of this thesis**

NCCM is a genetically and phenotypically heterogeneous cardiomyopathy and still not completely understood. To establish the genetic spectrum, and draw valid conclusion on genotype-phenotype correlations, a large study population was needed. We performed a multi-center study as presented in chapter 2. In chapter 3 we investigated the different left ventricular phenotypes of NCCM and the prevalence of familial disease. In chapter 5 we used the literature to confirm the findings of chapter 2 and 3. To establish phenotypic differences between genetic and sporadic patients using cardiac MRI we performed a study presented in chapter 4. In chapter 6 we broaden the genetic spectrum of NCCM. Concluding that this thesis focused on the following issues:

- What proportion of NCCM patients have a genetic cause.
- Are there phenotypical and clinical differences between genetic and sporadic NCCM
- Do NCCM genotypes correlated with distinct phenotypes and cardiac risks.

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# Chapter 1

## Genetics and family screening for noncompaction cardiomyopathy

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*Cardio- genetics and family screening of Noncompaction Cardiomyopathy.*
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**Abstract**

In at least half the patients diagnosed with noncompaction cardiomyopathy (NCCM) genetics plays an important role. In familial NCCM, like in other inherited cardiomyopathies, timely identification and treatment of relatives at risk is important. This chapter focusses on the process of identifying a genetic cause, predicting risk for relatives, and informing index cases and relatives on subsequent recommendations for family screening.

## Introduction

Noncompaction cardiomyopathy (NCCM) is characterized by endocardial hypertrabeculation of the myocardium of the left ventricle. In 1997 the first genetic cause for NCCM, a mutation in the X-linked *TAZ* gene, was identified in a family were six boys had Barth syndrome with hypertrabeculation of the left ventricle (6). The link of familial NCCM to defects in the sarcomere genes previously been linked to the more frequent hereditary hypertrophic (HCM) and dilated cardiomyopathies (DCM), came in 2007 by the report of *MYH7* mutations in NCCM and was followed by reports of other sarcomere gene mutations in familial NCCM (figure 1) (7, 58).

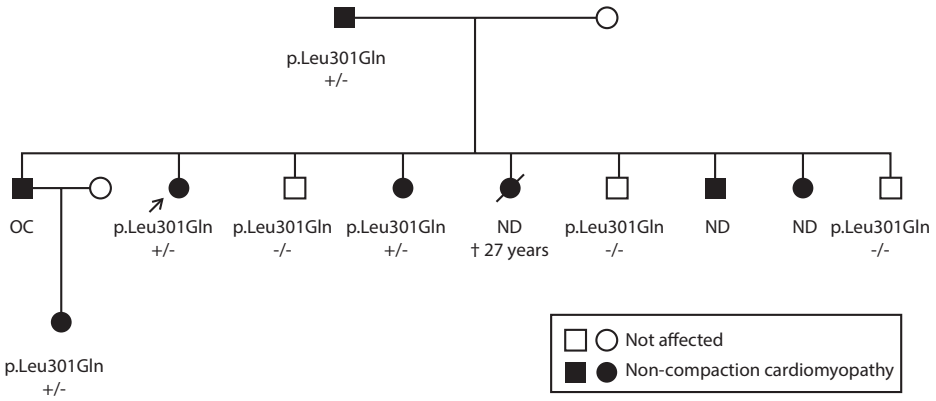


**Figure 1: Pedigree of family with a *MYH7* mutation.**
Pedigree of family A. The proband is indicated by the arrow. ND, not determined; OC, obligate carrier; +/–, heterozygous for the p.Leu301Gln MYH7 mutation; –/–, p.Leu301Gln absent. The figure was adapted from Hoedemaekers YM et al. Cardiac β-myosin heavy chain defects in two families with non-compaction cardiomyopathy: Linking non-compaction to hypertrophic, restrictive, and dilated cardiomyopathies. Eur Heart J. 2007;28(22):2732-7.

In NCCM the sarcomere genes are the most prevalent genetic causes. More recently the introduction of next generation sequencing (NGS ), allowing simultaneous analysis of panels of 50 or more cardiomyopathy genes, showed that around 35% of NCCM patients have a mutation, and that mutations occur more frequently in children diagnosed with NCCM than in patients diagnosed as adults (8, 10).

Overall, approximately 50% of NCCM patients are considered to have a genetic cause (8). Some because they have inherited a mutation in a cardiomyopathy gene, other patients have family members with a cardiomyopathy without

having a mutation in a known cardiomyopathy gene. In 45% of familial NCCM no mutation can be identified (8), indicating that many genetic causes for NCCM are still unknown. Overall, around 50% of cases diagnosed today with NCCM have no mutation in a cardiomyopathy gene or familial disease. In these -mostly adult patients- NCCM may be attributed to non-genetic, secondary causes for hypertrabeculation. Alternatively, these cases may have yet unknown (complex) genetic cause(s) carrying small risk for relatives (8). For NCCM, like for HCM and DCM, it is important for relatives of patients to be informed about the increased risk of having a cardiomyopathy.

For that reason referral of patients diagnosed with NCCM for genetic counseling, has become common practice (59). This allows, by taking family histories and performing DNA testing of the index case, to estimate the risk of having a cardiomyopathy for relatives. When there is a mutation, DNA testing for the familial mutation of first degree relatives is advised, with subsequent cardiologic screening of mutation carriers. In NCCM, the specific genetic defects may predict risk of having severe cardiac events (MACE). Some genes, like *MYH7,* carry lower risk for MACE than other genes. In this perspective DNA testing may help stratify risk for MACE of patient and relatives and help guide clinical management of genetic NCCM accordingly (8). For families of patients without a mutation, cardiologic screening of first degree relatives is recommended, also in absence of a family history of cardiomyopathy, because we cannot exclude that these patients may have an unknown genetic predisposition with low penetrance that conveys a small risk to relatives.

The aim of this chapter is to give an overview of the genetic causes for NCCM, and describe the routine of genetic diagnostics i.e. genetic counseling, DNA testing and initiating family screening. Illustrating in this way the importance of integrating genetic diagnostics to clinical management of NCCM patients by conveying appropriate information to patients and their families, in order to make early diagnosis and timely treatment accessible for the families of all NCCM patients.

*The genetics of NCCM*

Genetics plays a more important role in some patients with hypertrabeculation of the left ventricle than in others. Currently three main categories of genetic burden for noncompaction are recognized (figure 2). **1)** Patients with a genetic noncompaction cardiomyopathy. These are the patients with a mutation in a cardiomyopathy gene and/or relatives with a cardiomyopathy (familial cardiomyopathy). In genetic NCCM relatives have an increased risk of having a cardiomyopathy. In 45% of

familial NCCM no mutation is found (8). The majority of the genes associated with NCCM also play an important role in genetic hypertrophic (HCM) and dilated cardiomyopathy (DCM) (19, 54). For now, there is no explanation how overlapping genetic defects in these sarcomere genes cause the spectrum of phenotypes ranging from hypertrophic, dilated and noncompaction cardiomyopathy. **2)** Cardiomyopathy patients with noncompaction without a genetic cause; in these 'sporadic' NCCM cases no evidence for a genetic cause is found by DNA analysis, and the family history and/ or family screening are uninformative. These patients have similar cardiac outcomes as genetic NCCM patients. In sporadic patients NCCM may be the result of pathologic cardiac remodeling, activated by other (now unknown genetic or non-genetic) causes leading to hypertrabeculation. In these patients high incidences of left bundle branch blocks were identified (8). Also cardiac comorbidities like hypertension may play a role in these patients (8). We cannot exclude that apparently sporadic patient may have defect in a yet unknown cardiomyopathy gene, since not all cardiomyopathy genes have been identified yet. We know that at least one third of the NCCM patients with a mutation in a cardiomyopathy gene, did not report familial disease, indicating that negative family history does not exclude a genetic cause (8). Another possibility is that a group of apparently sporadic NCCM patients may have variants in known or unknown cardiomyopathy genes that have insufficient genetic effects and need additional interaction with other genetic or non-genetic factors to cause NCCM. **3)** Healthy individuals with a benign LV hypertrabeculation; large population based studies have reported that LV hypertrabeculation may occur as frequently as in 43% of the healthy adult population (60). A higher susceptibility for having more prominent trabeculations, without features of a cardiomyopathy was reported in blacks and athletes (27, 61). The cause might be a genetic or epigenetic regulation of gene expression or translation, activating similar pathways as mutations in sarcomere genes, causing hypertrabeculation without cardiomyopathy. The high incidence of hypertrabeculation supports that the currently used echo and MRI diagnostic criteria, relying on the ratio between noncompacted and compacted layer of myocardium, cannot distinguish pathologic noncompaction cardiomyopathy from benign, sometimes reversible, left ventricle hypertrabeculation without cardiomyopathy and therefore more sensitive diagnostic criteria are needed.

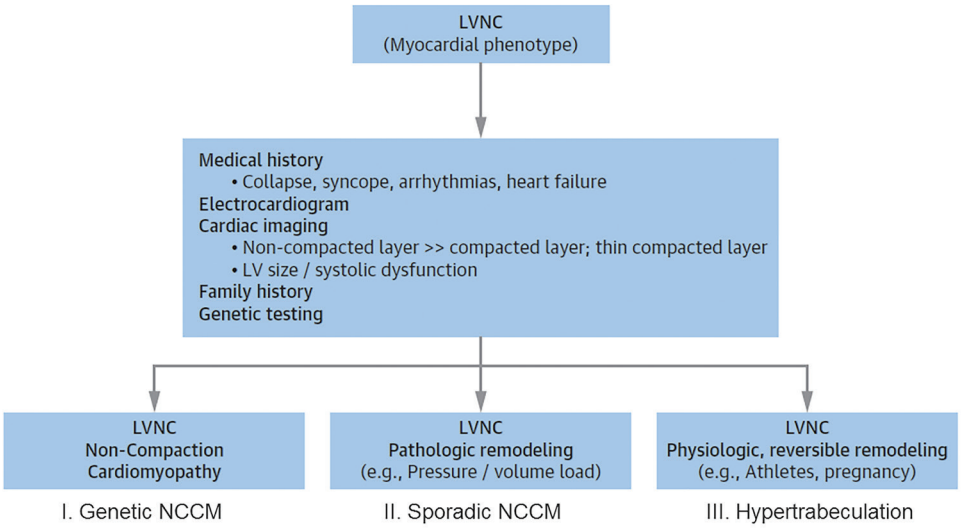


**Figure 2 : Etiology of NCCM.**
From the myocardial phenotype of LVNC to noncompaction cardiomyopathy, pathologic or physiologic reversible remodeling. LVNC: left ventricular noncompaction, NCCM: noncompaction cardiomyopathy. The figure was adapted from Oechslin E, Left Ventricular Noncompaction: From Physiologic Remodeling to Noncompaction Cardiomyopathy. J Am Coll Cardiol. 2018;71(7):723-6.

*NCCM genes*

In familial NCCM around 55% of NCCM patients have a mutation, indicating that the genetic cause has not been found for a large proportion of familial NCCM (8). In children and in adult patients the majority of the mutations occur in genes encoding for proteins of the cardiac sarcomere structure and function (figure 3) (8, 10). Less frequent genetic causes for NCCM are defects in genes encoding for intracellular signaling, homeostasis and cytoskeletal integrity associated with NCCM (62). Genetic causes are identified more frequently in patients diagnosed in childhood than in adults with NCCM (8). In figure 4 the frequency of all mutations reported in the literature are summarized. These observations show how little we understand about the development of the hypertrabeculation, because they suggest that the genetic effects might involve cardiac development as well as cardiac remodeling at older age.

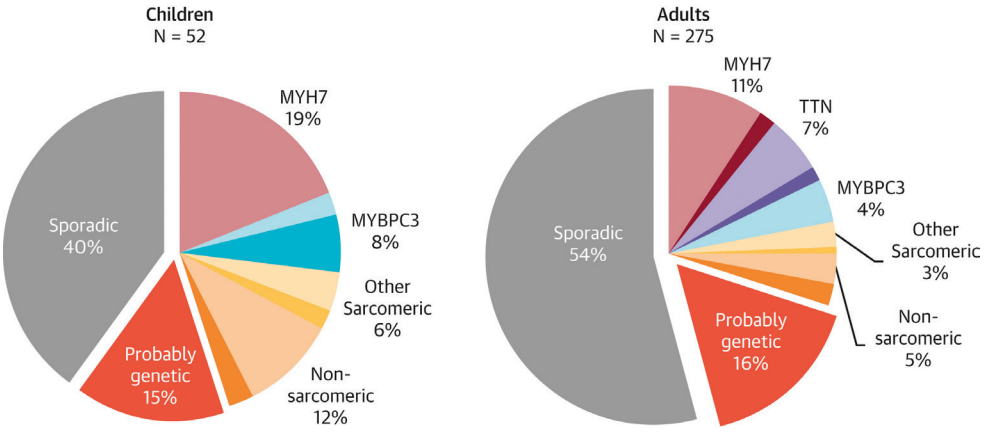


**Figure 3 : Genetics of NCCM.**
Darker shades indicate complex genotypes. DNA testing of approximately 45 cardiomyopathy genes and family histories showed that children were more likely to have a mutation (p=0.036). TTN occurred only in adult cases. Complex MYBPC3 genotypes occurred only in children (teal). The figure was adapted from van Waning JI, et al. Genetics, Clinical Features, and Long-Term Outcome of Noncompaction Cardiomyopathy. J Am Coll Cardiol. 2018;71(7):711-22.

*Genes for autosomal dominant inherited NCCM*

Defects in sarcomere genes are the most common genetic cause for NCCM (figure 3) (8, 10). These forms of NCCM have an autosomal dominant inheritance pattern. Patients (usually) inherited the mutation from one of the parents. Siblings and offspring of these patients have a 50% risk of having inherited the familial mutation. Reduced penetrance is a well-known feature of sarcomere mutations in genetic cardiomyopathies (63), meaning that for unknown reasons, around 30% (the percentage may vary by gene and variant) of the carriers (i.e. relatives with the familial mutation) do not have a cardiomyopathy. In a small proportion (4%) of the patients the mutation has occurred de novo (8, 64). In that case the mutation is not inherited from the parents and there is no increased risk for siblings, although risk for offspring of having the mutations remains 50%. Compound heterozygosity for sarcomere mutations, occurs when a patient inherited a (different) mutation from each parent. This is not uncommon, since sarcomere mutations are relatively frequent in the population (65). Patients with two sarcomere gene mutations may have more severe clinical features than their relatives with single mutations (66). In NCCM the most frequent genetic causes (71%) are defects in sarcomere genes: *MYH7 (58)*, *TTN (67)* and *MYBPC3 (8, 68)*. Less frequently (11%) affected are the other sarcomere genes: *ACTC1 (69)*, *LDB3 (70)*, *TNNC1 (71)*, *TNNI3 (72)* and *TNNT2 (73)*. Rare genetic causes are the other autosomal dominantly inherited cardiomyopathy genes *HCN4 (74)*, *KCNH2 (75)*, *KCNQ1(76)*, *RYR2* (77) and *SCN5A*

*(78),* involved in ion transport and genes affecting other cardiomyocyte functions or structure like, *DSP (79)*, *LMNA (80)*, *MIB1 (81)*, *MIB2* (82) and *PLN (8)*, occurring altogether in approximately 6% of the patients (8, 10).

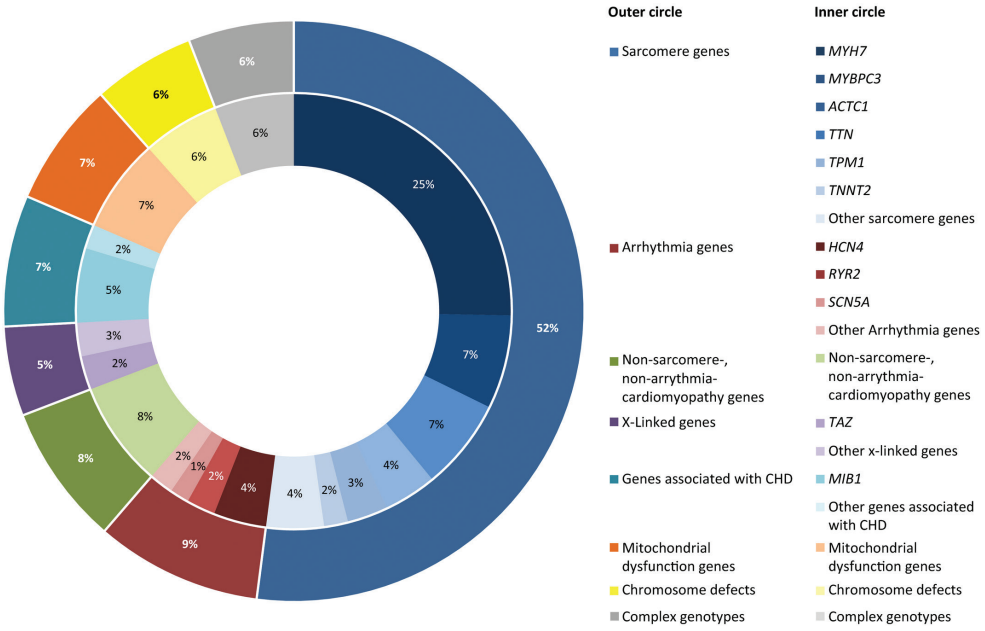


**Figure 4: Prevalence of mutations in the literature.**
Genetic noncompaction cardiomyopathy. Other sarcomere genes: ACTN2, DES, LDB3, MYL2, NEBL, OBSCN, TNNC1, and TNNI3. Other arrhythmia genes: ABCC9, ANK2, CACNA2D1, CASQ2, KCNE3, KCNH2, and KCNQ1. Non-sarcomere, non-arrhythmia-cardiomyopathy genes: DMPK, DSP, DTNA, FKTN, HFE, JUP, LMNA, PKP2, PLEC, PLN, PRDM16, RBM20, and SGCD. Other X-linked genes: DMD, FHL1, GLA, LAMP2, and RPS6KA3. Other genes associated with CHD:MIB2, NKX2.5, NOTCH1, NSD1, PTPN11, TBX20, and TBX5. Mitochondrial-functioning: HADHB, HMGCL, MIPEP, MLYCD, MT-ATP6, MT-CO1, MT-CO3, MTFMT, MT-ND1, MT-ND2, SDHA, SDHD, TMEM70, and VARS2. The figure was adapted from van Waning JI, et al. Meta-analysis of the genotype- phenotype correlation in noncompaction cardiomyopathy. J Am Heart Assoc. 2019 Dec 3;8(23):e012993.

### *Genes for X-linked inherited NCCM*

Defects of genes on the X chromosome affect only males and are inherited in an X-linked pattern. With this type of inheritance sons of unaffected female carriers have 50% risk of being affected. Daughters of patients or of female carriers have 50% risk of being an (unaffected) carrier and transmitting the trait to their sons. Barth syndrome is caused by defects in the *TAZ* gene on the X chromosome (6). Among the other X-linked causes for NCCM are some genes causing neuromuscular disorders; *DMD (83), FHL1(84), GLA (85), LAMP2 (86),*and rare neurodevelopmental

disorders caused by mutations in the *NONO (87),* and *RPS6KA3 (88)* genes.

*Genes for autosomal recessive inherited NCCM*

Recessive inherited NCCM is rare and was reported in single childhood cases with inborn errors of metabolism, related to a *FKTN (51)* or *SDHD (89)* mutation.

*Mitochondrial defects and NCCM*

Mitochondrial disorders are caused by defects in the mitochondrial (Mt) DNA or by a defect in nuclear DNA genes encoding mitochondrial structure or functioning. Defects in Mt genes are passed on cytoplasmatically in germ cells from mother to child. Defects in nuclear genes have dominant, recessive or X-linked inheritance pattern. Mutations in genes affecting the mitochondrial functioning lead to insufficient energy production required in various organs, particularly those with high energy demands, like the central nervous system, skeletal and cardiac muscles. These disorders present with a wide spectrum of clinical features including cardiomyopathy, visual impairment, deafness, stroke, epilepsy and diabetes. Mt genes linked to NCCM are *MT-ATP6, MT-ATP8, MT-CO1, MT-CO3, MT-CYB, MT-ND1, MT-ND2* and *MT-ND6* (90, 91). Nuclear genes coding for the mitochondria linked to NCCM are *DNAJC19* (92)*, GARS (93), HADHB (94), MIPEP (95), MTFMT (96)*and *NNT(97)*. To find Mt gene defects a specific analysis of the Mt DNA and nuclear DNA is needed, since these genes are not routinely sequenced in NGS cardiomyopathy gene panels.

*Chromosomal defects*

A number of chromosomal deletions and duplications have been associated with NCCM. These chromosomal defects are usually identified in children. Because they affect multiple genes they lead to complex congenital malformation syndromes. The 1p36 deletion syndrome is frequently reported presenting with NCCM, intellectual disability, delayed growth, hypotonia, seizures, limited speech ability, hearing and vision impairment and distinct facial features (98). Other chromosome anomalies linked to NCCM are deletions of 1q (99), 5q35 (100), 8p23.1 (101), 22q11 (102) and Xq28 (103). In addition NCCM has been observed in monosomy X (Turner syndrome) (104), trisomy 13 (105), trisomy 18 (106), trisomy 21 (107) and trisomy 22 (108) patients. To detect a small chromosome anomaly, an array analysis has to be performed, since these defects are not recognized by NGS sequencing of cardiomyopathy genes.

*Genetic Counseling and Genetic diagnosis of NCCM*

Genetic counseling is recommended for all patients fulfilling diagnostic criteria for NCCM to perform DNA analysis and detect familial disease. This information is needed to estimate risk for relatives, convey information on the risks to index cases and their families and subsequently initiate family screening. Like in HCM and DCM family screening for NCCM is recommended because it allows accurate and timely diagnosis of NCCM improving prognosis of patients in the family. To initiate genetic diagnostics for NCCM, index patients are counseled about the consequences of the results of DNA testing, and an informed consent for DNA testing is requested.

Genetic counseling involves communicating the goal of genetic testing and the explaining the importance of informing family members. Genetic counselors are trained to explain the clinical features of the disease and the inheritance pattern, to the index case and organize informing and screening family members. Genetic counseling has grown out of the need to personalize scientific information and to translate it into a user-friendly language that is accessible intellectually and emotionally for the patient and its family. Helping index cases and their relatives – if necessary-to handle the information on heredity, and discuss the subsequent risks and consequences, is an important part of the process of genetic counseling. The routine for genetic diagnosis and family screening for NCCM is summarized in figure 5. It is hereby the role of the genetic counselor to identify and help, during pre- and post-test counseling, coping with adverse feelings that some patients or relatives may experience like distress, anxiety or guilt, evoked by the possibility of a genetic cause for NCCM (109). It is important, in particular for asymptomatic relatives, to discuss that having a genetic risk and having a choice of predictive testing, whether by DNA analysis or cardiologic exam, may have medical implications, as well as psychological and socio -economic consequences. The genetic counselor may offer access to specialized psychologic support when needed by families.

*Family history*

At the departments of clinical genetics information on the occurrence of cardiomyopathies in the family of NCCM patients is obtained, and medical records of affected relatives are retrieved for verification of the diagnosis, when possible. Family history taking helps to determine if cardiomyopathy is familial and to identify the mode of inheritance (110). It is importance to acknowledge that an uninformative family history cannot completely exclude a genetic cause for NCCM. Because around 20% of NCCM patients without affected relatives may still

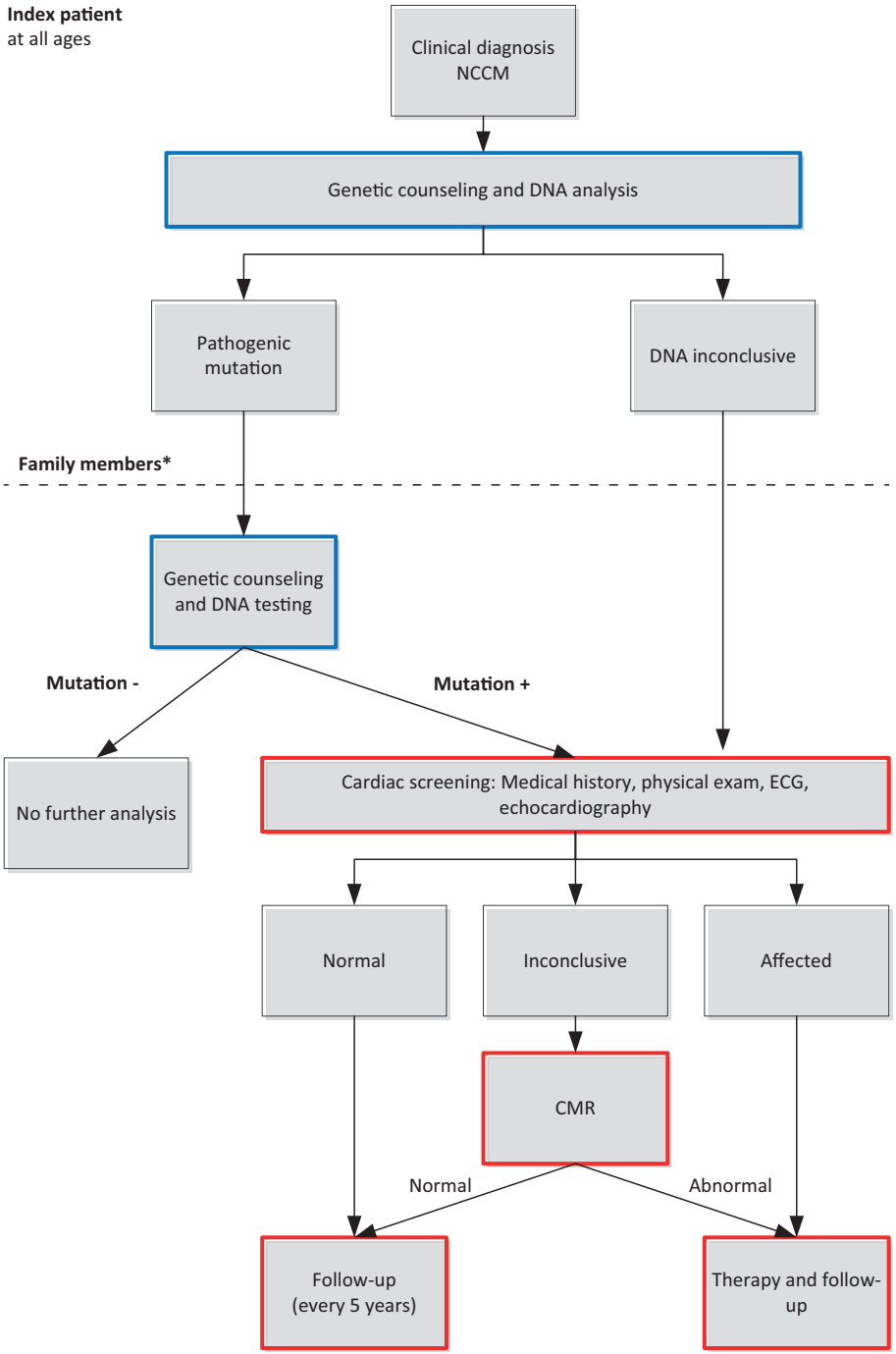


**Figure 5: Family screening if a cardiomyopathy is identified at all ages.**
*Presymptomatic DNA testing for relatives above 18 years. Cardiac screening for relatives from 10-12 years, without DNA testing. In blue: at the clinical genetics department. In red: at the cardiology department. CMR: cardiac magnetic resonance imaging.

have a mutation (8). The reasons for underreporting of familial cardiomyopathy might be that affected relatives might not have been diagnosed with NCCM. It is known that approximately 30% of the NCCM patients have a cardiomyopathy without the typical symptoms of cardiomyopathy at time of diagnosis (111). Also, like in HCM, non-penetrance occurs in around 30% of the carriers of a familial (sarcomere) mutations and these carriers do not have a cardiomyopathy (111). Another explanation for underreporting familial disease may be that family histories are not informative when families are small or patients have little information on relatives. Important questions when taking a family history for the purpose of establishing whether there is a familial cardiomyopathy is asking if relatives have had heart failure, arrhythmias, accidental or unexpected deaths, thromboses (including stroke), any kind of cardiac surgery, or if they had a congenital heart defect or neuromuscular disease. When family screening is performed the family histories are adjusted according to the results of the DNA and cardiac screening of relatives.

*DNA testing for NCCM*

The purpose of DNA testing - irrespective of the age of the patient - is to identify the genetic cause for NCCM (8, 112). An important aspect of DNA testing is that finding a mutation allows asymptomatic relatives to have a predictive DNA test that identifies accurately which relatives have a mutation and have an increased risk of developing a cardiomyopathy. In this way identifying the causative mutation facilitates genetic cascade screening. In families with a mutation, relatives who do not carry the familial mutation can be excluded from regular cardiac follow-up and can be reassured that there is no increased risk for their offspring. DNA testing may help to confirm the diagnosis for patient with borderline features of NCCM. In addition as we have shown recently, the genotype (specific genetic defect) may help to predict risk for ventricular systolic dysfunction and major cardiac adverse events for patients and guide clinical management accordingly (8), as discussed in more detail below in the paragraph on *genotype-phenotype correlations*.

*NGS Cardiomyopathy gene panels*

Since a large number of genes are involved in NCCM, the application of novel methods of DNA analysis like NGS and exome based testing improved the yield of genetic testing with the simultaneous analysis of panels with large numbers of cardiomyopathy genes (3). Current cardiomyopathy gene panels used in diagnostic and commercial laboratories may include the following genes: *ABCC9, **ACTC1**, **ACTN2**, ANKRD1, BAG3, CALR3, CRYAB, CSRP3, DES, **DMD**, DSC2, DSG2, **DSP**, **EMD**, **GLA**, JPH2, JUP, LAMA4, **LAMP2**, **LDB3**, **LMNA**, **MYBPC3**, MYH6, **MYH7**,*

*MYL2, MYL3,* ***MYPN****, MYOZ1, MYOZ2,* ***PKP2, PLN****, PRKAG2,* ***RBM20, RYR2, SCN5A,*** *SGCD,* ***TAZ****, TCAP, TMEM43,* ***TNNC1, TNNI3, TNNT2, TPM1, TTN*** *and VCL* (in bold the genes that were associated so far with NCCM). These genes encode proteins constituting structure and function of the sarcomere, cytoskeleton, desmosome, ion channels or nuclear lamina, and proteins participating in calcium (Ca2+) handling during contraction phase of action potential of the cardiomyocyte or affecting cardiac energy metabolism and are related to a large spectrum of cardiomyopathies. In case a cardiomyopathy gene panel is not available, DNA testing for NCCM of a smaller number of genes including *MYH7*, *MYBPC3* and *TTN,* which have a large proportion of the genetic defects in NCCM, is advised.

*Gene variant classification system*

For a correct interpretation of the results of DNA analysis stringent novel guidelines for classification of genetic variants are applied since 2015 (113). The outcome of DNA analysis for clinical purpose are currently classified into pathogenic variants (PV), likely pathogenic variants (LPV), variants of unknown clinical significance (VUS), likely benign or benign variants. This classification system for variants is based on in silico prediction of pathogenicity, population frequencies and previous reports providing (functional) evidence of the pathogenic nature of the specific variants (55). Variants classified as PV or LPV in sarcomere genes are usually nonsynonymous substitutions or deletions of a nucleotide classified as missense, nonsense, or frameshift mutations and have a deleterious effect on the protein. Older results of DNA testing, without current classification, should be re-evaluated, because some of the variants previously reported as (pathogenic) mutations may now be reclassified as not pathogenic. Application of novel classification system to a large number of variants in sarcomere genes in NCCM patients showed recently that 50% of variants previously reported to be pathogenic, were reclassified as VUS or benign variants (56). Similarly a large proportion of variants reported previously as mutations in sarcomere genes in HCM patients, were reclassified recently as VUS or benign variants (57). This endorses that the continuous surveillance of variant classification is needed, because new evidence on DNA variants like population frequencies, results of novel functional tests or in silico predictor tools becomes available(57). DNA testing of pre-symptomatic family members is only indicated when there is a PV or LPV in the family. Since the effect of VUS is not known, these variants cannot reliably predict risk for NCCM in relatives and therefor these variants are not of used for family screening.

*DNA testing of NCCM patients with Congenital Heart Defect, neuromuscular disease or NCCM with multiple congenital anomalies syndrome*

Around 10% of NCCM patients have a concomitant congenital heart defect (CHD) (22). Some families with NCCM and Ebstein anomaly, have a mutation in *MYH7* (23). There is little evidence that the combination of NCCM with other forms of CHD segregate in families or are caused by specific genetic defects. Thus it remains unknown if there are common (epi)genetic causes affecting embryologic cardiac development explaining the co-occurrence of NCCM and CHD, or that they co-occur by coincidence. NCCM in some patients represent cardiac manifestations of inherited neuromuscular disorders, for which specific diagnostic gene panels need to be analyzed since these genes are usually not included in the regular cardiomyopathy gene panels (114). Also NCCM patients with multiple congenital malformations, usually children, need additional DNA testing and/ or chromosome analysis (array), according to clinical features. These tests may include screening for mitochondrial defects or metabolic disorders occurring predominantly in childhood NCCM.

**Family screening**

*Risk for cardiomyopathy in relatives*

Overall affected relatives of NCCM patients have less severe cardiac features than the index cases, and relatives of index cases with a mutation have more risk of having a cardiomyopathy than the relatives of cases without a mutation. Because, at diagnosis affected relatives have usually less attenuated cardiac symptoms than the index case, independent of age at diagnosis, since most relatives are asymptomatic (111). And some of the index cases without a mutation may have a non-genetic, secondary cause for NCCM, with low risk for relatives. The risk for relatives of having a cardiomyopathy is furthermore related to the genetic defect in the index case, the mode of inheritance, the gene specific penetrance and the chance of having asymptomatic disease. These factors and also the age at diagnosis of the index case may help to determine the genetic risk for relatives. Also family history of cardiomyopathy or sudden cardiac death in the family may add information about the genetic risk for relatives. It is important for relatives to know that carriers of a familial mutation may have no signs of cardiomyopathy at cardiologic examination. Non-penetrance was observed in 17% of carriers of familial *MHY7* mutations, 33% of carriers of *MYBPC3* and 28% of carriers of *TTN* mutations (111). Intra-familial variability of cardiac features is a well-known feature of familial cardiomyopathies. The left ventricle (LV) dimension of the NCCM index case may be a predictor for disease severity in relatives. The dimension of the LV

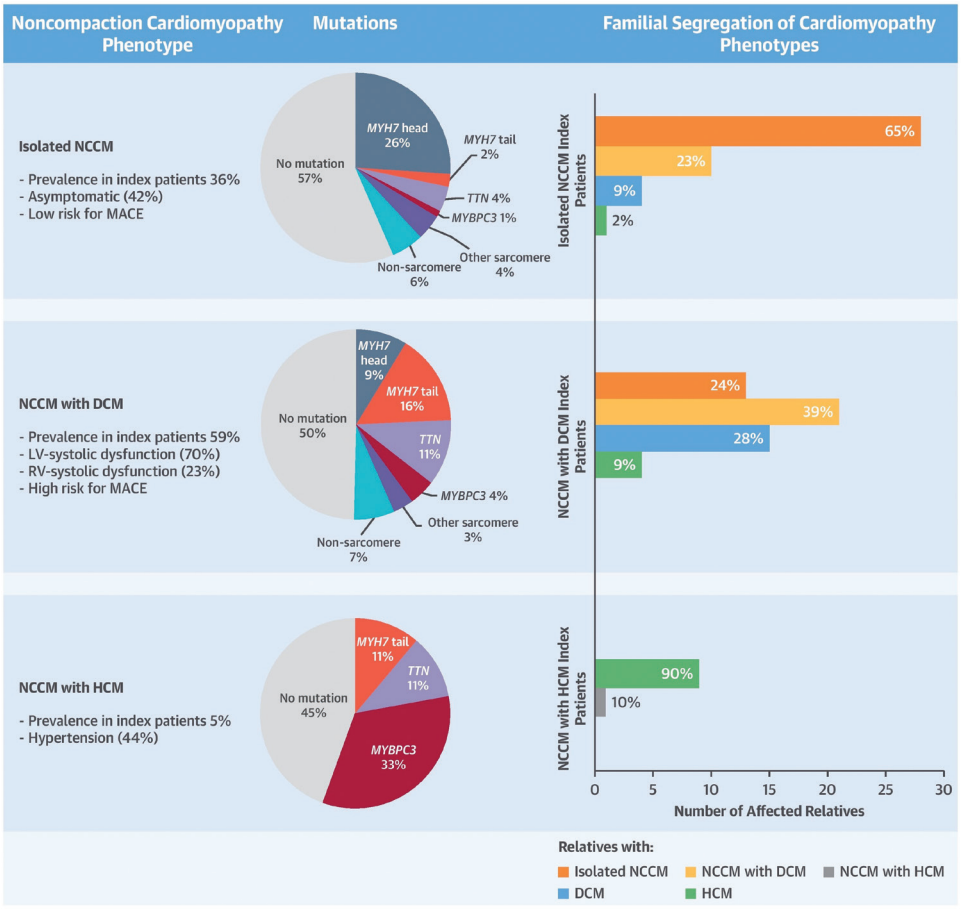


**Figure 6: Classification of NCCM according to cardiac phenotype.**
Classification of NCCM according to cardiac phenotype into isolated NCCM (51 index, 41 relatives), NCCM with DCM (84 index, 31 relatives) and NCCM with HCM (8 index and 1 relative) in 39 families with and 19 families without a mutation. Genotyping and family screening showed that isolated NCCM was linked to mutations in the head domain of MYH7 ($p<0.001$), isolated NCCM in relatives ($p<0.001$) and a lower risk for LV dysfunction ($p<0.001$). NCCM with DCM was linked to the MYH7 tail domain ($p<0.001$) and TTN and was associated with relatives with DCM without signs of noncompaction ($p=0.002$) and severe outcome ($p=0.016$). The HCM phenotype was linked to MYBPC3 in NCCM families ($p<0.001$) and HCM without signs of noncompaction in relatives ($p<0.001$). Factors reducing risk for relatives were absence of a mutation in index patients, non-penetrance of familial mutations or having asymptomatic disease. Underscoring that the NCCM phenotype of the index case and the genotype are important predictors of risk in relatives. The figure was adapted from van Waning JI et al. Cardiac Phenotypes, Genetics, and Risks in Familial Noncompaction Cardiomyopathy, J Am Coll Cardiol. 2019 Apr 9;73(13):1601-1611.

in NCCM relatives corresponded significantly with the LV phenotype of the index case (figure 6). In addition, since the LV dimension in NCCM patients was related to the course of the disease, the LV function may predict severity for relatives. Patients with NCCM and normal LV-dimensions, as observed in approximately 43% of the patients, had a mild course of the disease, with less frequent LV-systolic dysfunction or cardiac events. Patients with NCCM with a dilated LV-dimensions (like in DCM), occurring in approximately 53%, had a more severe disease course with frequent LV-systolic dysfunction and adverse events. The relatives these NCCM/DCM patients were more likely to have dilated LV. These patients were also more likely to have relatives with DCM without hypertrabeculation. In a small percentage of NCCM patients, (4%) there are concomitant signs of HCM. The relatives of the NCCM/HCM patients may have HCM without hypertrabeculation. In the families of NCCM patients, 20% of the affected relatives have HCM or DCM without signs of hypertrabeculation (111). In addition relatives of NCCM patients may have an increased risk for CHD, compared to population risk (111).

**Screening adult relatives of NCCM patients**

In families with a causative mutation, adult relatives can be offered predictive DNA testing. Predictive DNA testing of relatives can reliably identify which relatives carry a mutation and have an increased risk of developing a cardiomyopathy and thus need clinical surveillance. In addition since genotype are linked to the cardiac features and the outcome, genotyping of the index patient may help predict the cardiac phenotype and the risk of having a severe cardiomyopathy for the whole family (111). Relatives who do not carry the familial mutation can be excluded from regular cardiac follow-up and also can be reassured that there is no increased risk for cardiomyopathy for their offspring.

In families without a mutation, cardiologic family screening of first-degree relatives is recommended. Family screening can be initiated by asking the index patients to distribute a letter to their first and second-degree relatives with information on counseling for genetic risk for NCCM and recommendations for predictive DNA and/or cardiologic family screening. The legal framework for informing relatives varies. In most countries the index patient ( not the clinician) is expected to pass the information on genetic risk and screening to the family on behalf of the healthcare system (115). It is important that relatives consent and are correctly informed, *before* they are tested, about the risk of having a cardiomyopathy and about the eventual consequences when they are carriers of a familial mutation and/or signs of cardiomyopathy are detected at cardiologic exam. Diagnosis of a mutation or a cardiomyopathy, even when a relative is asymptomatic may

have medical, psychologically as well as socio- economically consequences. For instance regarding life insurance, pension, life style (sporting activities), and eligibility for fostering and adoption (116). Most relatives have no symptoms of cardiomyopathy and have not been diagnosed with a cardiomyopathy when they have a predictive DNA test or have the first cardiologic examination. The incentive for having a predictive test for some relatives is the wish to be in control of their life and gain clarity on the risk they and their offspring may be facing. For others, making a decision is more complicated and they prefer not knowing about the risk because they have a different perspective of the risk, giving the chance of being asymptomatic for years. A genetic counselor can help to guide in their decisions to have a pre-symptomatic test.

## Screening young relatives for NCCM

Like in other age dependent hereditary cardiomyopathies, the recommendations for pre-symptomatic screening are not the same for adults and children. Cardiologic screening is usually recommended from the age that first symptoms may appear. For instance for HCM, cardiologic screening starts around 10-12 years for asymptomatic children with unknown genetic status (116). In practice these guidelines are followed for NCCM as well. In families with a mutation, predictive DNA testing in children is usually postponed until the age that they can make an informed decision. Because the medical benefit of pre-symptomatic DNA diagnosis of having a familial mutation has not been established for children. The main advantage of pre-symptomatic DNA testing of children is that when a familial mutation can be excluded the child can be discharged from life-long follow-up. In contrast, for the asymptomatic children who are found to be carriers of a familial mutation, recommendations include regular cardiologic follow-up and address life style, like refraining from competitive sports (116). The burden for children of regular hospital visits, may have adverse psychological like anxiety or depression and may harm a child's self-esteem (117, 118). Another adverse effect of pre-symptomatic testing in children and adults alike are possible economic disadvantages like higher life insurance or mortgages later in life. For that reason predictive DNA testing for a familial mutation is usually performed in relatives above the age of 18 years. Clinical and/or genetic screening should be considered from younger age if the child has symptoms which can point to a cardiomyopathy or in families with a history of early-onset cardiomyopathy.

### Pregnancy and prenatal testing

An important aspect of the counseling and cardiologic care of young women with NCCM is to inform patients that a pregnancy may carry a risk for themselves

as well as for their offspring. For women with NCCM, the maternal risk in pregnancy for developing heart failure and/or arrhythmias or severe postpartum cardiomyopathy requires extensive follow-up during pregnancies as well as postpartum. Women with a cardiomyopathy who have symptoms before pregnancy have an increased risk and need specialized obstetric care (119). Women with asymptomatic cardiomyopathies usually tolerate pregnancy well and these women may have a spontaneous labor and vaginal delivery (120). NCCM patients have an increased risk of having a child with a cardiomyopathy. Depending on whether the patient has a mutation and the estimated risk for the child, prenatal diagnosis of NCCM (prenatal DNA testing and/or prenatal cardiac ultrasound of the fetus) can be discussed. Prenatal diagnostics for NCCM, however, are rarely requested, because the risk that a child has severe congenital NCCM is small, given that onset of symptoms of NCCM are age related, and patients/ carriers of mutations may not have symptoms. Unless there is an affected child in the family, in which case prenatal diagnostics for NCCM will be recommended.

The individual options and limitations of prenatal diagnosis of NCCM are discussed with NCCM patients with reproductive wishes. Pre- and post-test counseling is necessary because risks and prenatal testing in these pregnancies may evoke anxiety in parents and they may need help to make far reaching decisions during the pregnancy. It is important to acknowledge the likelihood that testing may cause distress, meaning that steps should be taken to minimize distress and provide support, not that testing should be denied.

For prenatal testing for NCCM the familial mutation is important. In families with a mutation, prenatal DNA testing can be performed. We have the choice of a DNA testing in chorionic villus sampling (conducted at 10-12 weeks of gestation) or amniocentesis (conducted at 14-20 weeks of gestation). The DNA test results are known within 2 – 3 weeks, well within the legal framework in most countries for terminating a pregnancy affected with a severe disorder. The parents need to be informed that these interventions carry a risk for the mother and fetus including miscarriage (121). If the child is shown to have the familial mutation that may causes (severe) childhood cardiomyopathy, parents may choose to terminate the pregnancy or have additional prenatal echocardiography for structural defects and assessment of cardiac function to detect a congenital cardiomyopathy (122). Prenatal cardiac sonography is performed in specialized tertiary prenatal centers, and allows to detect fetal cardiac malformations, cardiomyopathies, systolic and diastolic function and arrhythmia in the second –and third trimester of pregnancy. Prenatal cardiac sonography is also the method of choice for prenatal screening

of NCCM when there is no mutation in the family. A major limitation of prenatal sonography for NCCM is that little is known about the onset and prenatal development of NCCM, and we do not know in which NCCM patients we may and in which we cannot find prenatally signs of noncompaction and in which trimester the first cardiac signs of noncompaction be may observed. There are few reports, showing early prenatal onset of NCCM in cases with a *MYH7* mutation (123). Since prenatal diagnosed NCCM may remain asymptomatic after birth, prediction of disease severity from the results of prenatal testing remains difficult (124). However, prenatal testing does have a role in assessment which pregnancy may need perinatal cardiac monitoring.

**Psychological impact of genetic testing for index and for relatives**

Having a genetic cardiomyopathy implies that your children and other family members may have an increased risk of having a cardiomyopathy. For patients, this knowledge may add to the burden of having a cardiomyopathy. For insight in the psychological effects of genetic testing, we depend on studies focusing on familial cardiomyopathies or other genetic disorders. The studies looking at the impact of having a genetic cardiomyopathy showed that overall the burden of cardiac symptoms had greater psychosocial impact than the burden of the condition being genetic (109, 125). Index cases might be pressured by their relatives to have genetic testing. But this did not negatively affect satisfaction with the genetic counseling process or getting the results of DNA testing (125). Overall clinical symptoms are the principal source of concern: index cases showed more distress when having a diagnostic DNA test than relatives having a predictive DNA test, probably because the index cases had a cardiomyopathy, while the relatives usually are asymptomatic (126). Predictive testing can evoke anxiety about risk of being affected and transmitting the predisposition for disease to offspring, but it may also bring clarity about a subject that has been on the mind for a significant time. Overall relatives at risk for hereditary cardiac diseases did not have more emotional distress the normal population (127). Understandably, relatives with a positive genetic test showed more distress than relatives where a familial mutation could be excluded (126). Despite the result of the genetic test, the vast majority (80%) of the patients was satisfied with the decision of undergoing a genetic test (126). Patients who had less understanding of carriership of the mutation or had stronger belief in serious consequences had more symptoms of depression (125). High levels of anxiety were linked to a younger age, females, less formal education and fewer social contacts (125, 128). From these studies we have learned the importance of focusing during pre- and post-test counseling on the individual perception of risk and disease and helping the patients to cope with the consequences of the test result. The way of

giving the test result, by telephone or by a face to face appointment, did not have an impact on contentment of the patient (125).

*Cardiologic family screening*
Cardiologic screening is o recommended for the relatives from a family with a mutation, who were shown to have the familial mutation as well as the relatives who choose not to have a predictive DNA test. Likewise, in families were no mutation is found, relatives are advised to have a cardiologic examination from age of 10 years onwards. Cardiologic screening may be initiated before the age of 10 years if the child is planning to engage in competitive sports, or there is a family history of sudden cardiac death. Cardiologic screening in family members includes a physical examination, twelve-lead electrocardiography and echocardiography. When abnormalities are found the cardiac work up should be expanded. For example when signs of a cardiomyopathy on echocardiography are identified, additionally cardiac magnetic resonance imaging (CMR) can be performed. A 48-hour ambulatory electrocardiography should be performed when patients have palpitations or there are other indications for an arrhythmia.

In familial NCCM, relatives have less severe cardiac features than the index cases. Early detection in relatives is important and allows treatment and prevention of severe complications. Another reason why cardiologic family screening is recommended for the families of all cases, is that in this way asymptomatic relatives with a cardiomyopathy even in families without evidence for genetic disease can be detected. Family screening may thus help to identify familial NCCM and stratify risk for relatives into a high genetic risk.

**Cardiologic follow-up**
In general, the diagnosis NCCM requires lifelong follow-up to detect changes in symptoms, risk for adverse events, LV function and cardiac rhythm. Prevalence of LV systolic dysfunction and atrial arrhythmias increases with age (129, 130). The frequency of monitoring is determined by the severity of disease, age and symptoms. A clinical examination, including 12-lead ECG and TTE, should be performed every 1 to 2 years or sooner if patients have new cardiac symptoms (116). In 50-70% of the mutation carriers a cardiomyopathy is identified by first screening, 30% of these patients are asymptomatic (111, 131). These asymptomatic carriers with a phenotype should also have follow-up every 1 to 2 years. The clinical significance of mild morphological and functional abnormalities is uncertain but probably minor in most (131, 132). In 30% of the adult relatives with a mutation no cardiomyopathy at first cardiologic screening is identified, these

are mutation carriers without a phenotype (111). Studies suggest a benign clinical course for mutation carriers without a phenotype in HCM families (133, 134). However, a proportion of mutation carriers without a phenotype will develop a cardiomyopathy later in life, because of age-related increase in penetrance (63). According to current insight, mutation carriers without a phenotype should have cardiac examination at least every five years (116). Also first degree family members without a phenotype in familial NCCM s without a mutation cannot be discharged from medical follow-up and should also be screened at least every 5 years (116). Similarly adult relatives of cases without a family history or mutation, who do not show signs of cardiomyopathy will be screened until the age that occurrence of a signs of a genetic cardiomyopathy is small. This is because we cannot fully exclude a genetic cause even when the index has no mutation and no other cases of NCCM occur in the family.

**Genotype-phenotype correlation**

Knowing the genetic cause for familial NCCM may help to predict the outcome. Specific genetic defects were associated with the phenotype, and associated clinical features, including risk for major adverse cardiac events for the index case and affected relatives. In other words, complementing cardiologic diagnosis with genetic status may allow tailoring clinical management and follow-up of familial NCCM according to genetic burden. In the future the associations between specific mutations and clinical features or risks may become clearer, by more extended methods of DNA testing and the analysis of the features large numbers of patients. Although specific genotype based cardiomyopathy treatments are not available, the established genotype-phenotype correlations for NCCM can help to guide clinical management of the patients.

**Genetic versus sporadic NCCM**

There are distinct differences between the genetic (the NCCM patients with a mutation and/or patients with a family history of cardiomyopathy) and the patients without a mutation or family history, the sporadic cases (8). Sporadic patients presented more often at adult age (figure 7). In children, genetic NCCM was associated with severe outcome and cardiac symptoms, LV systolic dysfunction, and a high risk for major adverse cardiac events (MACE). In contrast to sporadic children, who had a good prognosis, with a mild clinical course and low risk for complications. In severe forms of NCCM occurring in childhood the possibility there may be a complex genotype. For that reason if a child is diagnosed with a cardiomyopathy in the family of an adult NCCM case, we recommend to perform a full panel of genes testing instead of only testing for the familial mutation.

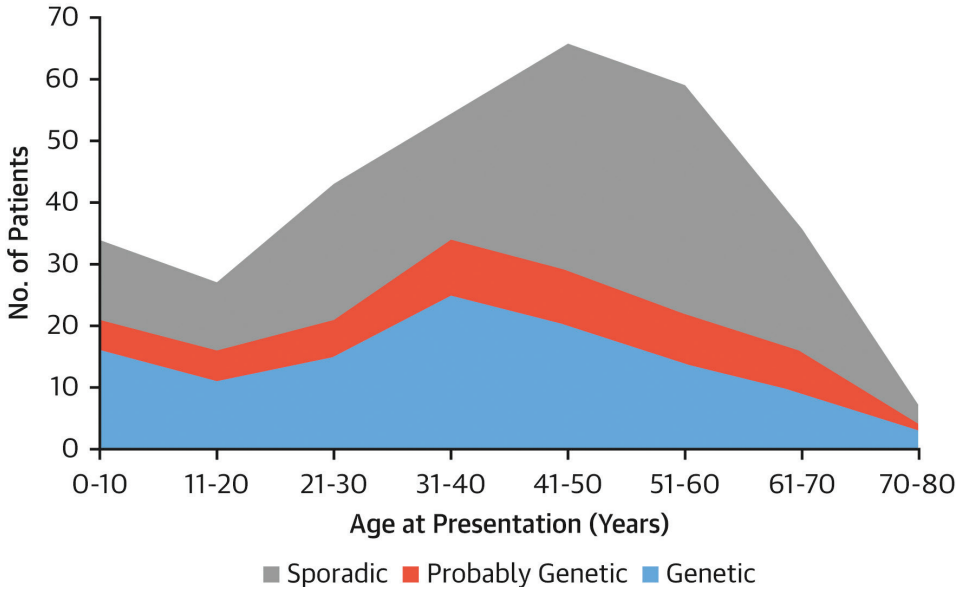


**Figure 7: Age at Diagnosis in 327 Dutch NCCM Cases.**
In blue the genetic patients, these are the patients with a mutation. In red the probably genetic patients, these are the patients without a mutation, but with familial cardiomyopathy. In grey the sporadic patients, these are the patients without a mutation and without a familial cardiomyopathy. Chance of having a mutation decreased with age (odds ratio: 0.983 per year; 95% confidence interval: 0.97 to 0.99; p=0.01). The figure was adapted from van Waning JI, et al. Genetics, Clinical Features, and Long-Term Outcome of Noncompaction Cardiomyopathy. J Am Coll Cardiol. 2018;71(7):711-22.

Adults with a mutation had high risk for LV and RV systolic dysfunction (8). Prognosis in adult NCCM patients with a mutation was correlated with left ventricular function (figure 8). Adult NCCM patients with a mutation and a persevered LV ejection fraction had a good prognosis. In contrast to adults with a mutation and LV systolic dysfunction, who had worse outcome. In sporadic adult patients prognosis was not related to LV systolic function. The sporadic NCCM patients had frequently hypertension, suggesting an acquired form of NCCM, which eventually, may have consequences for recommendations of family screening

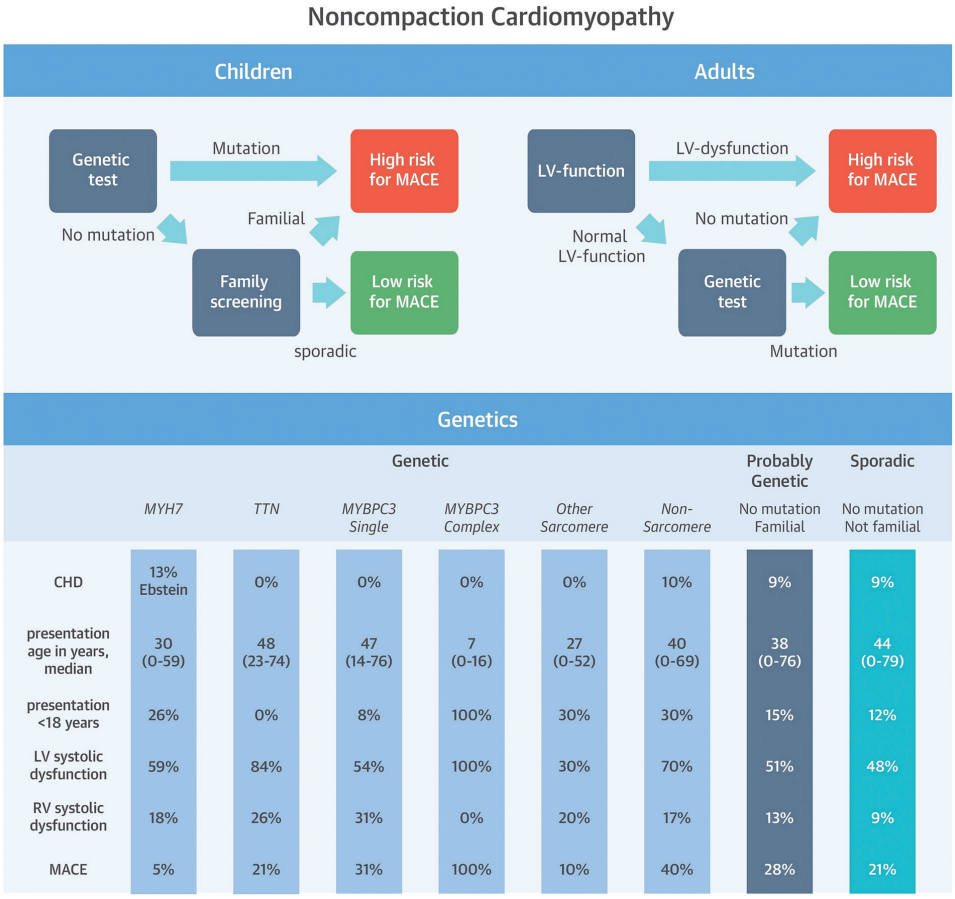


**Figure 8: Overview of genetics, clinical features and outcome in NCCM.**
Noncompaction cardiomyopathy (NCCM) is a heterogeneous condition, and genetic stratification has a role in clinical care. In children, genetic causes with severe outcome are more common than in adults. In non-genetic NCCM, which occurs frequently in adults, acquired causes for NCCM may be involved. In adults with a normal left ventricular (LV) systolic function, genetics helps to predict cardiac events. MYH7, MYBPC3, and TTN mutations were the most common in genetic NCCM. Patients with MYH7 mutations had low risk for cardiac events. Distinguishing genetic from non-genetic NCCM complements prediction of outcome and allows tailoring management to genetic status. MACE: major adverse cardiac events; RV: right ventricular. The figure was adapted from van Waning JI, et al. Genetics, Clinical Features, and Long-Term Outcome of Noncompaction Cardiomyopathy. J Am Coll Cardiol. 2018;71(7):711-22.

*Sarcomere genes*

*MYH7* gene mutations are the most prevalent genetic cause for NCCM are associated with a relatively milder course of disease with low risk for complications, compared to other genetic causes (figure 8)(8). The prognosis for patients with mutations in the head of the *MYH7* gene was better than for patients with mutations in the tail of the gene (111). Mutations in the head of *MYH7* were associated with NCCM with normal dimensions of the LV and a milder course of the disease (figure 9). Mutations in the tail had high incidence of LV dilatation and LV systolic dysfunction. An explanation for the association between mutations in the tail and the dilated subtype could be that tail mutations may infer with the binding site for *TTN*, and thus may have a similar effect as the effect of *TTN* mutations. Similar to NCCM patients with a *TTN* mutation, the mutations in the tail of *MYH7* were associated with DCM without hypertrabeculation in relatives. Another feature of the *MYH7* was that this is one of the rare sarcomere genes that was observed in families where Ebstein anomaly occurred in NCCM patients (23). The *TTN* gene, which is a major cause of DCM, is also a frequent cause of NCCM, predominantly in adult NCCM patients (figure 8) (8). This could indicate that younger *TTN* carriers are not symptomatic, which may be important information for relatives (children) who are carriers of a *TTN* mutation. The adult NCCM patients with *TTN* mutations had high prevalence of LV systolic dysfunction and LV dilatation, similar to DCM patients with *TTN* mutations (111). *MYBPC3* (compound) homozygous mutations were observed in NCCM cases with a severe phenotype and major cardiac events at young age (66). NCCM patients with a single *MYBPC3* mutation had high prevalence of RV systolic dysfunction and an increased risk for LV hypertrophy (HCM) (8). In the families of NCCM patients with a *MYBPC3* mutation, HCM without signs of hypertrabeculation may occur in relatives (111).

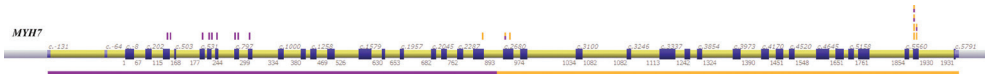


**Figure 9: *MYH7* mutations in NCCM.**
Head, purple, families with isolated NCCM. Tail, yellow, families with NCCM with DCM. Mixed purple and yellow mark, families with both isolated NCCM and NCCM with DCM. Mutations in the head, isolated NCCM phenotype vs in the tail, NCCM with DCM ($p<0.001$). Figure adapted from Alamut® visual -interactive biosoftware, 12-2016. The figure was adapted from Waning JI et al. Cardiac Phenotypes, Genetics, and Risks in Familial Noncompaction Cardiomyopathy, J Am Coll Cardiol. 2019 Apr 9;73(13):1601-1611.

*Other cardiomyopathy genes*

Mutations in *HCN4* were associated with NCCM with bradycardia (135). Mutations in *RYR2* lead to catecholaminergic polymorphic ventricular tachycardia (CPVT) and may also cause NCCM, especially variants in exon 3 (136). *LMNA* and *RBM20* are rare causes for NCCM and may be associated with worse outcome, like in DCM (10). *SCN5A* was reported to be a genetic modifier, increasing the risk for arrhythmias in NCCM (78).

*Future directions of genetic diagnosis and family screening for NCCM*

The application of whole exome or genome sequencing of NCCM patients in the near future will reveal novel genetic causes or genetic interactions with modifiers, some of which may explain cardiac remodeling into different cardiomyopathy phenotypes within families. A disease model may be developed to obtain functional evidence of the deleterious effect of genetic variants for better understanding and a more accurate classification of the DNA variants in cardiomyopathy genes, especially for the variants that are now regarded as variants of unknown significance. The expected broad application in the general population of predictive DNA testing for genetic susceptibilities for a large range of disease, may achieve a change in attitude towards and the perception of having a genetic susceptibility. Because it is clear that all of us are carrying genetic defects for one disease or another. This awareness hopefully leads to ban the discriminatory socio economic sanctions experienced currently when revealing personal genetic burden.

Prospective large follow up studies are needed to confirm the genotype-phenotype correlations in NCCM, and adjust guidelines for clinical follow up of patients and at risk relatives accordingly. Leading eventually to family- and gene tailored follow-up and management of NCCM patients and their families. The risk for cardiomyopathy for relatives of sporadic NCCM patients seems low, also risk for mutation carriers without a phenotype seems low. Follow-up studies of these relatives at low risk are needed to establish guidelines for the follow-up screening of these groups and to confirm the low risk. When al NCCM genes are known, excluding a genetic predisposition in a proportion of patients may be achieved, thus allowing making the important distinction between genetic and non-genetic NCCM, and design follow-up strategies according to genetic burden. In the distant future genetic classification may lead to the development of genotype specific treatment and eventually gene therapy for NCCM.

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# Chapter 2

## Genetics, clinical features and long-term outcome of noncompaction cardiomyopathy: A Dutch multicenter study

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*Genetics, Clinical Features, and Long-Term Outcome of Noncompaction Cardiomyopathy, J Am Coll Cardiol. 2018 Feb;71(7):711-722*

**Abstract**

Children were more likely to have a mutation, whereas the majority of the patients diagnosed in adulthood were 'sporadic' cases without a mutation or familial disease. In carriers of mutations risk of major adverse cardiac events was related to left ventricular systolic dysfunction. In contrast, in sporadic cases left ventricular function did not determine risk of complications. Patients with the most prevalent genetic cause, *MYH7* mutations, had low risk for adverse events. These findings highlight the heterogeneity of hypertrabiculation and show that genetic testing and taking family histories may have important implications for the management and prediction of adverse events for patients and their relatives.

## Introduction

Noncompaction cardiomyopathy (NCCM), also known as left ventricular noncompaction (LVNC), is a cardiomyopathy with excessive trabeculations of predominantly the left ventricle with a more than twofold thickening of the endocardial noncompacted (NC) layer compared to the epicardial compacted (C) layer of the myocardium(NC/C>2)(2, 21, 137)Initially referred to as 'spongy' heart, NCCM has gained attention with the improvements in cardiac imaging allowing more detailed visualization and increasing clinical awareness(138, 139). Clinical symptoms range from severe prenatal manifestations to asymptomatic cardiomyopathy presenting at adult age(4, 5).

Genetics play an important role in NCCM since 17-50%(140, 141) of the patients have a family member with a cardiomyopathy and the yield of DNA testing ranges from 17 to 41% depending on patient selection and the number of genes screened(7, 54). In most families, an autosomal dominant pattern of inheritance is observed with variable penetrance(142). The majority of the genetic defects associated with NCCM has also been reported in hypertrophic (HCM) and dilated cardiomyopathy (DCM)(19, 54). In NCCM, mutations in the sarcomere genes, particular in *MYH7,* are the most common (7, 54). However, the role of the sarcomere gene defects in the development of the cardiac hypertrabeculation has not been established yet. The diagnosis of NCCM requires genetic counseling because timely screening and diagnosis of at risk relatives is important. Finding a mutation allows to distinguish accurately the relatives at risk for NCCM. As NCCM is not as common as HCM and DCM, associations between mutations in cardiomyopathy genes, family history and the age of onset, left ventricular (LV) systolic dysfunction and long term outcome have not been investigated in detail before.

We conducted a large multicenter study in four cardiogenetic centers in the Netherlands to investigate the role of genetics in NCCM. The focus of the study was to investigate the relationship between clinical and cardiologic features at diagnosis, the risk of LV systolic dysfunction and occurrence of major adverse cardiac events (MACE) during follow-up in NCCM patients diagnosed in childhood or adulthood. These insights may help to improve management of NCCM patients and their families.

## Methods

### *Study Population*

The retrospective study consisted of 327 unrelated NCCM patients referred to one of the participating departments of Clinical Genetics for genetic counseling and DNA testing (with informed consent) between January 2005 and January 2016. Sixteen patients who did not have DNA testing were excluded (see online methods). Diagnosis of NCCM was based on consensus of re-evaluated echocardiographic and MRI images according to the Jenni and Petersen criteria by JVW and a dedicated participating cardiologist(21, 139). Echocardiographic and MRI data were available for all patients except for 34 with only MRI and 80 with only echocardiographic data. One patient was diagnosed at autopsy.

### *Genetics*

Specifics on the genes tested and methods of classification of variants are described in detail in the online methods. The core-panel of tested cardiomyopathy genes included 45 cardiomyopathy genes. All variants were evaluated according to the current Dutch guidelines and classification of each variant was achieved with consensus of the participating molecular geneticists. Patients were classified as genetic if they had a (likely) pathogenic mutation (online table 1a, 2a, 1b and 2b). Variants of unknown clinical significance (VUS) were reported in online table 3. Patients with only a VUS were not classified as genetic because these variants have not been proven to be pathogenic. Patients were classified as probably genetic if they had a family history of cardiomyopathy and DNA testing did not identify a mutation. Patients were classified as sporadic if patients had no mutation or family history of cardiomyopathy (figure 1).

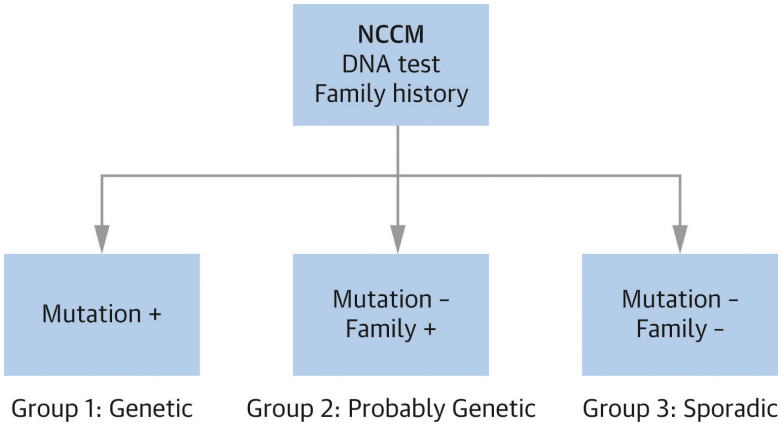


**Figure 1: Noncompaction cardiomyopathy study population.**

*Clinical data*
Clinical data were retrieved retrospectively from the medical records, including age, gender, cardiac diagnosis, ECG, echocardiography and cardiac magnetic resonance imaging (MRI) when available. In case the images of the first echocardiographic examination were unavailable, more recent echocardiographic imaging was used.

*Ventricular function*
LV systolic dysfunction was defined as LV ejection fraction < 45% on MRI or fractional shortening < 19% in men and < 21% in women on echocardiography if MRI images (n=80) were missing(143). Abnormal right ventricular (RV) systolic function was defined as a tricuspid annular plane systolic excursion (TAPSE) <17 mm on echo or RV ejection fraction <45% on MRI(144). For children dimensions of the ventricles of more than two standard deviations from reference range were classified as abnormal.(145, 146).

*Adverse events*
Information on the occurrence of clinical events at follow-up was collected from the medical records. Heart failure requiring hospitalization was defined as new-onset or worsening signs of heart failure requiring therapy and hospitalization. Sustained VT/VF was identified when at least 30 seconds of hemodynamically stable VT or hemodynamically unstable VT/VF of any duration was documented by electrocardiograms, pacemaker or defibrillator data. We used a combined end-point for our hazard models, because of low incidence of death. The occurrence of cardiac death, implantation of a left ventricular assistance device (LVAD), heart transplantation, (aborted) sudden cardiac death, appropriate ICD shock, or ischemic stroke were classified as major adverse cardiac events (MACE). Seventeen patients were lost to follow-up. Information on vital status was confirmed by checking municipal registries for all patients.

*Family history*
Family histories were ascertained at the departments of Clinical Genetics. Patients were classified as familial, if at least one first-degree or two second- degree relatives were reported with a cardiomyopathy. None of the patients had only one affected second degree relative. Medical records confirmed the diagnosis of 82% of the relatives reported to have a cardiomyopathy; in 18% of the affected relatives, no medical records were available. The occurrence of (aborted) sudden cardiac death (SCD) at age <50 years in the family of the index patients was recorded. In families with multiple cases of NCCM, only one case per family, the first diagnosed was included.

*Statistical analysis*

Categorical data were compared with Pearson Chi-Squared test or Fisher's exact test. For continuous variables, unpaired t-tests were used for two groups and ANOVA tests for more than two groups. Logistic regression was used to find associations between genetic status and left ventricular dysfunction at baseline. Kaplan–Meier survival curves were estimated and differences between groups were assessed by the log-rank test, using time at diagnosis as time zero. Risk factors for MACE, was calculated by Cox proportional hazards regression analysis. Patients lost to follow-up were considered at risk until the date of last contact, at which time-point they were censored. Analysis was performed with SPSS statistical software, version 21.0(SPSS Inc., Chicago, IL).

## Results

*NCCM genetics*

The 327 NCCM patients were categorized into the three groups; 104 genetic (32%; 81 adults, 23 children), 53 probably genetic (16%; 45 adults, 8 children) and 164 sporadic (52%; 149 adults, 21 children; table 1, figure 2). The complete list of mutations in children and adult NCCM patients with reference to previous reported variants, the reported cardiomyopathy (NCCM, HCM or DCM) and the yield per tested gene are presented in the online tables 1a, 2a and figure 1) In addition, 192 VUS in cardiomyopathy genes were identified in 111 patients (Online table 3); thirteen (12%) patients with a mutation had an additional VUS. Of the patients without a mutation 98 (30%) had a VUS. Forty-one percent (n=71) of the sporadic cases had a VUS, compared to 63% of the probably genetic cases (n=27, p=0.013).

From the 104 genetic cases, 82% involved a sarcomere gene (figure 2), the majority (71%) had a mutation in *MYH7*, *MYBPC3* or *TTN* and 11% in *ACTC1, ACTN2, MYL2, TNNC1, TNNT2* or *TPM1.* Mutations were more frequent in children (44%) than in adults (30%; p=0.036)(table 1). *MYH7* was the most frequently mutated gene; in 19% (n=10) of children and 11% (n=29) of adults. *TTN* occurred frequently in adults (7%, n=18) not in children. Non-sarcomere gene mutations were detected in *DES, DSP, FKTN, HCN4, KCNQ1, LAMP2, LMNA, MIB1, NOTCH1, PLN, RYR2, SCN5A* and *TAZ.* One patient classified as genetic had a 1p36 deletion. Mutations occurred more frequently in female patients irrespective of age at diagnosis (male 27% vs female 38%, p=0,039). The yield per tested gene was highest for *MYH7* (13%), *TTN* (11%) and *MYBPC3* (5%).

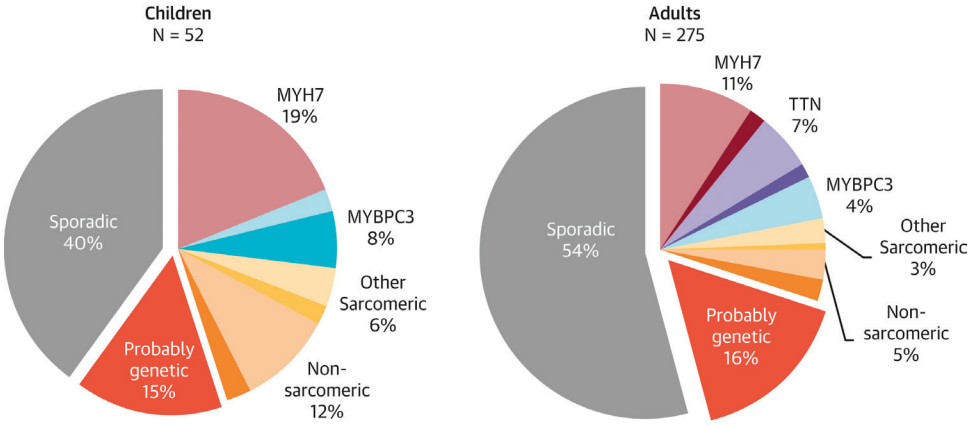


**Figure 2: Genetics of noncompaction cardiomyopathy.**
Darker shades indicate complex genotypes. DNA testing of approximately 45 cardiomyopathy genes and family histories showed that children were more likely to have a mutation (p=0.036). TTN occurred only in adult cases. Complex MYBPC3 genotypes occurred only in children (dark green)

*Complex genotypes*

Complex genotypes (multiple mutations in one patient) in cardiomyopathy genes were more prevalent in children (10%) than in adults (3%) (p=0.038; table1, online Tables 1b and 2b). Three children had complex *MYBPC3* mutations presenting with severe clinical phenotypes. Complex *MYBPC3* genotypes were not observed in adults. Four adult patients had the same two *MYH7* mutation in cis (c.1633G>A and c.2863G>A), that were not considered as complex genotypes, and are expected to have a common ancestor. Three adult patients had the combination of a *MIB1* and a *TTN* mutation. One patient had three pathogenic mutations in three different genes; *FKTN*, *RBM20* and *HCN4*. This patient was 53 years old when he was first admitted to the hospital for bradycardia. He had no structural heart defect and did experience serious adverse effects at end of follow-up (58 years).

**Table 1: Baseline characteristics and clinical features at diagnosis of 327 noncompaction cardiomyopathy (NCCM) patients.**

| | Children n=52* | | | | Adults n=275 | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Mutation n=23 (45%) | No mutation n=29 (55%) | | | Mutation n=81 (30%) | No mutation n=194 (70%) | | | |
| | | Familial n=8 (15%) | Not familial n=21 (40%) | | | Familial n=45 (16%) | Not familial n=149 (54%) | | |
| | Genetic | Probably genetic | Sporadic | Total | Genetic | Probably genetic | Sporadic | Total | p value |
| **Male** | 8 (35%) | 4 (50%) | 15 (71%) | 27 (52%) | 39 (48%) | 39 (62%) | 80 (54%) | 148 (54%) | NS |
| **Median age yrs (IQR)** | 5 (0-14) | 5 (0-13) | 8 (1-15) | 7 (0-14) | 41 (31-54) | 45 (34-57) | 47 (35-57) | 45 (33-56) | NS |
| Genetics | | | | | | | | | |
| Complex genotype | 5 (22%) | | | 5 (10%) | 9 (11%) | | | 9 (3%) | 0.038† |
| Familial cardiomyopathy | 13 (57%) | 8 (100%) | | 21 (40%) | 54 (67%) | 45 (100%) | | 99 (36%) | <0.001‡ |
| SCD before age 50 years in family | 1 (4%) | 1 (13%) | | 2 (4%) | 7 (9%) | 1 (2%) | 13 (9%) | 21 (8%) | NS |
| Sanger, no NGS cardiopanel | 15 (65%) | 3 (38%) | 3 (14%) | 21 (40%) | 36 (40%) | 16(40%) | 45 (31%) | 97 (35%) | NS |
| **Co-morbidity** | | | | | 19 (23%) | 16 (35%) | 49 (33%) | 84 (31%) | NS |
| Coronary artery disease | | | | | 1 (1%) | 2 (4%) | 9 (6%) | 12 (4%) | NS |
| Hypertension | | | | | 12 (15%) | 12 (27%) | 38 (26%) | 62 (23%) | 0.047‡ |
| Diabetes | 1 (4%) | | | 1 (2%) | 3 (4%) | 3 (7%) | 6 (4%) | 12 (4%) | NS |
| Hypercholesterolemia | | | | | 5 (6%) | 5 (11%) | 11 (7%) | 21 (8%) | NS |
| COPD | | | | | 6 (7%) | 5 (11%) | 9 (6%) | 20 (7%) | NS |
| **CHD** | 5 (22%) | 2 (25%) | 7 (33%) | 14 (27%) | 2 (3%) | 3 (7%) | 9 (6%) | 14 (5%) | NS |
| ASD | 2 (9%) | | 4 (19%) | 6 (12%) | | 1 (2%) | 5 (3%) | 6 (2%) | NS |
| VSD | 2 (9%) | 2 (25%) | 4 (19%) | 8 (15%) | | | 3 (2%) | 3 (1%) | NS |
| Ebstein anomaly | 2 (9%) | | | 2 (4%) | 2 (3%) | | | 2 (1%) | NS |
| BAV | 1 (4%) | | | 1 (2%) | | 2 (4%) | 2 (1%) | 4 (2%) | NS |
| CoA | 1 (4%) | | 1 (5%) | 2 (4%) | | | 1 (1%) | 1 (0%) | NS |
| **LBBB** | 1 (6%) | | | 1 (2%) | 7 (10%) | 4 (11%) | 40 (27%) | 51 (21%) | 0.010§ |
| **LV systolic dysfunction** | 16 (70%) | 4 (50%) | 2 (11%) 1 (5%) | 22 (42%) | 49 (60%) | 23 (51%) | 80 (54%) | 152 (58%) | 0.001# |
| **RV systolic dysfunction** | 4 (17%) | | | 5 (10%) | 17 (21%) | 7 (16%) | 15 (10%) | 39 (22%) | 0.029‡ |
| **LV and RV systolic dysfunction** | 4 (17%) | | | 4 (8%) | 16 (20%) | 5 (11%) | 11 (7%) | 32 (12%) | 0.020‡ |

*diagnosis before the age of 18 years, †significant for children compared to adults ‡significant for adults with mutation compared to adults without a mutation §significant for (probably) genetic compared to sporadic adults #Significant for children with mutation compared to children without a mutation
ASD: atrial septal defect, BAV: bicuspid aortic valve, CHD: Congenital heart disease, CoA: coactation of the aorta, LV: left ventricular, NGS: Next-generation sequencing, RV: right ventricular, SCD: sudden cardiac death, VSD: ventricular septal defect.

*De novo mutations*

*De novo* mutations involving *DES* and *PLN* were observed in two children and *PRDM16* and *MYH7* in two adult patients. The children had a severe heart failure and had a heart transplantation at young age (the *DES* patient at the age of 10 years, the *PLN* patient at age 17 years). In contrast, the adult patients with a *de novo* mutation had a mild course of the disease without severe complications.

*Family history*

Forty percent (n=21) of the children had a family history of cardiomyopathy and 36% (n=99) of the adults (table 1). Of the 120 familial cases, 56% (n=67) had a (likely) pathogenic mutation. Of the 207 patients without a family history of cardiomyopathy 18% (n=37) had a mutation (p<0.001). Overall, NCCM occurred in 23% (n=76) of the affected relatives in 12% (n=39) of the families DCM was reported, in 3% (n=11) HCM, and in one family a relative was diagnosed with Arrhythmogenic Cardiomyopathy. Sudden death (SD) below the age of 50 of a relative was reported by 7% (n=23) of the patients; in 8% (n=21) of the families of adult index cases and 4% (n=2) of the families of pediatric index cases.

*Age at diagnosis*

The study population included 16% (n=52) pediatric patients diagnosed before the age of 18 years, and 84% (n=275) adult patients (Figure 3) the median age at diagnosis of NCCM was 41 years (range 0-79 year, IQR 27-54 years), 44 years (range 18-79 IQR 33-56) for adult patients and 8 years (range 0-17 IQR 0-14) for children (table 1). Thirty percent (n=16) of the children were diagnosed before the age of 1 year. Age at diagnosis was inversely associated with the probability of finding a mutation (OR 0.983 per year; CI 95% 0.97-0.99; p=0.01).

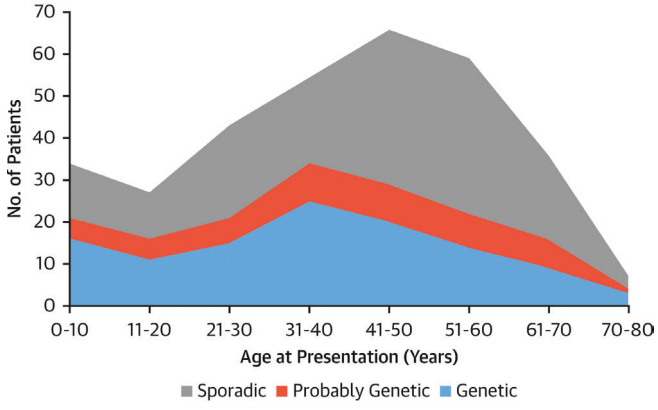


**Figure 3: Age at diagnosis in 327 Dutch noncompaction cardiomyopathy cases.**
Chance of having a mutation decreased with age (OR 0.983 per year; CI 95% 0.97-0.99; p=0.01).

*Congenital heart defect (CHD)*
CHD was observed in 9% of the patients (n=28). In particular children (p=0.027; table 1) had more ASD (p=0.005) and VSD (p<0.001). Six of the 16 patients diagnosed before the age of one year had a congenital heart defect. Two children and two adults) had Ebstein anomaly and an *MYH7* mutation (figure 4). Familial segregation of NCCM and Ebstein anomaly was observed in one family. *MYH7* was the only sarcomere gene associated with CHD. Two children with a CHD had a chromosomal defect: one with a 1p36 deletion had an ASD, multiple VSD's and an open ductus arteriosus and a trisomy 21 patient with a *MYH7* mutation had an ASD and a VSD. The other congenital heart defects were observed in patients with defects in non-sarcomere genes, in probably genetic and in sporadic cases.

*Clinical features*
Among children 83% and 85% of the adults were symptomatic at presentation. Heart failure (27%) and arrhythmias (26%) were the most common presentations in children and adults (online figure 2). In 4% (n=14) the primary presentation was a cardiac arrest. Thrombo-embolic events were the first sign of NCCM in 3% (n=10). Three patients had a CHD. Seven patients presenting with a thrombo-embolic event had a stroke, two a kidney infarction and one a mesenteric occlusion. Thirty patients, of which 16 had a mutation, were identified through family cardiologic family screening for i.e. HCM, DCM, SCD, familial hypertension, or hemochromatosis. Cardiologic screening for other reasons g identified 42 patients, of which 12 had a mutation. , Thirty-four percent (n=24) of these 42 patients were asymptomatic, seven asymptomatic cases had a mutation.

Hypertension occurred frequently in adults (n=62, 23%), in particular adults without a mutation (p=0.047, table 1). Also a left bundle branch block (LBBB) was significantly more common in sporadic adult patients (27%) compared to the adult genetic (10%) and probably genetic cases (11%, p=0.01; table 1). Information on echocardiographic, ECG and CMR parameters of the NCCM patients is presented in online table 4.

**Table 2: Clinical features at long-term follow-up of pediatric and adult patients with noncompaction cardiomyopathy (NCCM).**

| | Children n=52 | | | | Adults n=275 | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Mutation n=23 (45%) | No mutation n=29 (55%) | | | Mutation n=81 (30%) | No mutation n=194 (70%) | | | |
| | | Familial n=8 (15%) | Not familial n=21 (40%) | | | Familial n=45 (16%) | Not familial n=149 (54%) | | |
| | Genetic | Probably genetic | Sporadic | Total | Genetic | Probably genetic | Sporadic | Total | p value |
| **Median follow up (IQR), months** | 81 (13-114) | 24 (1-102) | 40 (19-120) | 60 (18-113) | 26 (6-69) | 29 (10-71) | 22 (4-49) | 25 (4-58) | |
| **Heart failure** | | | | | | | | | |
| Heart failure requiring hospitalization | 9 (39) | 3 (38) | 1 (5) | 13 (25) | 20 (25) | 9 (20) | 30 (20) | 59 (21) | |
| **Thrombo-embolic events** | | | | | | | | | |
| TIA | | | | | 2 (2) | 1 (2) | 5 (3) | 8 (3) | |
| Stroke | 1 (4) | | | 1 (2) | 1 (2) | 4 (9) | 12 (8) | 17 (6) | |
| Peripheral Thrombo-embolism | 1 (4) | | | 1 (2) | 4 (5) | 1 (2) | 6 (4) | 11 (4) | |
| **Arrhythmias** | | | | | | | | | |
| Atrial fibrillation | 3 (13) | 1 (13) | 1 (5) | 5 (10) | 15 (19) | 8 (18) | 21 (14) | 44 (16) | |
| Sustained VT/VF | 1 (4) | 2 (25) | 1 (5) | 3 (6) | 3 (4) | 2 (4) | 11 (7) | 16 (6) | |
| **ICD** | 5 (22) | 2 (25) | 2 (10) | 9 (17)* | 34 (42) | 15 (33) | 57 (38) | 106 (39) | 0.004* |
| Secondary prevention (% of ICD) | | 1 (50) | | 1 (11) | 3 (9) | 2 (13) | 5 (9) | 12 (4) | |
| Appropriate shock (% of ICD) | | | | | 1 (3) | 3 (20) | 6 (11) | 10 (9) | |
| **LVAD** | 1 (4) | | | 1 (2) | 2 (2) | | 1 (1) | 3 (1) | |
| **Heart transplant** | 4 (17) | | | 4 (8) | 2 (2) | 2 (4) | 2 (1) | 6 (2) | |
| **Death** | 4 (17) | 3 (38) | 1 (5) | 8 (16) | 3 (4) | 5 (11) | 8 (5) | 16 (6) | |
| **MACE in patients with LV systolic dysfunction** | 8 (35) | 3 (38) | 0 (0) | 11 (21) | 12 (15) | 6 (13) | 18 (12) | 36 (13) | |
| **MACE in patients with normal LV-function** | 1 (4) | 1 (13) | 1 (5) | 3 (6) | 1 (1) | 5 (11) | 16 (11) | 22 (8) | 0.027† |

* significant for children vs adults, †significant for mutation carriers vs no mutation, MACE marked in blue, ICD: implantable cardioverter defibrillator, LV: Left ventricular, LVAD: Left ventricular assist device, RV: right ventricular, TIA: Transient ischemic attack, VF: ventricular fibrillation, in blue, risk factors included in MACE.

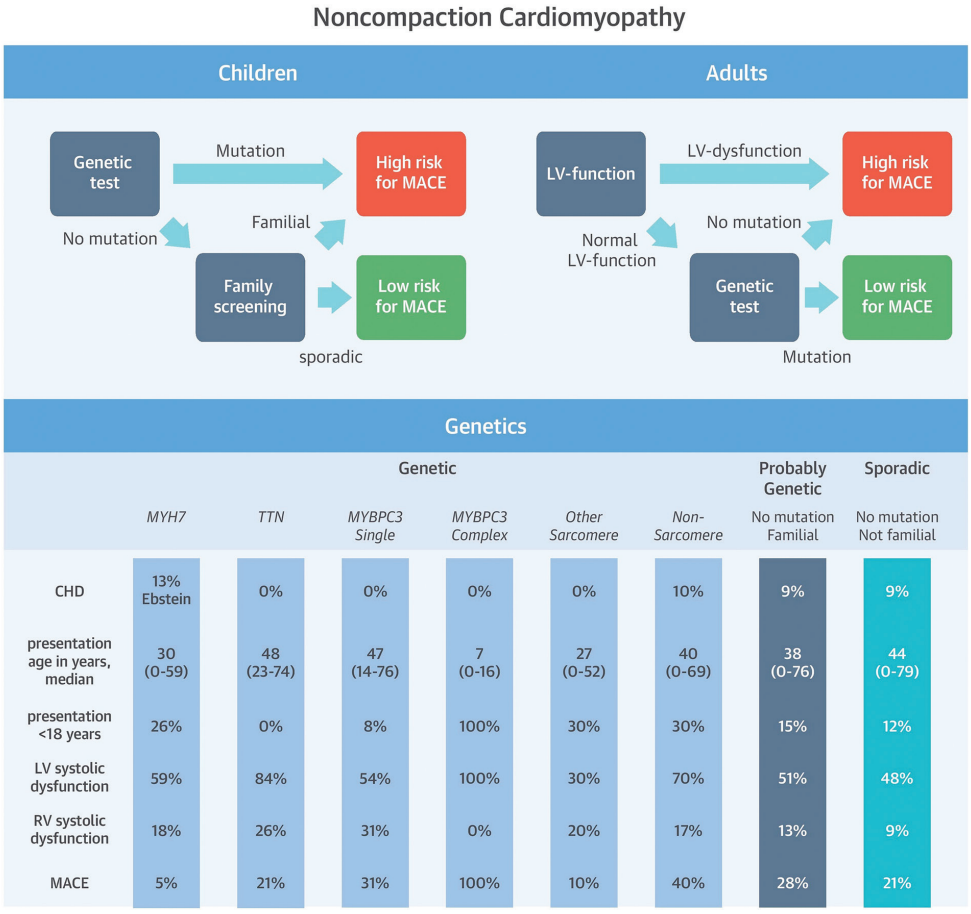


**Figure 4: Central illustration: Noncompaction cardiomyopathy.**
NCCM is a heterogeneous condition and genetic stratification has a role in clinical care. In children, genetic causes with severe outcome are more common than in adults. In non-genetic NCCM, occurring frequently in adults, acquired causes for NCCM may be involved. In adults with a normal LV systolic function, genetics helps to predict cardiac events. MYH7, MYBPC3 and TTN mutations were the most common in genetic NCCM. Patients with MYH7 mutations had low risk for cardiac events. Distinguishing genetic from non-genetic NCCM complements prediction of outcome and allows tailoring management to genetic status.

**Table 3: Risk for ventricular dysfunction in noncompaction cardiomyopathy.**

| | Reduced LV function OR (95% CI) | p value | Reduced RV function OR (95% CI) | p value |
|---|---|---|---|---|
| Genetic | 1.73 (1.07-2.78) | 0.024 | 2.55 (1.28-5.06) | 0.008 |
| *MYH7* | 1.30 (0.66-2.55) | 0.456 | 1.74 (0.66-4.53) | 0.258 |
| *MYBPC3 single* | 1.02 (0.34-3.10) | 0.855 | 5.33 (1.15-24.79) | 0.033 |
| *TTN* | 5.03 (1.44-17.61) | 0.012 | 2.19 (0.70-6.92) | 0.180 |
| Other sarcomere | 0.36 (0.10-1.43) | 0.148 | 1.51 (0.28-8.03) | 0.632 |
| Non-sarcomere | 2.10 (0.84-5.24) | 0.113 | 1.53 (0.46-5.13) | 0.491 |
| Complex genetic defect | 8.24 (1.03-65.78) | 0.047 | 2.54 (0.41-15.69) | 0.316 |
| Probably genetic | 0.89 (0.49-1.60) | 0.698 | 0.87 (0.36-2.16) | 0.770 |
| Sporadic | 0.67 (0.43-1.03) | 0.069 | 0.45 (0.23-0.90) | 0.024 |
| LBBB | 6.04 (2.73-13.38) | <0.001 | 0.77 (0.27-2.16) | 0.620 |
| Presentation under 1 year | 1.48 (0.53-4.18) | 0.456 | 0.74 (0.08-6.46) | 0.781 |

CI: confidence interval, LBBB: Left bundle branch block, LV: left ventricular, OR: odds ratio, RV: right ventricular.



*Reduced ventricular systolic function*

The risk of having a LV systolic dysfunction was higher for genetic patients compared to the probably genetic and sporadic cases (p=0.024), with highest risk for patients with multiple mutations and *TTN* mutations (table 3 and in figure 4). LV systolic dysfunction occurred in 44% of the children and 58% of the adult patients (p=0.067).LV dysfunction was observed more often than dysfunction of the right ventricle (RV 14% and LV 53 %). The RV function was measured in 31 of the 52 pediatric patients (12 genetic, 5 probably genetic, and 14 sporadic) at admission, detecting reduced RV function in four genetic cases (with a *DES de novo, PLN de novo*, homozygous *MYL2*, and *MYH7* defect) with a LV systolic dysfunction and in one sporadic patient with a normal LV function. The risk for having a reduced right ventricular function was increased for genetic patients (p=0.008).

*Major adverse cardiac events ( MACE)*

During median follow-up (FU) of 60 months (IQR 18-113), MACE occurred in 14 (27%) children and 58 ( 21%) of the adults during a median FU of 25 months (IQR 4-58 months; table 2). An increased risk for MACE was observed in children with (probably) genetic NCCM (p=0.025; figure 5A), children diagnosed before the age of 1 year (HR 2.1; 95% CI 1.0-4.4; p=0.048; table 4) and children with multiple mutations in *MYBPC3* (HR 5.2; 95% CI 1.62-16.5; p=0.006) and risk for MACE in

children was associated with LV systolic dysfunction (HR 7.7; 95% CI 1.70-34.7; p=0.008). The risk for adverse events in sporadic children was low (HR 0.1; 95% CI 0.02-0.93; p=0.043). No difference in risk for MACE was observed between adults with genetic, probably genetic and sporadic NCCM (Figure 5B).

LV systolic dysfunction was associated with increased risk for MACE (HR 1.7; 95% CI 1.1-2.8; p=0.028). In line with these observations, genetic patients with a good left ventricular function had low risk of adverse cardiac events (p=0.002; figure 5C). For patients with an *MYH7* mutation, low risk for MACE was observed (HR 0.17; 95% CI 0.04-0.69; p=0.013; table 4 and figure 4). The reduced risk for MACE in patients with a MYH7 mutation remained after correction for LV systolic function. In sporadic patients risk for MACE was not related to LV function (figure 5D); patients without a mutation and a normal LV-function had similar risk of MACE as patients with LV systolic dysfunction.

Cardiac arrest occurred significantly more in female patients than in male patients (11%, vs 2%, p=0,003). More adult females had an ischemic stroke than males (9%, vs 3%, p=0,045). Patients with congenital heart defects were more often associated with stroke (with 56% vs without CHD 5% p=0,001).

**Table 4: Risk for major adverse cardiac events in noncompaction cardiomyopathy.**

| | All patients HR (95% CI)† | p value |
|---|---|---|
| Genetic | 0.83 (0.50-1.37) | 0.459 |
| MYH7 | 0.17 (0.04-0.69) | 0.013 |
| MYBPC3 single | 1.44 (0.52-3.94) | 0.482 |
| MYBPC3 complex | 5.17 (1.62-16.48) | 0.006 |
| TTN | 1.02 (0.37-2.79) | 0.973 |
| Other sarcomere | 0.41 (0.06-2.94) | 0.374 |
| Non-sarcomere | 1.60 (0.77-3.34) | 0.211 |
| Complex genetic defect | 2.11 (0.85-5.24) | 0.108 |
| Probably genetic | 1.29 (0.73-2.29) | 0.375 |
| Sporadic | 1.01 (0.63-1.61) | 0.971 |
| LBBB | 1.20 (0.65-2.22) | 0.563 |
| Reduced LV | 1.72 (1.06-2.80) | 0.028 |
| Reduced RV | 1.73 (0.94-3.20) | 0.080 |
| Presentation under 1 year* | 2.11 (1.01-4.41) | 0.048 |

*Presentation under 1 year HR in children, †HR (95% CI) for Composite Endpoint, CI: confidence interval, HR: hazard ratio, LBBB: Left bundle branch block, LV: left ventricular, RV: right ventricular.

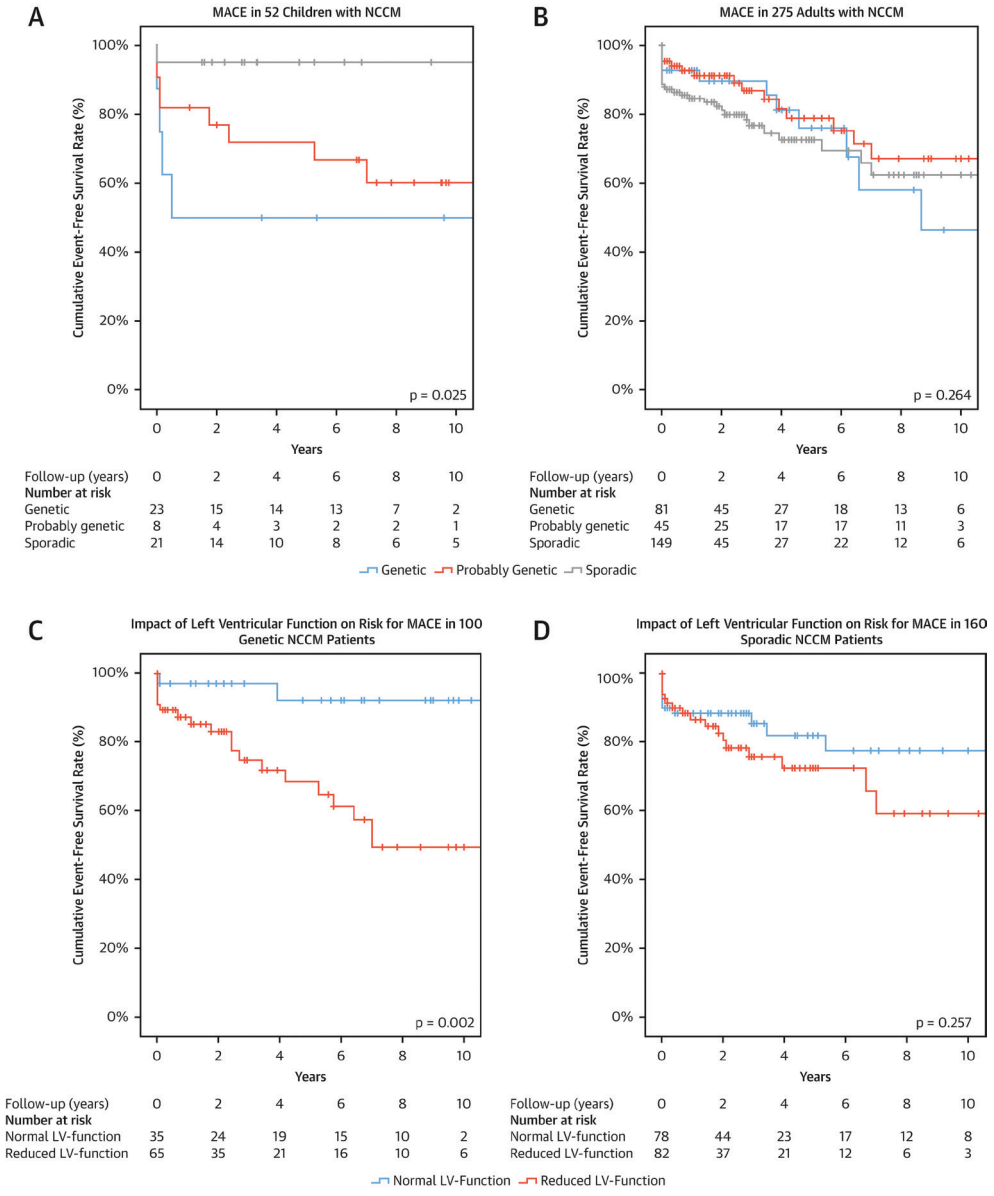


**Figure 5: Risk for major adverse cardiac events (MACE) in NCCM.**
Kaplan Meier curves of MACE; (A) genetics and freedom from MACE in children. (B) genetics and freedom from MACE in adults. (C) left ventricular systolic dysfunction and freedom from MACE in genetic NCCM. (D) left ventricular systolic dysfunction and freedom from MACE in sporadic NCCM.

## Discussion

In this large cohort of NCCM patients we investigated the correlations between genetics, clinical presentation and the long-term outcome. We showed that nearly one third of the NCCM patients had a mutation in a cardiomyopathy gene. In this heterogeneous cardiomyopathy, age at diagnosis, left ventricle systolic dysfunction and risk for MACE were linked to genetic status. Children diagnosed with NCCM had more often a genetic cause than adults. Left ventricular systolic dysfunction at presentation and long-term outcome were related to genetics.

It is important to distinguishing genetic NCCM because genetic status may add to prediction of risk for major adverse cardiac events and may guide clinical management and intensity of follow-up for specific groups of patients and their relatives. Children with a mutation were diagnosed frequently before the age of 1 year, and had cardiac symptoms, LV systolic dysfunction, and a high risk for MACE. In contrast, children with sporadic NCCM were diagnosed incidentally, had normal cardiac function and low risk for MACE. In adults with a mutation high risk for MACE was strongly correlated to left ventricular systolic dysfunction. However, risk of MACE in sporadic adults was not determined by LV systolic dysfunction.

In approximately half (48%) of the NCCM patients genetics played a role; 32% of the patients had a mutation and 16% of the patients had familial disease without a mutation. Sporadic NCCM was more prevalent in adults than in children. These results suggest that apart from genetic causes for NCCM, acquired (non-genetic) causes for hypertrabiculation may play a role, particularly in the sporadic adult cases(11). In addition our results endorse the heterogeneity of NCCM and the importance of genetics, simultaneously evoking questions on different etiologies for hypertrabicularisation in children and adults.

Three of the 22 different cardiomyopathy genes were not reported previously in NCCM, expanding the genetic spectrum of NCCM with the *DES, PLN* and *RBM20* genes. In the genetic cases mutations in *MYH7*, *MYBPC3*and *TTN* were the most frequent. *MYH7* was the most affected gene, as described previously(7,10). The risk for MACE was lower in *MYH7* patients (5%). *MYH7* was the only sarcomere gene associated with congenital heart defects, four patients with *MYH7* mutation had Ebstein anomaly(23). Compound heterozygosity of *MYBPC3* was associated with severe early onset NCCM. In three patients TTN mutations co-occurred with MIB1 mutations, endorsing the hypothesis that a TTN-mutation may not be a sufficient genetic cause for NCCM(147). *TTN* defects were not observed in pediatric cases.

High prevalence of *TTN* mutations in women with a peripartum cardiomyopathy and in chemotherapy induced cardiomyopathy(148-150), also suggests involvement of co-factors accumulated during life in the development of *TTN* associated cardiomyopathies.

Acquired causes for NCCM are expected in a large proportion of patients, the sporadic adults. The role of acquired causes in late onset NCCM challenge the general assumption of the embryological nature of hypertrabeculation. If NCCM is a developmental disorder, a higher rate of diagnosis would be expected shortly after birth or in childhood than the observed 16% of childhood cases in our cohort. Late onset NCCM may be explained by an enhancement of a latent asymptomatic congenital defect by disruptions of the cardiac homeostasis later in life. Recent studies endorse the hypothesis that acquired causes may lead to characteristic hypertrabeculation. Conditions with an increased cardiac preload which are associated with hypertrabeculation included sickle cell anemia, pregnancy and intensive sports(24-26)(61, 151, 152). Our observation that LBBB was more prevalent in adults with sporadic NCCM may be explained by a role of increased cardiac preload in the development of hypertrabeculation. LBBB leads to higher end-diastolic volumes, which leads to an increased cardiac preload(153). Hypertension may also lead to increased preload and may be another secondary cause, as it was also more frequent in sporadic patients(154).

One third of the mutations were found in patients who did not report relatives with a cardiomyopathy, i.e. when family history was negative. This illustrates that DNA testing should not be restricted to cases with a positive family history, and that DNA testing of patients without a family history is as important. DNA testing is important because when a mutation is found it allows families to have DNA testing and identify accurately which relatives have a mutation and an increased risk. In this way identifying the causative mutation facilitates genetic cascade screening. Also relatives who do not carry the mutation can be excluded from regular cardiac follow-up and can be reassured that there is no increased risk for their offspring.

The proportion of genetic patients is expected to be higher than 48% because in thirty percent of the patients without a mutation, Sanger sequencing of a small number of cardiomyopathy genes was performed and these may have a mutation in a cardiomyopathy gene that was not tested. In 71 (41%) of the sporadic cases had a VUS that may be reclassified in the future as 9likely pathogenic. Moreover, cardiomyopathy genes, that have not been identified yet, and may play a role in the familial cases without a mutation as well as in the sporadic cases.

*Study Limitations*

A referral bias of more severe cases cannot be excluded. Consequently, asymptomatic or mildly affected cases may be underrepresented leading to an overestimation of severe clinical features. In addition we might have introduced a selection bias of symptomatic NCCM by including only index cases (to control for overrepresentation of genetic causes). By doing so we might have missed asymptomatic cases that would only be recognized by family screening. We may have underestimated the role of genetic causes for NCCM because not all patients were tested for the complete genetic cardio-panel(~45 genes). Some patients had a rare variant of unknown significance, that are for now not classified as disease causing, but these may in the future be reclassified as likely pathogenic. On the other hand, some variants, although they were classified by current stringent criteria as (likely) pathogenic, could in the future turn out to be benign.

We used the current widely used diagnostic criteria for NCCM, despite the fact that they lack specificity and novel criteria, preferably using both morphologic and genetic data are needed. We cannot rule out for now to have included patients with benign hypertrabeculation as NCCM. Family screening and obtaining accurate diagnosis of relatives is more difficult in families of adult patients, this might be one of the causes of the high prevalence of sporadic NCCM in adults. In addition given the retrospective design clinical data may have been missing. We may have an underestimation of heart failure related events because we selected only heart failure requiring hospitalization.

**Conclusion**

NCCM is a heterogeneous condition and genetic stratification has a role in clinical care. Distinguishing genetic from non-genetic NCCM may complement prediction of outcome and subsequently guide management of patients with follow-up tailored to genetic status.

**Perspectives**

**Competency in medical knowledge:** NCCM is heterogeneous and genetics plays a more important role in children than in adults**.** Mutations and family history carry information that may complement cardiologic management and predict prognosis of NCCM, since high risk for cardiac events was related to left ventricular systolic dysfunction in mutation carriers, but not in sporadic cases. Hence, showing the

importance of genetic testing in NCCM in addition to cardiomyopathy family screening.

**Translational outlook:** Taking family histories, DNA testing and family screening are important for a correct genetic classification of NCCM. Prospective large follow up studies are needed confirm the genotype-phenotype correlations, determine the effects of risk related follow-up strategies and design gene tailored management of NCCM patients and their families.

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## Supplementary methods

*Patient selection*

The current study used retrospective cardiologic and DNA data of noncompaction cardiomyopathy patients referred from 2005 – 2016 for genetic counseling to the four participating Departments of Clinical Genetics. All consecutive NCCM patients who fulfilled Jenni or Petersen diagnostic criteria, were referred for genetic counseling and had a DNA test were selected. For this study the diagnosis of NCCM was reconfirmed by re-evaluation of the echocardiograms or/and MRI of all patients by JVW and a dedicated (pediatric) cardiologist specialized in cardiomyopathy of the participating cardiogenetic centers. Only index cases with a DNA test were included in this study to avoid overrepresentation of specific genetic defects, affected family members were excluded.

From the 394 patients selected for the study 67 were excluded (online figure 3).

- 16 because they did not have a DNA test.
- 7 because the echo and MRI images were missing.
- 44 (14 children and 30 adults) because the diagnosis of NCCM was rejected after careful re-examination of the echocardiograms and/or MRI by JVW and an experienced cardiologist.

*Genetic testing*

DNA was isolated from peripheral blood of the patients to analyze coding regions of the tested genes, with informed consent of the patients. From 2012 on, next generation sequencing of a targeted panel of 45 cardiomyopathy genes was gradually introduced in the participating clinical genetic centers. Currently, the national core panel of cardiomyopathy genes consists of at least the following genes: *ABCC9, ACTC1, ACTN2, ANKRD1, BAG3, CALR3, CRYAB, CSRP3, DES, DMD, DSC2, DSG2, DSP, EMD, GLA, JPH2, JUP, LAMA4, LAMP2, LMNA, MYBPC3, MYH6, MYH7, MYL2, MYL3, MYPN, MYOZ1, MYOZ2, PKP2, PLN, PRKAG2, RBM20, RYR2, SCN5A, SGCD, TAZ, TCAP, TMEM43, TNNC1, TNNI3, TNNT2, TPM1, TTN, VCL and LDB3.*

*Target enrichment and sequencing*

Targeted enrichment was performed according to manufacturer's instructions on different machines in the different medical centers. Information is available upon request.

*Variant Filtering and Classification*

Assessment of the pathogenic effect of genetic variants was performed with of Alamut Interactive Biosoftware (Rouen, France). This software incorporates

SpliceSiteFinder-like, MaxEntScan, NNSPLICE, GeneSplicer, and Human Splicing Finder for the prediction of splicing variants and the programs Align GVGD, SIFT, Polyphen-2 and Mutation Taster for *in silico* prediction of the effect of amino acid changes. Additionally, it gives population frequencies for dbSNP, ESP, ExAC and GoNL and shows whether or not a variant has been reported before in the Human Gene Mutation Database (HGMD). The classification system of variants was adapted from the sequence variation classification proposed by Plon et al. (113). The criteria for classification of variants included the allele frequency in the dbSNP/ESP/ExAC/GoNL (cutoff 0.01, in at least 300 ethnically matched control alleles), predicted effects on splicing, the *in silico* prediction of effect on the protein and previously described links to disease. For each variant present in HGMD the supporting evidence was reviewed and previous reports evaluated whether linking specific variants to cardiomyopathy were supported by co-segregation data, data on the identification of the variant in multiple independent cases, functional studies and/or expression assays. Additionally, a variant only predicted by *in silico* prediction to be pathogenic would not automatically be classified as such because of lack of additional evidence. This resulted in categorizing variants into five classes: pathogenic, likely pathogenic, unknown significance (VUS), likely benign, and benign (Richards et al. (55). All variants classified as pathogenic and probably pathogenic were reported in this paper. In addition online table 3 reports all the variants of unknown clinical significance observed in the study population. A single previous description of a variant in a patient was not considered as sufficient evidence for causation and these variants were classified as variants of unknown significance instead of likely pathogenic. Familial segregation of the variants with NCCM in families was examined when affected relatives were available and consented for DNA testing. All four participating centers reached consensus on the classification of all variants.



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**Online Table 1a: (Likely) pathogenic variants in 52 children with noncompaction cardiomyopathy (NCCM).**

| Gene | NM-Number GRCh37 (hg19) | Nucleotide substitute | Amino acid substitute | Families | CM in family | Complex* | CHD | Outcome | Reference | mutated gene/ patients analyzed |
|---|---|---|---|---|---|---|---|---|---|---|
| **ACTC1** | NM_005159.4 | c.811A>G | p.(Met271Val) | 1 | | | | | | 1/38 (3%) |
| **DES** | NM_001927.3 | c.1222C>G | p.(Leu408Val) | 1 | De novo | | | | | 1/33 (3%) |
| **LAMP2** | NM_002294.2 | c.966dup | p.(Ala323Cysfs*27) | 1 | | | | | | 1/30 (3%) |
| **KCNQ1** | NM_000218.2 | c.973G>A | p.(Gly325Arg) | 1 | | 5 | | | | |
| **MYBPC3** | NM_000256.3 | c.442G>A | p.(Gly148Arg) | 2 | HCM, NCCM | 1, 2 | | 1. AF, Death 2. LVAD, Htx, Stroke | DCM (1) | 4/43 (9%) |
| | | c.932C>A | p.(Ser311*) | 1 | HCM | 1 | | | HCM (2, 3) | |
| | | c.2373dupG | p.(Trp792Valfs*41) | 2 | HCM | 2, 3 | | 3. Death | HCM (4-8) | |
| | | c.2827C>T | p.(Arg943*) | 2 | HCM | 3 | | | HCM (5-9) | |
| **MYH7** | NM_000257.2 | c.495G>A | p.(Met165Ile) | 1 | NCCM | | | Htx | | 10/48 (21%) |
| | | c.689T>C | p.(Phe230Ser) | 1 | NCCM | | Ebstein anomaly | | | |
| | | c.728G>A | p.(Arg243His) | 1 | NCCM | | | | | |
| | | c.732+1G>A | p.(?) | 1 | NCCM | | | | NCCM (3, 10-12) | |
| | | c.798T>A | p.(Tyr266*) | 1 | NCCM | | | | | |
| | | c.976G>C | p.(Ala326Pro) | 1 | | | Trisomy 21, ASD, VSD | | HCM (13-15) | |
| | | c.1106G>A | p.(Arg369Gln) | 1 | | | | | NCCM (16-18), DCM (17) | |
| | | c.2713T>C | p.(Cys905Arg) | 1 | DCM | | | | NCCM (19) | |
| | | c.3113T>C | p.(Leu1038Pro) | 1 | NCCM | | Ebstein anomaly | | DCM (20) | |
| | | c.5773C>G | p.(Arg1925Gly) | 1 | NCCM | | | | | |
| **MYL2** | NM_000432.3 | c.403-1G>C | p.(?) | 1 | | 4 | | Death | HCM (21, 22) | 1/42 (3%) |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **NOTCH1** | NM_017617.3 | c.3787C>T | p.(Arg1263Cys) | 1 | DCM | | CoA, BAV | | | 1/2 (50%) |
| **PLN** | NM_002667.3 | c.25C>T | p.(Arg9Cys) | 1 | De novo, HCM | | | | DCM (23-27) | 1/40 (3%) |
| **SCN5A** | NM_198056.2 | c.3784G>A | p.(Gly1262Ser) | 1 | | 6 | | AF, kidney infarct | | 2/32 96%) |
| | | c.4978A>G | p.(Ile660Val) | 1 | | 5 | | SCD | | |
| **1p36** | | deletion | | 1 | | | ASD, VSD, PDA | | | |

* patient number in online table 1b, ASD: atrial septal defect, BAV: Bicuspid Aortic Valve, CHD: congenital heart disease, CoA: Coarctation of the aorta, DCM: dilated cardiomyopathy, HCM: hypertrophic cardiomyopathy, Htx: heart transplant, LVAD: left ventricular assistant device, NCCM: noncompaction cardiomyopathy, PDA: patent ductus arteriosus, SCD: sudden cardiac death, VSD: ventricular septal defect, WPW: Wolff-Parkinson-White syndrome.

**Online Table 2a: (Likely) pathogenic variants in 275 adult NCCM patients.**

| Gene | NM-Number GRCh37 (hg19) | Nucleotide substitute | Amino acid substitute | Families (+)† | CM in family | Complex[a] | CHD | Outcome | Reference | mutated gene/ patients analyzed |
|---|---|---|---|---|---|---|---|---|---|---|
| **ACTN2** | NM_001103.2 | c.574C>T | p.(Arg192*) | 1 | | 1 | | | | 3/184 (2%) |
| | | c.586delG | p.(Asp196fs*14) | 1 | HCM | | | | | |
| | | c.909G>A | p.(Trp303*) | 1 | NCCM | | | | | |
| **DES** | NM_001927.3 | c.1193T>C | p.(Leu398Pro) | 1 | | | | | | 1/205 (1%) |
| **DSP** | NM_004415.2 | c.6687delA | p.(Arg-2229Serfd*32) | 1 | NCCM, DCM | | | | | 2/178 (1%) |
| | | c.3084+1G>A | p.(?) | 1 | | | | ICD shock | | |
| **FKTN** | NM_001079802.1 | c.1112A>G | p.(Tyr371Cys) | 1 | | 2 | | | | 1/43 (2%) |
| **HCN4** | NM_005477.2 | c.1241C>G | p.(Ala414Gly) | 1 | NCCM | | | AF | NCCM (28) | 2/24 (8%) |
| | | c.1441T>C | p.(Tyr481His) | 1 | | 2 | | | NCCM (28) | |
| **LMNA** | NM_170707.3 | c.1608+5G>C | p.(?) | 1 | NCCM | | ASD | ICD shock | | 1/224 (0%) |
| **MIB1** | NM_020774.2 | c.1096_1097del | p.(Leu366fs) | 1 | | 8 | | | | 4/127(3%) |
| | | c.2589_2590dupTG | p.(Glu864fs*31) | 1 | DCM | 9 | | | | |
| | | c.2701C>T | p.(Arg901*) | 1 | | 7 | | | | |
| | | c.2827G>T | p.(Val943Phe) | 1 | | | | | NCCM (29) | |
| **MYBPC3** | NM_000256.3 | c.772G>A | p.(Glu258Lys) | 1 | | | | Sudden death | HCM | 12/254 (5%) |
| | | c.932C>A | p.(Ser311*) | 1 (+1) | DCM | | | | HCM (2, 3) | |
| | | c.1484G>A | p.(Arg495Gln) | 1 | | | | | HCM (4, 11, 30) | |
| | | c.1831G>A | p.(Glu611Lys) | 1 | | | | | DCM (31) HCM (32, 33) | |
| | | c.2373dupG | p.(Trp-792Valfs*41) | 5 (+1) | NCCM, HCM | | | LVAD, death, AF | HCM (4-8) | |
| | | c.2827C>T | p.(Arg943*) | 2 (+1) | | | | Death | HCM (5) | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **MYH7** | NM_000257.2 | c.266A>G | p.(Asp89Gly) | 1 | | | | | 29/263 (11%) |
| | | c.415G>T | p.(Val139Leu) | 1 | NCCM | | | | DCM (35) |
| | | c.715_717delGAC | p.(Asp239del) | 1 | NCCM | | | | NCCM (10, 12) |
| | | c.689T>C | p.(Phe230Ser) | 1 | NCCM | | | | |
| | | c.732+1G>A | p.(?) | 1 | NCCM | | | | NCCM (10, 19) |
| | | c.847T>G | p.(Tyr283Asp) | 1 | NCCM | | Ebstein anomaly | | NCCM, Ebstein (36) |
| | | c.902T>A | p.(Leu301Gln) | 1 | NCCM | | | | NCCM (37) |
| | | c.1208G>C | p.(Arg403Pro) | 1 | NCCM | | | | |
| | | c.1325G>A | p.(Arg442His) | 1 | | | Pulmonary stenosis | | DCM (38, 39) |
| | | c.1633G>A | p.(Asp545Asn) | 4 | NCCM, DCM | 3, 4, 5, 6 | | | NCCM (37) DCM (40) |
| | | c.1915A>G | p.(Lys639Glu) | 1 | NCCM | | | | |
| | | c.2085_2097dup | p.(Glu700Glnfs*37) | 1 | | | | | |
| | | c.2678C>T | p.(Ala893Val) | 1 | NCCM | | | | DCM (41) |
| | | c.2710C>T | p.(Arg904Cys) | 1 | NCCM, DCM | | | | DCM (42) |
| | | c.2863G>A | p.(Asp955Asn) | 4 | NCCM | 3, 4, 5, 6 | | | NCCM (37) DCM (40) |
| | | c.3100-2A>C | p.(?) | 1 | | | | | |
| | | c.3113T>C | p.(Leu1038Pro) | 1 (+1) | DCM | | | | DCM (20) |
| | | c.4075C>T | p.(Arg1359Cys) | 1 | | | | | NCCM (10, 12) |
| | | c.4125T>A | p.(Tyr1375*) | 1 | ARVC | | | | |
| | | c.5754C>G | p.(Asn1918Lys) | 6 | NCCM, DCM | | Ebstein anomaly | SCD | NCCM Ebstein (36), DCM (40) |
| | | c.5773C>G | p.(Arg1925Gly) | 2 | NCCM, DCM | | | | NCCM (43) |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **PLN** | NM_002667.3 | c.40_42delAGA | p.(Arg14del) | 1 | NCCM | | | Htx | DCM (40) ACM (44) | 2/220 (1%) |
| **PRDM16** | NM_22114.3 | c.56delA | p.(Asn19Ilefs*114) | 1 | | | | Sudden death | | 3/107 (3%) |
| | | c.676+1G>A | p.(?) | 1 | De novo | | | | | |
| | | c.2848C>T | p.(Arg950*) | 1 | NCCM | | | | | |
| **RBM20** | NM_001134363.1 | c.846_853delT-TACGGAC | p.(Tyr283Gl-nfs*14) | 1 | | 2 | | | | 2/178 (1%) |
| | | c.1900C>T | p.(Arg634Trp) | 1 | DCM | | | | DCM (45) | |
| **RYR2** | NM_001035.2 | c.169-198_273+-823del | p.(?) | 1 | | | CPVT | | CPVT(46) | 2/80(1%) |
| | | c.4299+1delG | p.(?) | 1 | | 1 | Brugada syndrome | | Brugada Syndrome (50) | |
| **TAZ** | NM_000116.3 | c.646G>A | p.(Gly216Arg) | 1 | | | Barth syndrome | | Barth (51-53) | 1/217 (1%) |
| **TNNC1** | NM_003280.2 | c.149A>G | p.(Gln50Arg) | 1 | | | | | DCM (40) | 1/212 (1%) |
| **TNNT2** | NM_000364.2 | c.629_631delAGA | p.(Lys210del) | 1 | DCM | | | | DCM (55) | 1/238 (0%) |
| **TPM1** | NM_001018005.1 | c.250G>A | p.(Asp84Asn) | 2 | NCCM, DCM | | | | NCCM (54) | 2/229 (1%) |
| **TTN** | NM_001267550.1 | c.4459G>T | p.(Glu1487*) | 1 | | 7 | | | | 19/156 (12%) |
| | | c.4583G>A | p.(Trp1528*) | 1 | NCCM | | | | | |
| | | c.7967delG | p.(Gl-y2656Alafs*6) | 1 | DCM | 9 | | | | |
| | | c.8853C>A | p.(Tyr2951*) | 1 | NCCM | | | AF | | |
| | | c.47352T>G | p.(Tyr15784*) | 1 | | | | | | |
| | | c.37195C>T | p.(Arg12399*) | 1 | DCM | | | | | |
| | | c.71602C>T | p.(Arg23868*) | 1 | DCM | | | | | |
| | | c.48313-1G>A | p.(?) | 1 | NCCM | | | | | |
| | | c.53656_53663del8 | p.(Pro17886*) | 1 | DCM | | | Death | | |
| | | c.78593_78594del | p.(Lys26198I-lefs*16) | 1 | DCM | | | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | c.69192dupC | p.(Glu23066G-lyfs*8) | 2 | NCCM | | | AF |
| | c.74666dupA | p.(Tyr24889*) | 1 | De novo | | | |
| | c.77254_77255delAA | p.(Lys25752fs*18) | 1 | | 8 | | AF, Htx |
| | c.80514delA | p.(Val26839fs*5) | 1 | NCCM | | WPW | SCD |
| | c.82309_82312dup4 | p.(Asn27438fs*2) | 1 | NCCM | | | |
| | c.89165delC | p.(Pro29722fs) | 1 | NCCM, DCM | | | |
| | c.94816C>T | p.(Arg31606*) | 1 | DCM | | | |
| | c.104421_104425del5 | p.(Arg34807fs*8) | 1 | DCM | | | |

*(+ )= occurring in patients < 18 years, †: patient number in online tabel 2b, AF: atrial fibrillation, ASD: atrial septal defect, BAV: Bicuspic Aortic Valve, CHD: congenital heart disease, CoA: Coarctation of the aorta, CPVT: catecholaminergic polymorphic ventricular tachycardia, DCM: dilated cardiomyopathy, HCM: hypertrophic cardiomyopathy, Htx: heart transplant, LVAD: left ventricular assistant device, NCCM: noncompaction cardiomyopathy, SCD: sudden cardiac death, VSD: ventricular septal defect, WPW: Wolff-Parkinson-White syndrome.

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**Online Table 2b: Complex genotypes in adults with NCCM.**

| Patient number in online table 2a | Mutation 1 | Mutation 2 | Mutation 3 |
|---|---|---|---|
| 1 | ACTN2 c.574C>T, p.(Arg192*) | SCN5A c.4299+1delG p.(?) | |
| 2 | HCN4 c.1441T>C , p.(Tyr481His) | FKTN c.1112A>G, p.(Tyr371Cys) | RBM20 c.846_853delTTACGG-AC p.(Tyr283Glnfs*14) |
| 3, 4, 5, 6 | MYH7 c.2863G>A, p.(Asp955Asn)† | MYH7 1633G>A, p.(Asp545Asn)† | |
| 7 | TTN c.4459C>T, p.(Glu1487*) | MIB1 c.2701C>T, p.(Arg901*) | |
| 8 | TTN c.77254_77255delAA p.(Lys25752fs*18) | MIB1 c.1096_1097del, p.(Leu366fs) | |
| 9 | TTN c.7967delG , p.(Gly2656fs) | MIB1 c.2589_2590dupTG, p.(Glu864fs) | |

†in cis.

**Online Table 3: Variants of unknown significance in 327 NCCM patients.**

| Gene | Nucleotide substitute | Amino acid substitute | NM-Number GRCh37 (hg19) |
|---|---|---|---|
| ABCC9 | c.3238C>A | p.Pro1080Thr) | NM_020297.2 |
| ACTC1 | c.635G>A | p.(Arg212His) | NM_0051594.4 |
| ACTN2 | c.2569G>C | p.(Asp857His) | NM_001103.2 |
| ALPK3 | c.830C>A | p.(Thr277Asn) | NM_020778.4 |
| ANKRD1 | c.222dupA | p.(Leu75fs*8) | NM_014391.2 |
| CALR3 | c.-14C>T | p.(?) | NM_145046.4 |
| CALR3 | c.564delT | p.(Gln189Serfs*8) | NM_145046.4 |
| CALR3 | c.403G>A | p.(Asp135Asn) | NM_145046.4 |
| CTNNA3 | c.1507A>C | p.(Ile503Leu) | NM_013266.3 |
| DES | c.1009G>A | p.(Ala337Thr) | NM_001927.3 |
| DES | c.1123C>T | p.(Arg375Trp) | NM_001927.3 |
| DES | c.934G>A | p.(Asp312Asn) | NM_001927.3 |
| DSC2 | c.1231A>G | p.(Lys411Glu) | NM_024422.3 |
| DSC2 | c.2126G>C | p.(Cys709Ser) | NM_024422.3 |
| DSC2 | c.865C>T | p.(Pro289Ser) | NM_024422.3 |
| DSG2 | c.1003A>G | p.(Thr335Ala) | NM_001943.3 |
| DSG2 | c.1912G>A | p.(Gly638Arg) | NM_001943.3 |
| DSG2 | c.2140A>G | p.(Met714Val) | NM_001943.3 |
| DSP | c.1138C>A | p.(Gln380Lys) | NM_004415.2 |
| DSP | c.3121C>T | p.(Leu1041Phe) | NM_004415.2 |

| | | | |
|---|---|---|---|
| DSP | c.423G>T | p.(Arg141Ser) | NM_004415.2 |
| DSP | c.5676_5678del | p.(Lys1892del) | NM_004415.2 |
| FHL1 | c.944C>T | p.(Thr315Ile) | NM_001159702.2 |
| FLNC | c.2617G>A | p.(Glu873Lys) | NM_001458.4 |
| FLNC | c.6397C>T | p.(Arg2133Cys) | NM_001458.4 |
| JPH2 | c.2001_2006dup | p.(Val668_Glu669dup) | NM_020433.4 |
| JPH2 | c.572C>G | p.(Pro191Arg) | NM_020433.4 |
| LAMA4 | c.*7A>G | p.(?) | NM_001105206.1 |
| LAMA4 | c.2900C>T | p.(Ser967Leu) | NM_001105206.1 |
| LAMA4 | c.3868_3870del | p.(Thr1290del) | NM_001105206.1 |
| LDB3 | c.1145C>A | p.(Ala382Glu) | NM_001171610.1 |
| LDB3 | c.664G>A | p.(Ala222Thr) | NM_007078.2 |
| LDB3 | c.98C>T | p.(Thr33Ile) | NM_007078.2 |
| LMNA | c.215G>A | p.(Arg72His) | NM_170707.3 |
| LMNA | c.71C>T | p.(Thr24Ile) | NM_170707.3 |
| MYBPC3 | c.2330C>T | p.(Ala777Val) | NM_000256.3 |
| MYBPC3 | c.1246G>A | p.(Gly416Ser) | NM_000256.3 |
| MYBPC3 | c.131G>A | p.(Arg44His) | NM_000256.3 |
| MYBPC3 | c.2164G>A | p.(Glu722Lys) | NM_000256.3 |
| MYBPC3 | c.2479C>A | p.(Gln827Lys) | NM_000256.3 |
| MYBPC3 | c.3425A>C | p.(Gln1142Pro) | NM_000256.3 |
| MYBPC3 | c.529C>T | p.(Arg177Cys) | NM_000256.3 |
| MYBPC3 | c.713G>A | p.(Arg238His) | NM_000256.3 |
| MYBPC3 | c.814C>T | p.(Arg272Cys) | NM_000256.3 |
| MYBPC3 | c.2441_2443del | p.(Lys814del) | NM_000256.3 |
| MYH6 | c.1545C>T | p.(=) | NM_002471.3 |
| MYH6 | c.2827C>T | p.(Arg943Cys) | NM_002471.3 |
| MYH6 | c.5026G>A | p.(Val1676Met) | NM_002471.3 |
| MYH7 | c.2384T>C | p.(Val795Ala) | NM_000257.2 |
| MYH7 | c.1245_1247dup | p.(Gln415_Asn416insLys) | NM_000257.2 |
| MYH7 | c.2453T>A | p.(Ile818Asn) | NM_000257.2 |
| MYH7 | c.2945T>C | p.(Met982Thr) | NM_000257.2 |
| MYH7 | c.345C>T | p.(=) | NM_000257.2 |
| MYH7 | c.4048_4050del | p.(Glu1350del) | NM_000257.2 |
| MYH7 | c.4076G>A | p.(Arg1359His) | NM_000257.2 |
| MYH7 | c.4377G>T | p.(Lys1459Asn) | NM_000257.2 |
| MYH7 | c.541G>A | p.(Gly181Arg) | NM_000257.2 |

| | | | |
|---|---|---|---|
| MYH7 | c.5534G>A | p.(Arg1845Gln) | NM_000257.2 |
| MYH7 | c.644C>T | p.(Thr215Ile) | NM_000257.2 |
| MYH7 | c.2704G>A | p.(Glu902Lys) | NM_000257.2 |
| MYH7 | c.4463A>G | p.(Tyr1488Cys) | NM_000257.2 |
| MYH7 | c.2453T>A | p.(Ile818Asn) | NM_000257.2 |
| MYH7 | c.3928C>G | p.(Gln1310Glu) | NM_000257.2 |
| MYH7 | c.698C>T | p.(Ala233Val) | NM_000257.3 |
| MYL2 | c.263A>C | p.(Glu88Ala) | NM_000432.3 |
| MYL2 | c.421G>A | p.(Ala141Thr) | NM_000432.3 |
| MYL2 | c.247C>T | p.(Leu83Phe) | NM_000432.3 |
| MYL3 | c.*8G>A | p.(?) | NM_000258.2 |
| MYL3 | c.463C>T | p.(His155Tyr) | NM_000258.2 |
| MYOZ1 | c.167G>C | p.(Gly56Ala) | NM_021245.3 |
| MYOZ2 | c.148C>T | p.(Leu50Phe) | NM_016599.4 |
| MYOZ2 | c.488T>C | p.(Leu163Ser) | NM_016599.4 |
| MYPN | c.1012C>T | p.(Arg338Cys) | NM_032578.3 |
| MYPN | c.2428C>T | p.(Arg810Cys) | NM_032578.3 |
| MYPN | c.3046A>G | p.(Asn1016Asp) | NM_032578.3 |
| MYPN | c.3046A>G | p.(Asn1016Asp) | NM_032578.3 |
| NEXN | c.1878_1880del | p.(Glu626del) | NM_144573.3 |
| NEXN | c.1090delA | p.(Ile364fs*19) | NM_144573.3 |
| PKP2 | c.1652A>G | p.(Asp551Gly) | NM_004572.3 |
| PKP2 | c.464G>C | p.(Ser155Thr) | NM_004572.3 |
| PKP2 | c.1048G>A | p.(Glu350Lys) | NM_004572.3 |
| PRDM16 | c.1040C>T | p.(Thr347Met) | NM_022114.3 |
| PRDM16 | c.1633G>A | p.(Ala545Thr) | NM_022114.3 |
| PRDM16 | c.1840G>A | p.(Asp614Asn) | NM_022114.3 |
| PRDM16 | c.567T>G | p.(Ser189Arg) | NM_022114.3 |
| RBM20 | c.2737G>A | p.(Glu913Lys) | NM_001134363.1 |
| RBM20 | c.2761A>T | p.(Ile921Phe) | NM_001134363.2 |
| RBM20 | c.2986G>T | p.(Asp996Tyr) | NM_001134363.1 |
| RBM20 | c.3115C>T | p.(Pro1039Ser) | NM_001134363.2 |
| RBM20 | c.929C>T | p.(Pro310Leu) | NM_001134363.2 |
| RYR2 | c.11084T>C | p.(Met3695Thr) | NM_001035.2 |
| RYR2 | c.12047T>A | p.(Phe4016Tyr) | NM_001035.2 |
| RYR2 | c.12341G>A | p.(Arg4114Gln) | NM_001035.2 |
| RYR2 | c.13291G>A | p.(Glu4431Lys) | NM_001035.2 |

| | | | |
|---|---|---|---|
| RYR2 | c.13822C>T | p.(Arg4608Trp) | NM_001035.2 |
| RYR2 | c.1827+140_1961+426del | p.(?) | NM_001035.2 |
| RYR2 | c.8147A>T | p.(Lys2716Ile) | NM_001035.2 |
| SCN5A | c.-27_-15del | p.(?) | NM_198056.2 |
| SCN5A | c.1735G>A | p.(Gly579Arg) | NM_198056.2 |
| SCN5A | c.1993G>T | p.(Ala665Ser) | NM_198056.2 |
| SCN5A | c.5692C>T | p.(Arg1898Cys) | NM_198056.2 |
| SCN5A | c.1735G>A | p.(Gly579Arg) | NM_198056.2 |
| SCN5A | c.665G>A | p.(Arg222Gln) | NM_198056.2 |
| TBX1 | c.199C>A | p.(Pro67Thr) | NM_080647.1 |
| TBX5 | c.556G>A | p.(Val186Met) | NM_000192.3 |
| TCAP | c.209G>A | p.(Arg70Gln) | NM_003673.3 |
| TMEM43 | c.1178G>A | p.(Arg393Gln) | NM_024334.2 |
| TMEM43 | c.206C>T | p.(Ser69Leu) | NM_024334.2 |
| TMEM43 | c.862C>T | p.(His288Tyr) | NM_024334.2 |
| TNNC1 | c.205A>G | p.(Ser69Gly) | NM_003280.2 |
| TNNI3 | c.539A>G | p.(Asp180Gly) | NM_000363.4 |
| TNNT2 | c.652A>T | p.(Ile218Phe) | NM_001001430.1 |
| TNNT2 | c.230C>T | p.(Pro77Leu) | NM_001001430.1 |
| TNNT2 | c.381C>T | p.(=) | NM_001001430.1 |
| TNNT2 | c.431G>A | p.(Arg144Gln) | NM_001001430.1 |
| TNNT2 | c.522G>T | p.(Lys174Asn) | NM_001001430.1 |
| TNNT2 | c.40G>T | p.(Glu14*) | NM_000364.3 |
| TPM1 | c.850A>G | p.(Met284Val) | NM_000366.5 |
| TTN | c.102155G>A | p.(Arg34052Gln) | NM_001267550.1 |
| TTN | c.105383C>G | p.(Ala35128Gly) | NM_001267550.1 |
| TTN | c.106784A>T | p.(Lys35595Ile) | NM_001267550.1 |
| TTN | c.106975G>A | p.(Asp35659Asn) | NM_001267550.1 |
| TTN | c.107723T>C | p.(Ile35908Thr) | NM_001267550.1 |
| TTN | c.13006_13008del | p.(Glu4336del) | NM_001267550.1 |
| TTN | c.13241T>G | p.(Leu4414Arg) | NM_001267550.1 |
| TTN | c.18470T>C | p.(Ile6157Thr) | NM_001267550.1 |
| TTN | c.23581C>A | p.(His7861Asn) | NM_133378.4 |
| TTN | c.26047C>T | p.(Leu8683Phe) | NM_001267550.1 |
| TTN | c.26949A>T | p.(Leu8983Phe) | NM_001267550.1 |
| TTN | c.28786C>T | p.(Leu9596Phe) | NM_001267550.1 |
| TTN | c.31594G>A | p.(Val10532Ile) | NM_001267550.1 |

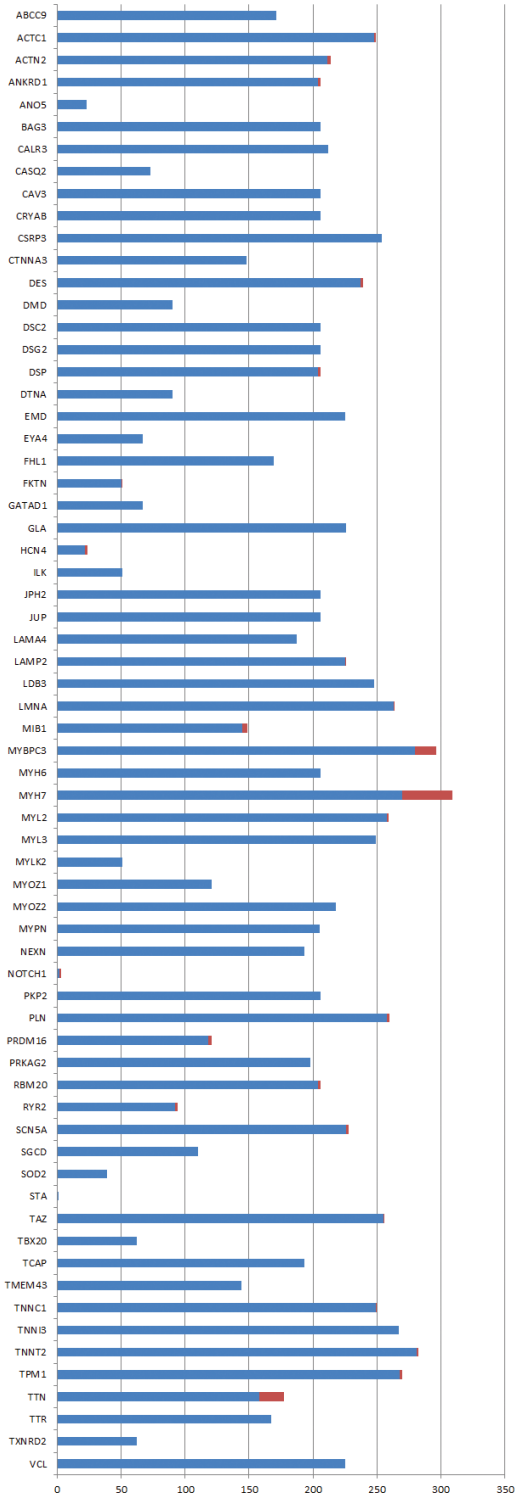
| | | | |
|---|---|---|---|
| TTN | c.33911-6_33911-5insG | p.(?) | NM_001267550.1 |
| TTN | c.38214A>T | p.(Glu12738Asp) | NM_001267550.1 |
| TTN | c.39643+5G>C | p.(?) | NM_133432.3 |
| TTN | c.44586G>C | p.(Gln14862His) | NM_001267550.1 |
| TTN | c.45508G>A | p.(Asp15170Asn) | NM_001267550.1 |
| TTN | c.46296A>C | p.(Glu15432Asp) | NM_001267550.1 |
| TTN | c.49165G>A | p.(Glu16389Lys) | NM_001267550.1 |
| TTN | c.49322C>T | p.(Pro16441Leu) | NM_001267550.1 |
| TTN | c.54313C>T | p.(Arg18105Cys) | NM_001267550.1 |
| TTN | c.56654C>G | p.(Pro18885Arg) | NM_001267550.1 |
| TTN | c.61699C>T | p.(Leu20567Phe) | NM_001267550.1 |
| TTN | c.62317C>G | p.(Leu20773Val) | NM_001267550.1 |
| TTN | c.62546C>T | p.(Thr20849Met) | NM_001267550.1 |
| TTN | c.62567A>G | p.(Tyr20856Cys) | NM_001267550.1 |
| TTN | c.65144G>T | p.(Arg21715Leu) | NM_001267550.1 |
| TTN | c.659G>A | p.(Arg220Gln) | NM_133378.4 |
| TTN | c.69116A>T | p.(Asp23039Val) | NM_001267550.1 |
| TTN | c.70710A>C | p.(Glu23570Asp) | NM_001267550.1 |
| TTN | c.73073A>T | p.(Tyr24358Phe) | NM_001267550.1 |
| TTN | c.73567C>G | p.(Pro24523Ala) | NM_001267550.1 |
| TTN | c.75259G>A | p.(Ala25087Thr) | NM_001267550.1 |
| TTN | c.77894T>C | p.(Ile25965Thr) | NM_001267550.1 |
| TTN | c.82001T>C | p.(Leu27334Pro) | NM_001267550.1 |
| TTN | c.84353G>T | p.(Arg28118Leu) | NM_001267550.1 |
| TTN | c.84652G>A | p.(Gly28218Ser) | NM_001267550.1 |
| TTN | c.8641A>G | p.(Thr2881Ala) | NM_133378.4 |
| TTN | c.87041G>A | p.(Arg29014Gln) | NM_001267550.1 |
| TTN | c.88037A>T | p.(Asp29346Val) | NM_001267550.1 |
| TTN | c.92267T>C | p.(Leu30756Pro) | NM_001267550.1 |
| TTN | c.9226A>G | p.(Met3076Val) | NM_001267550.1 |
| TTN | c.92451G>T | p.(Glu30817Asp) | NM_001267550.1 |
| TTN | c.93775C>T | p.(Arg31259Cys) | NM_133378.4 |
| TTN | c.94827C>T | p.(=) | NM_001267550.1 |
| TTN | c.96449G>C | p.(Arg32150Thr) | NM_001267550.1 |
| TTN | c.96544G>A | p.(Glu32182Lys) | NM_001267550.1 |
| TTN | c.97604T>C | p.(Ile32535Thr) | NM_001267550.1 |
| TTN | c.99779A>T | p.(Gln33260Leu) | NM_001267550.1 |

| | | | |
|---|---|---|---|
| TTR | c.190T>C | p.(Phe64Leu) | NM_000371.3 |
| TXNRD2 | c.1575A>C | p.(*525Tyrext*?) | NM_006440.4 |
| VCL | c.2046A>T | p.(Leu682Phe) | NM_014000.2 |
| VCL | c.1534C>T | p.(Arg512Cys) | NM_014000.2 |
| VCL | c.20G>T | p.(Arg7Leu) | NM_014000.2 |

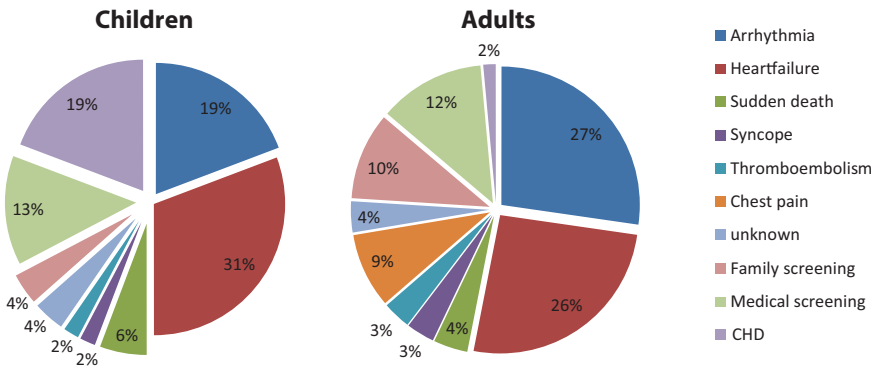
**Online Table 4: Characteristics and clinical features at diagnosis of 327 noncompaction cardiomyopathy (NCCM) patients.**

| | Mutation n=23 (45%) | No mutation n=29 (55%) | | Mutation n=81 (30%) | No mutation n=194 (70%) | | |
|---|---|---|---|---|---|---|---|
| | | Familial n=8 (15%) | Not familial n=21 (40%) | | Familial n=45 (16%) | Not familial n=149 (54%) | |
| | Genetic | Probably genetic | Sporadic | Genetic | Probably genetic | Sporadic | p value |
| **ECG** | | | | | | | |
| BPM | 98 ±41 | 74 ±12 | 89 ±46 | 71 ±16 | 71 ±18 | 72 ±19 | ns |
| PQ | 161 ±48 | 152 ±18 | 125 ±28 | 169 ±28 | 161 ±37 | 161 ±28 | ns |
| QRS | 98 ±34 | 97 ±12 | 90 ±20 | 101 ±21 | 103 ±21 | 115 ±32 | 0,002† |
| QTc | 418 ±47 | 411 ±12 | 416 ±31 | 414 ±27 | 417 ±37 | 434 ±41 | 0,001† |
| Bradycardia (<50) | | | 3 (14%) | 4 (4%) | 3 (7%) | 7 (5%) | ns |
| AV-block (1st degree) | 1 (4%) | | 1 (5%) | 5 (6%) | 4 (10%) | 8 (6%) | ns |
| RBBB | 2 (9%) | | 3 (14%) | 3 (3%) | 3 (7%) | 11 (8%) | ns |
| LBBB | 1 (6%) | | | 7 (10%) | 4 (11%) | 40 (27%) | 0,01† |
| **Echocardiography** | | | | | | | |
| LA diameter mm (±SD) | 32 ±13 | 30 ±20 | 30 ±5 | 39 ±8 | 40 ±8 | 40 ±8 | ns |
| LV-EDD mm (±SD) | 50 ±19 | 40 ±21 | 45 ±9 | 57 ±8 | 57 ±9 | 57 ±10 | ns |
| LV-ESD mm (±SD) | 38 ±16 | 26 ±14 | 30 ±8 | 44 ±10 | 44 ±10 | 44 ±12 | ns |
| LV FS % (±SD) | 20 ±8 | 23±15 | 34 ±7 | 23 ±8 | 25 ±8 | 25 ±10 | ns |
| LV EF % (±SD) | 39 ±17 | 30 ±12 | 61±11 | 39 ±13 | 39 ±13 | 43 ±17 | ns |
| RV TAPSE mm (±SD) | 17±6 | 19 ±3 | 22±6 | 21 ±6 | 22 ±6 | 23 ±5 | ns |
| NC/C | 2,6 ±0,6 | 2,6 ±0,4 | 2,5 ±0,6 | 2,5 ±0,8 | 2,3 ±0,7 | 2,5 ±0,07 | ns |
| **MRI** | 12 (57%) | 4 (50%) | 14 (64%) | 63 (79%) | 33 (74%) | 118 (78%) | ns |
| LV-EDV | 252 ±130 | 175 ±68 | 144 ±52 | 226 ±77 | 207 ±55 | 213 ±68 | ns |
| LV-ejection fraction % (±SD) | 37 ±14 | 59 ±12 | 53 ±9 | 40 ±13 | 41 ±15 | 43 ±14 | ns |
| RV-ejection fraction % (±SD) | 29 ±18 | 56 ±6 | 54 ±9 | 45 ±13 | 47 ±12 | 52 ±9 | ns |
| Delayed Enhancement | 1/6 (17%) | 0/4 (0%) | 0/5 (0%) | 9/53 (17%) | 3/22 (14%) | 14/78 (18%) | ns |

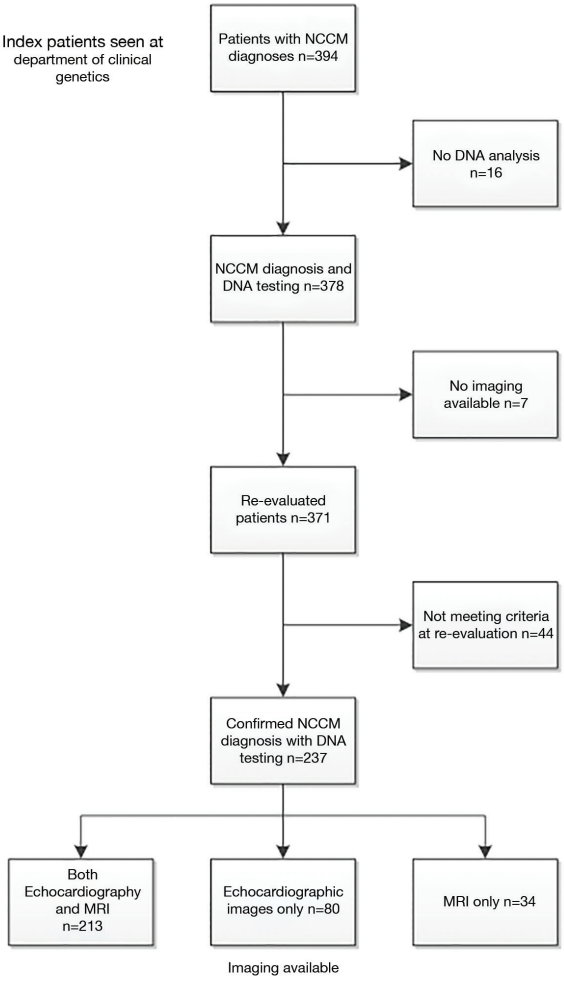
*diagnosed before the age of 18 years, †significant for (probably) genetic compared to sporadic adults, AV-block: Atrioventricular block, BPM: beats per minute, ECG: Electrocardiogram, LA diameter: Left atrial diameter, LBBB: Left bundle branch block, LV: Left ventricle, LV-EDV: Left ventricular end diastolic volume, LVEDD: Left ventricular end diastolic diameter, LVESD: Left ventricle ends-systolic diameter, RBBB: Right bundle branch block, RV: Right ventricle, TAPSE: tricuspid annular plane systolic excursion.




Online figure 1: Yield of genetic testing.




**Online figure 2: Clinical presentation.**




**Online figure 3: Patient inclusion.**

# Chapter 3

## Cardiac phenotypes, genetics, and risks in familial noncompaction cardiomyopathy

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*Cardiac Phenotypes, Genetics, and Risks in Familial Noncompaction Cardiomyopathy, J Am Coll Cardiol. 2019 Apr 9;73(13):1601-1611*

**Abstract**

Half of the NCCM index cases had at least one relative with a cardiomyopathy. NCCM with DCM was associated with LV systolic dysfunction, increased risk for MACE, mutations in the tail of *MYH7*, and DCM without NCCM in relatives. Isolated NCCM was associated with a milder course, mutations in the head of *MYH7*, asymptomatic NCCM and isolated NCCM in relatives. NCCM with HCM was associated with *MYBPC3* and HCM without NCCM in relatives. These findings highlight that phenotype of relatives may be predicted by NCCM phenotypes and the mutation of index patients.

## Introduction

Noncompaction cardiomyopathy (NCCM), also known as left ventricular noncompaction (LVNC), is a cardiomyopathy characterized by excessive trabeculations of the left ventricle (LV)(1, 2). Current imaging diagnostic criteria, including the most frequent used echocardiographic Jenni criteria, are based on the ratio between a severely thickened myocardium, with an noncompacted layer which is at least twice as thick as the compacted layer, measured in systole in the short axis view(21). Clinical features of NCCM range from asymptomatic patients with noncompaction of the left ventricle to patients with or without a mutation in a cardiomyopathy gene with symptoms of heart failure, arrhythmias or major adverse cardiac events (MACE)(8).



In approximately 50% of the NCCM patients there is evidence for a genetic cause; because there is a mutation in a cardiomyopathy gene and/or at least one family member with a non-ischemic cardiomyopathy(8, 155). Mutations in mostly sarcomere genes explain approximately 32% of NCCM. In 15% of the patients familial disease occurs without a mutation, indicating that many genetic causes are still unknown. Novel genetic causes conveying small risk for relatives or alternatively non-genetic, secondary causes for noncompaction of the left ventricle are expected in NCCM cases without a mutation and without familial disease. Among the NCCM genes *MYH7*, *MYBPC3* and *TTN* are the most prevalent and are also frequent causes for hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM)(156, 157). Previous family studies of NCCM reported occasionally relatives with HCM or DCM apparently without noncompaction in familial NCCM(54, 80, 158-160).

NCCM patients may have additional ventricular dilatation or septal hypertrophy (68, 161-164) similar to HCM and DCM. Subsequently, NCCM can be classified phenotypically into isolated NCCM, NCCM with DCM, and NCCM with HCM(165). In children subtyping of NCCM according to cardiac phenotype was a good predictor for adverse events(166). Prediction of phenotype and associated clinical features for relatives is important, from the point of view of informing family members of NCCM patients and eventually guiding family screening and follow-up of relatives according to estimated risk.

In this study we examined the clinical features, outcome, genetics, and familial recurrence of NCCM phenotypes (isolated NCCM, NCCM with DCM and NCCM with HCM), by using the results of cardiac and genetic family screening comprising

of 473 family members of 143 NCCM index patients. Our goal was to investigate if we could predict risk for relatives of NCCM patients by establishing if cardiac NCCM phenotypes were related to outcome and to genotype, and if NCCM phenotype of the index case was related to cardiac features in relatives.

## Methods

### *Study population*

The retrospective study population consisted of the families of 143 index patients diagnosed with NCCM between January 2005 and January 2017 at the Department of Cardiology and referred for genetic counseling to the Department of Clinical Genetics of the Erasmus Medical Center Rotterdam (EMC) in the Netherlands. Genetic screening included DNA testing of a cardiomyopathy gene panel, ascertainment of family history and initiating cardiologic family screening.

### *Family screening*

Family screening was initiated by asking the index patients to distribute a letter with information on heritability of NCCM and the recommendations for family screening(54, 167). In families with a mutation, adult relatives were counseled about predictive DNA testing. For children at risk, cardiologic screening from the age of 10 years was recommended, with DNA testing in case of a cardiomyopathy. Cardiac screening for relatives consisted of physical examination, a twelve-lead electrocardiography and echocardiography. In families with a mutation, cardiologic screening was offered to relatives with the mutation and to adult relatives refusing DNA testing. In families without a mutation, relatives had cardiologic screening. Specific on the DNA diagnostics for NCCM were described previously(8). Online table S1shows the list of mutations in the participating NCCM patients.

### *Diagnostic criteria*

NCCM diagnosis was based on consensus of evaluated echocardiographic and Cardiovascular Magnetic Resonance (CMR) images according to the Jenni and Petersen criteria for NCCM by JIVW and a dedicated cardiomyopathy cardiologist (KC, MM, AFLS, MDH)(21, 139). All patients had echocardiographic images and CMR data were available for 176 (82%) NCCM patients. Patients were classified according to cardiac phenotype into isolated NCCM, NCCM with DCM or NCCM with HCM. The NCCM with DCM phenotype was diagnosed in NCCM patients according to dilatation criteria for DCM on echocardiography and was defined as a LV end diastolic dimension (LVEDD) of greater than 112% of predicted values (168). Predicted LVEDD was calculated according to the formula of Henry:

LVEDD = (45.3 x body surface area$^{0.3}$) - (0.03 x age) - 7.2 (169). The NCCM with HCM phenotype was diagnosed in NCCM patients using the hypertrophic cardiomyopathy (HCM) criteria for adult family members, maximum LV wall thickness of ≥13mm, not explained by loading conditions(170). For children we used either ventricular septal or left ventricular (LV) posterior wall thickness for body-surface area more than 2 standard deviations different from the value for a normal population of children with similar body-surface area, or the presence of localized LV hypertrophy in children(171). Patients were categorized in the NCCM with HCM category despite of left ventricular dilatation. The diagnosis of DCM or HCM in relatives without hypertrabeculation was made according to current European guidelines(1, 170).



*Ventricular function and adverse events*

LV systolic dysfunction was defined as LV ejection fraction < 45% on CMR. Alternatively LV systolic dysfunction was measured on echocardiography by using a wall motion score index that was lower than mildly reduced, for patients without CMR imaging (n=39). Systolic dysfunction was visually assessed using the wall motion score index on echocardiography and was described as normal (≥55%), mildly reduced (45% to 54%), moderately reduced (30% to 44%), or poor (<30%) according to the echocardiography guidelines(172). Abnormal right ventricular (RV) systolic function was defined as RV ejection fraction <45% on CMR. For patients without CMR imaging (n=39) tricuspid annular plane systolic excursion (TAPSE) <17 mm on echocardiography was used to define RV systolic dysfunction(144). For children dimensions of the ventricles of more than two standard deviations from reference range were classified as abnormal(145, 146). We used the same definition for adverse cardiac events as described before(8). The occurrence of cardiac death, implantation of a LV assistance device, heart transplantation, (aborted) sudden cardiac death, appropriate implantable cardioverter-defibrillator (ICD) shock, or ischemic stroke were classified as major adverse cardiac events (MACE). For the hazard models a combined endpoint for MACE was used because of the low incidence of death. Information on vital status of patients was retrieved from municipal registries.

*Statistical analysis*

Categorical data were compared with Pearson Chi-Squared test or Fisher's exact test. For continuous variables, unpaired t-tests were used for two groups and ANOVA for more than two groups. Odds ratios were calculated using binary logistic regression. Statistics for variables at follow-up were compared using the log-rank test, using time at diagnosis as time zero. Hazard ratios for major adverse

cardiac events (MACE) were calculated by Cox proportional hazards regression analysis, and presented as MACE per 100 patients years. Follow-up data were obtained in July 2017, five patients were lost to follow-up. Patients ( lost to follow-up) were considered at risk until the date of last contact, at which time-point they were censored. Statistical analysis was performed with SPSS statistical software, version 21.0 (SPSS Inc., Chicago, IL).

## Results

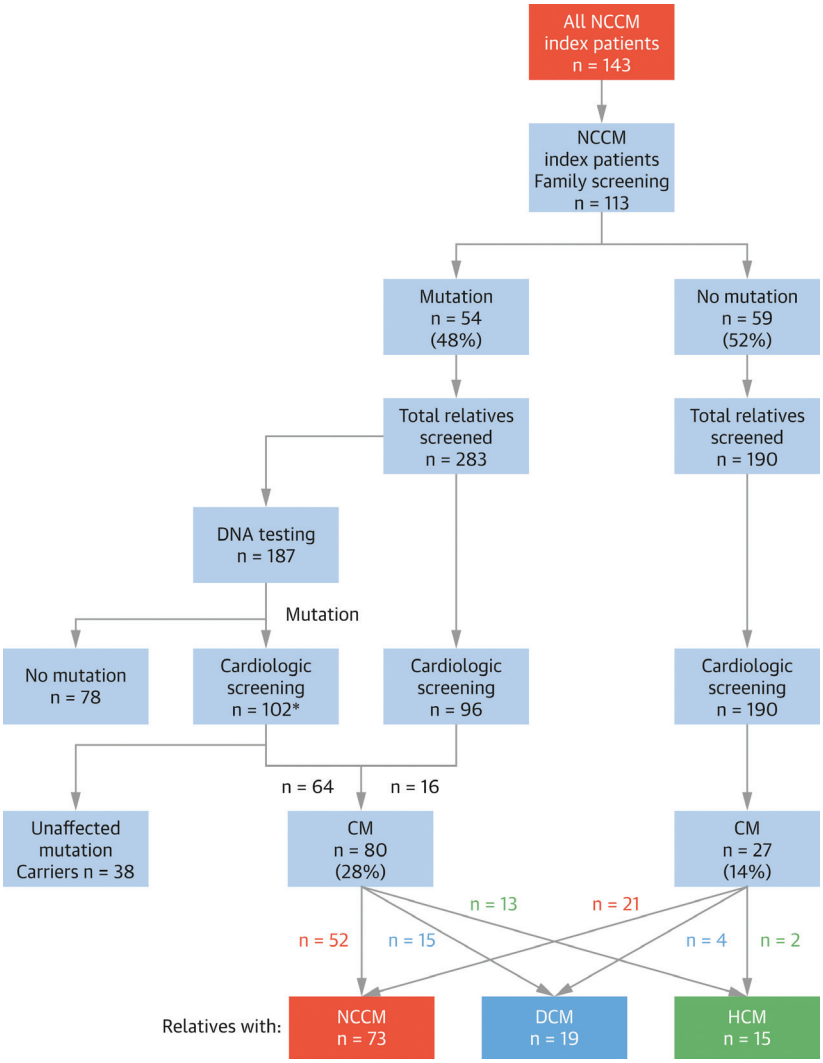



**Figure 1: Family screening for noncompaction cardiomyopathy (NCCM) .**
In 113 of 143 families (54 with a mutation) cardiologic screening identified 107 relatives with a cardiomyopathy; 73 with NCCM, 19 with DCM without noncompaction and 15 with HCM without noncomapction. Thirty-eight carriers of a familial mutation were unaffected..

*Family screening*

Seventy nine percent (113/143) of the families of NCCM index cases participated in genetic and cardiologic family screening (figure 1). In total 473 relatives were screened; 286 (60%) first-degree relatives, and 187 (40%) second degree or more distantly related relatives. We found a mutation in 54/113 (48%) of the index patients. Subsequently 187/283 (66%) relatives of the index cases with a mutation had a genetic testing, showing that 109 (58%) of the tested relatives had a mutation. In 78 relatives a mutation was excluded. Cardiologic screening was performed in 102 of the 109 relatives with a mutation, showing that 64 (63%) relatives with a mutation had a cardiomyopathy, and 38 (37%) did not have a cardiomyopathy. In addition 16 relatives, who refused DNA testing, from the families with a mutation, were diagnosed with a cardiomyopathy. In 39 of the 54 families with a mutation (72%), 267 of 283 participating relatives had a cardiologic examination, showing that 80 of the examined relatives were affected (29%). For 15/54 (28%) of the families with a mutation, family screening was inconclusive. Family screening of 59 families without a mutation identified a cardiomyopathy in 27/190 (14%) relatives from 19 families. In total family screening showed familial cardiomyopathy in 58 of the screened families with 107 (23%) affected relatives. In 34 families all affected family members had NCCM. In 17 families there were relatives with DCM without noncompaction, and in 7 families relatives with HCM without noncompaction were observed. In families with a mutation the yield of the family screening was higher than in families without a mutation (mutation 72%, without mutation 32%; p<0.001; central illustration; online table S2).

*Characteristics and outcome of the NCCM phenotypes; isolated NCCM, NCCM with DCM and NCCM with HCM*

The 216 patients diagnosed with NCCM were classified according to NCCM phenotype into; 92 isolated NCCM patients (51 index cases and 41 relatives), 115 NCCM with DCM patients (84 index cases and 31 relatives) and 9 NCCM with HCM patients (8 index cases and 1 relative; table 1). Affected relatives with NCCM had less severe clinical features at diagnosis and follow-up compared to NCCM index patients (table 1 and 2): 48% of the relatives with NCCM were asymptomatic compared to 24% of the index patients (p<0.001). The NCCM with DCM phenotype was more frequent in index cases (p=0.010) and LV systolic dysfunction was more frequent (p<0.001) in index cases than in affected relatives (table 1). Patients with isolated NCCM had less RV- and LV systolic dysfunction than NCCM patients with DCM or HCM (p=0.023, p<0.001; table 1). Patients with NCCM and HCM had more often hypertension (p=0.014). During a median follow-up of 44 months (IQR: 9 to 93 months), MACE occurred in 45 patients (table 2). The hazard ratios at follow-up showed that NCCM with DCM had highest risk for MACE (HR 2.29, CI95% 1.17-4.47, p=0.016; figure 2, table 3).

**Table 1: Characteristics of noncompaction cardiomyopathy (NCCM) phenotypes.**

| | All NCCM (n= 216) | NCCM index patients (n=143) | Relatives with NCCM (n=73) | p value | NCCM phenotypes | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Isolated NCCM (n=92) | NCCM with DCM (n=115) | NCCM with HCM (n=9) | p value |
| | | Genetic | Probably genetic | Sporadic | Genetic | Probably genetic | Sporadic | p value |
| Index patient (%) | 116 (54) | 143 | 73 | | 51 (55) | 84 (73) | 8 (89) | 0.010 |
| Male (%) | 116 (54) | 76 (53) | 40 (55) | NS | 44 (48) | 66 (57) | 6 (67) | NS |
| <18 age at presentation (%) | 35 (16) | 25 (17) | 10 (14) | NS | 15 (16) | 18 (16) | 2 (22) | NS |
| Median age at presentation (IQR) | 38 (23-52) | 40 (24-54) | 35 (22-48) | NS | 34 (22-48) | 40 (26-56) | 36 (33-45) | NS |
| Mutation (%) | 104 (48) | 63 (44) | 41 (56) | NS | 41 (45) | 58 (50) | 5 (56) | NS |
| Congenital heart defect (%) | 15 (7) | 9 (6) | 6 (8) | NS | 6 (7) | 7 (6) | 2 (22) | NS |
| Comorbidity† (%) | 48 (22) | 33 (23) | 15 (21) | NS | 15 (16) | 18 (16) | 5 (56) | 0.009 |
| Asymptomatic‡ (%) | 69 (32) | 34 (24) | 35 (48) | <0.001 | 39 (42) | 28 (24) | 2 (22) | 0.018 |
| Right bundle branch block (%) | 8 (4) | 6 (4) | 2 (3) | NS | 3 (3) | 5 (4) | 0 | NS |
| Left bundle branch block (%) | 27 (13) | 25 (17) | 2 (3) | 0.002 | 7 (8) | 18 (16) | 2 (22) | NS |
| Left atrial diameter >45 mm (%) | 39 (20) | 32 (25) | 7 (10) | 0.013 | 9 (12) | 26 (24) | 4 (44) | 0.019 |
| RV systolic dysfunction (%) | 38 (18) | 28 (20) | 10 (14) | NS | 9 (10) | 26 (23) | 3 (33) | 0.023 |
| LV systolic dysfunction (%) | 113 (52) | 87 (61) | 26 (36) | <0.001 | 25 (27) | 81 (70) | 7 (78) | <0.001 |

IQR: Interquartile range, NS: non-significant,

† Hypertension, hypercholesterolemia, coronary artery disease and diabetes

‡ s at presentation.

**Table 2: Follow-up of noncompaction cardiomyopathy (NCCM) phenotypes.**

| | All NCCM (n= 216) | NCCM index patients (n=143) | Relatives with NCCM (n=73) | p value | NCCM phenotypes | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Isolated NCCM (n=92) | NCCM with DCM (n=115) | NCCM with HCM (n=9) | p value |
| | | Genetic | Probably genetic | Sporadic | Genetic | Probably genetic | Sporadic | p value |
| Median follow-up months (IQR) | 44 (9-93) | 42 (9-85) | 50 (8-106) | NS | 51 (10-93) | 38 (8-93) | 80 (55-120) | NS |
| Stroke (%) | 8 (4) | 5 (3) | 3 (4) | NS | 4 (4) | 4 (3) | 0 | NS |
| Peripheral embolism (%) | 10 (5) | 10 (7) | 0 (0) | 0.010 | 4 (4) | 6 (5) | 0 | NS |
| (paroxysmal) Atrial fibrillation (%) | 25 (12) | 22 (15) | 3 (4) | 0.004 | 6 (7) | 15 (13) | 4 (44) | 0.034 |
| Ventricular tachycardia (%) | 25 (12) | 23 (16) | 2 (3) | 0.001 | 8 (9) | 16 (14) | 1 (11) | NS |
| Sustained VF/VT (%) | 13 (6) | 12 (8) | 1 (1) | NS | 4 (4) | 9 (8) | 0 | NS |
| Heart failure requiring hospitalization (%) | 42 (20) | 38 (27) | 4 (6) | <0.001 | 7 (8) | 33 (29) | 2 (22) | <0.001 |
| ICD (%) | 71 (33) | 57 (40) | 14 (19) | 0.001 | 19 (21) | 51 (44) | 1 (11) | <0.001 |
| Secondary prevention (%)* | 11 (16) | 11 (19) | 0 (0) | NS | 3 (16) | 8 (16) | 0 | NS |
| Appropriate shock (%)* | 5 (7) | 5 (9) | 0 (0) | NS | 0 | 5 (10) | 0 | NS |
| Heart transplant (%) | 7 (3) | 7 (5) | 0 (0) | 0.043 | 1 (1) | 6 (5) | 0 | NS |
| Deceased (%) | 20 (9) | 13 (9) | 7 (10) | NS | 4 (4) | 14 (12) | 2 (22) | NS |
| MACE (%) | 45 (21) | 35 (24) | 10 (14) | NS | 12 (13) | 31 (27) | 2 (22) | 0.040 |

IQR: inter quartile range,, VF: Ventricular fibrillation, VT: ventricular tachycardia
* ICD carriers.

| NCCM phenotype | MACE /100 patient years | HR (95% CI) | p-value |
|---|---|---|---|
| Isolated NCCM | 2.80 | 1.00 | NA |
| NCCM with DCM | 6.77 | 2.29 (1.17 - 4.47) | 0.016 |
| NCCM with HCM | 3.44 | 1.30 (0.29 - 5.81) | 0.733 |

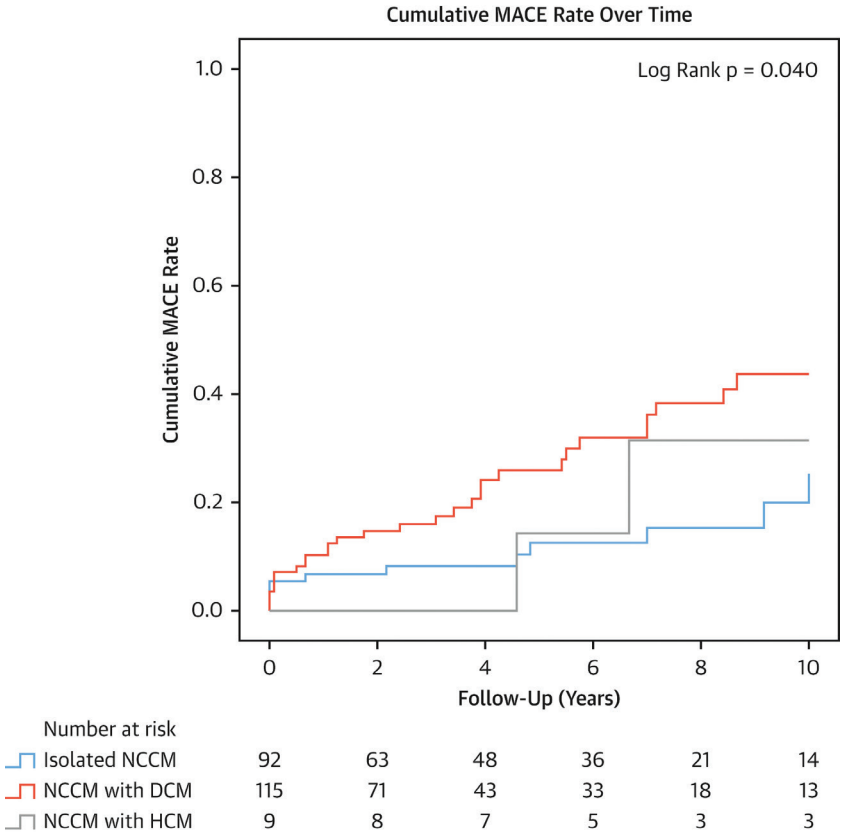



**Figure 1: Family screening for noncompaction cardiomyopathy (NCCM) .**
In 113 of 143 families (54 with a mutation) cardiologic screening identified 107 relatives with a cardiomyopathy; 73 with NCCM, 19 with DCM without noncompaction and 15 with HCM without noncomapction. Thirty-eight carriers of a familial mutation were unaffected..

**Table 3: MACE in Noncompaction cardiomyopathy (NCCM).**

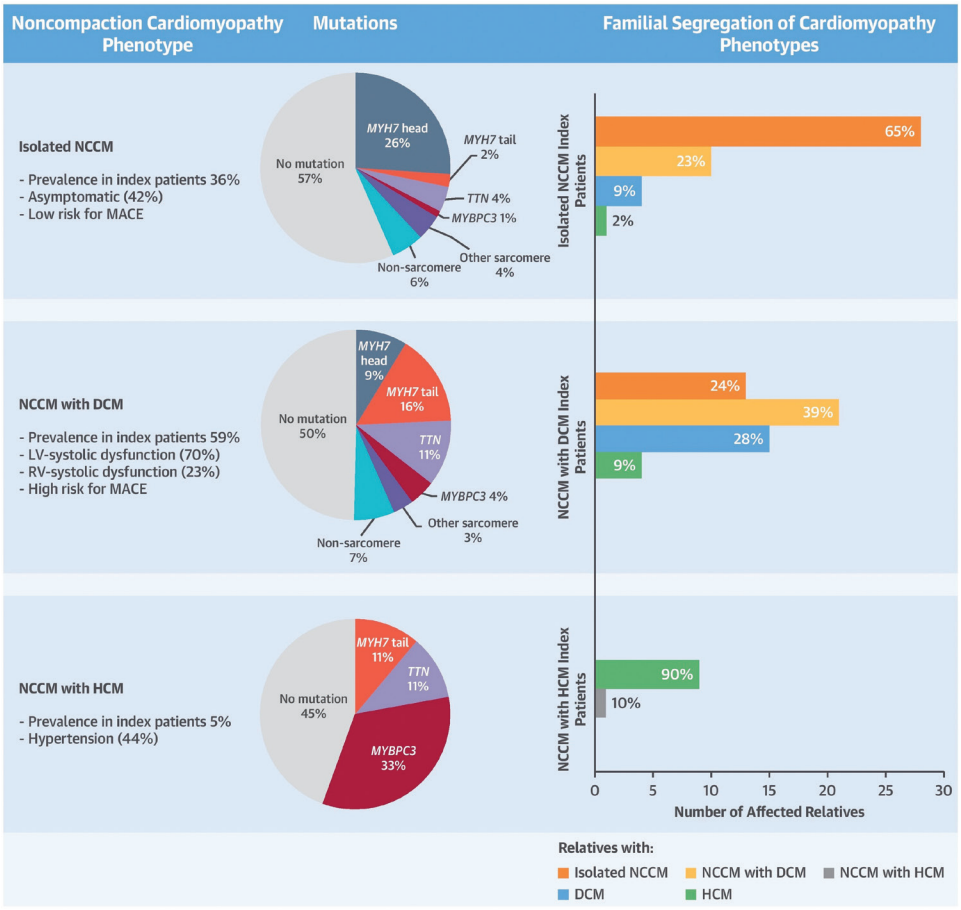
| Univariate analysis | MACE /100 patient years | HR (CI 95%) | p-value |
|---|---|---|---|
| Isolated NCCM | 2.80 | 1.00 | NA |
| NCCM with DCM* | 6.55 | 2.29 (1.17-4.47) | 0.016 |
| NCCM with HCM* | 3.44 | 1.30 (0.29-5.81) | 0.733 |
| Index patient | 5.69 | 1.95 (0.96-3.95) | 0.065 |
| Male | 4.44 | 0.91 (0.51-1.65) | 0.763 |
| <18 years at presentation | 3.24 | 1.46 (0.65-3.28) | 0.365 |
| Mutation | 4.93 | 1.2 (0.63-2.08) | 0.646 |
| Congenital heart disease | 1.02 | 0.21 (0.03-1.53) | 0.123 |
| Cardiovascular co-morbidities† | 8.24 | 2.08 (1.09-3.97) | 0.027 |
| Asymptomatic at presentation | 2.32 | 0.40 (0.18-0.90) | 0.027 |
| Right bundle branch block | 0 | 0.05 (0.00-14.32) | 0.292 |
| Left bundle branch block | 6.47 | 1.56 (0.75-3.24) | 0.239 |
| Left atrial diameter >45 mm | 6.39 | 1.55 (0.79-3.05) | 0.204 |
| Reduced LV systolic function | 7.34 | 3.16 (1.60-6.27) | 0.001 |
| Reduced RV systolic function | 8.36 | 2.47 (1.29-4.73) | 0.006 |

* Reference was isolated NCCM, † Hypertension, hypercholesterolemia, coronary art disease and diabetes.



*Familial segregation of NCCM phenotypes*

Of the 107 relatives diagnosed with a cardiomyopathy 73 had NCCM. Fifty relatives had the same NCCM phenotype as the index patient in the family. The risk of having isolated NCCM was higher for relatives of index patients with isolated NCCM than for relatives of NCCM with DCM index cases (p<0.001)(central illustration). NCCM with DCM in relatives occurred in families of index cases with isolated NCCM and NCCM with DCM. These patterns of familial segregation of NCCM phenotypes were observed in families with and in families without a mutation. In some families, all patients had the same phenotype; in 8 families all patients had isolated NCCM, in 9 families all had NCCM with DCM and in 1 family all had NCCM with HCM. In 17 families relatives were diagnosed with DCM or HCM without noncompaction. Relatives with DCM (without noncompaction) were most frequently observed in the families of the NCCM with DCM index cases (p=0.049). Relatives with HCM (without noncompaction) occurred only in families of index cases with NCCM with HCM (p<0.001;central illustration). Mutations in the *MYH7* head domain predicted isolated NCCM (OR 7.5 95%CI 2.9-19.5; p<0.001), while *MYH7* tail domain mutations predicted NCCM with DCM (OR 9.8 95%CI 2.8-34.0; p<0.001). *TTN* mutations predicted risk for DCM without noncompaction in relatives (OR 10.9 95%CI 2.3-51.1; p=0.002). Risk for HCM without noncompaction in relatives was increased in families with *MYBPC3* mutations (OR 585.0 95%CI 49.5-6915.4; p<0.001). In families of index cases without a mutation, fewer relatives were diagnosed with a cardiomyopathy, because there are probably fewer cases with a genetic cause in this group of patients.




**Central illustration.**
Classification of NCCM according to cardiac phenotype into isolated NCCM (51 index, 41 relatives), NCCM with DCM (84 index, 31 relatives) and NCCM with HCM (8 index and 1 relative) in 39 families with and 19 families without a mutation. Genotyping and family screening showed that isolated NCCM was linked to mutations in the head domain of MYH7 ($p<0.001$), isolated NCCM in relatives ($p<0.001$) and a lower risk for LV dysfunction ($p<0.001$). NCCM with DCM was linked to the MYH7 tail domain ($p<0.001$) and TTN and was associated with relatives with DCM without signs of noncompaction ($p=0.002$) and severe outcome ($p=0.016$). The HCM phenotype was linked to MYBPC3 in NCCM families ($p<0.001$) and HCM without signs of noncompaction in relatives ($p<0.001$). Factors reducing risk for relatives were absence of a mutation in index patients, non-penetrance of familial mutations or having asymptomatic disease. Underscoring that the NCCM phenotype of the index case and the genotype are important predictors of risk in relatives.

**Phenotypes of *MYH7* mutations**

In total 69 *MYH7* mutation carriers were identified of which 55 (23 index) had NCCM; 34 patients had a mutation in the head domain and 21patients had a mutation in the tail of *MYH7* (table 4). Nearly half (n=24, 44%) of the NCCM patients with a *MYH7* mutation were asymptomatic. Non-penetrance was observed in 12 (17%) of the *MYH7* mutation carriers (50% with a mutation in the head and 50% in the tail domain). NCCM with DCM was associated with, mutations in the tail domain of *MYH7* (outside of the *MYH7* p-loop) (29% isolated NCCM vs 86% NCCM with DCM, p<0.001; figure 3). Two relatives with a mutation in the tail domain of *MYH7* had DCM without noncompaction. Overall patients with a mutation in the *MYH7* head were younger at presentation (p=0.036). Mutations in the *MYH7* tail were associated with RV-dysfunction (6% vs. 33%, p=0.01). There was no difference in risk for MACE for patients with mutations in the head and tail domain of *MYH7*. Ebstein anomaly occurred in 4/34 of the patients with a *MYH7* mutation in the head domain; two NCCM and Ebstein patients from one family, and one family with a NCCM with Ebstein patient and a relative with Ebstein without noncompaction.

**Phenotypes of *TTN* mutations**

*TTN* mutations occurred in 30 cases; 18 (15 index) with NCCM, and 12 relatives with DCM. Ten (67%) of the mutations occurred in the A-band (table 4). Of the five patients with a mutation outside of the A-band, two had a complex genotype involving a *MIB1* mutation. Nine of the 14 relatives with a familial *TTN* mutation had no signs of a cardiomyopathy. For mutations outside of the A-band non penetrance (57% vs 6%, p=0.004), asymptomatic disease (2/3) and older age at diagnosis was observed (non-A-band 59 years vs A-band 39 years, p=0.006).

*Phenotypes of **MYBPC3** mutations*

Eleven *MYBPC3* mutations in 8 families with 9 NCCM patients were observed. Three NCCM index patients had two *MYBPC3* mutations (table 4). Three patients with a *MYBPC3* mutation had NCCM with HCM. Five patients with a *MYBPC3* mutation had NCCM with DCM and 1 had isolated NCCM, 7 (78%) had LV-dysfunction and 5 (56%) had a MACE. Eleven (33%) of the relatives with a mutation, 5 with the Dutch founder mutation c.2373dupG, had no signs of cardiomyopathy.

**Table 4: Mutations in *MYH7*, *TTN* and *MYBPC3* in familial non compaction cardiomyopathy (NCCM).**

| | MYH7 | | | | TTN | | | | *MYBPC3* |
|---|---|---|---|---|---|---|---|---|---|
| **Mutation carriers (n)** | **Head* (40)** | **Tail* (29)** | **Total (69)** | **p-value** | **Non A-band (14)** | **A-band (16)** | **Total (30)** | **p- value** | **(33)** |
| **NCCM index patient** | **11** | **12** | **23** | | **5** | **10** | **15** | | **8** |
| Isolated NCCM | 24 | 2 | 26 | | 2 | 2 | 4 | | 1 |
| NCCM with DCM | 10 | 18 | 28 | | 3 | 10 | 13 | | 5 |
| NCCM with HCM | 0 | 1 | 1 | | 0 | 1 | 1 | | 3 |
| DCM | 0 | 2 | 2 | | 1 | 2 | 3 | | 0 |
| HCM | 0 | 0 | 0 | | 0 | 0 | 0 | | 13 |
| Non-penetrance | 6 | 6 | 12 | | 8 | 1 | 9 | | 11 |
| **Patients with NCCM** | 34 | 21 | 55 | | 5 | 13 | 18 | | 9 |
| Age at presentation yrs | 28 | 38 | 32 | 0.036 | 59 | 39 | 45 | 0.006 | 34 |
| Asymptomatic (%) | 16 (47) | 8 (38) | 24 (44) | NS | 1 (20) | 2 (15) | 3 (17) | NS | 3 (33) |
| LV systolic dysfunction (%) | 14 (41) | 14 (67) | 28 (51) | NS | 4 (80) | 10 (77) | 14 | NS | 7 (78) |
| RV systolic dysfunction (%) | 2 (6) | 7 (33) | 9 (17) | 0.020 | 0 | 6 (15) | 6 (33) | NS | 0 |
| MACE (%) | 3 (9) | 3 (14) | 6 (11) | NS | 0 | 5 (38) | 5 (28) | NS | 5 (56) |

DCM: dilated cardiomyopathy, HCM: hypertrophic cardiomyopathy, LV: left ventricular, MACE: major adverse cardiac events, NCCM: noncompaction cardiomyopathy, RV: right ventricular. *head is the p-loop of MYH7 ending at c.2523, the tail was the rest of the MYH7 gene.

## Discussion

Prediction of risk for a cardiomyopathy phenotype and associated risk for adverse events is important for counseling the relatives of NCCM patients and eventually tailoring cardiologic family screening. This study showed that the cardiac features and the genetic defect of index cases may help to predict the cardiomyopathy phenotype and associated risk for relatives. Familial segregation of isolated NCCM, NCCM with DCM, or NCCM with HCM was observed as well as families in which a range of different NCCM phenotypes occurred. When the index case had isolated NCCM, relatives were more likely to have similarly isolated NCCM, linked to a better prognosis with less RV- and LV systolic dysfunction and low risk for MACE. Almost half of the isolated NCCM patients had a mutation, predominantly in the head domain of *MYH7*. NCCM with DCM was overall the most frequent NCCM phenotype and was associated with mutations in the tail domain of *MYH7* and in *TTN* with increased risk for LV systolic dysfunction and MACE in patients. Relatives of index cases with NCCM with DCM had an increased risk of having DCM without hypertrabiculation, compared to the relatives of the index cases with other NCCM features. For relatives of index cases with NCCM with HCM risk for HCM without noncompaction was increased.



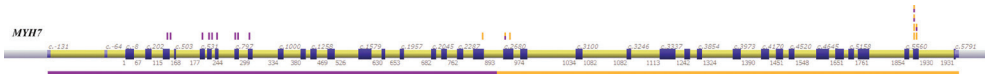



**Figure 3: MYH7 mutations in NCCM.**
Head, purple, families with isolated NCCM. Tail, yellow, families with NCCM with DCM. Mixed purple and yellow mark, families with both isolated NCCM and NCCM with DCM. Mutations in the head, isolated NCCM phenotype vs in the tail, NCCM with DCM ($p<0.001$). Figure adapted from Alamut® visual -interactive biosoftware, 12-2016.

### *NCCM phenotypes*

The cardiomyopathy phenotypes in relatives included DCM without signs of NCCM, in particular in families of index cases with NCCM with DCM. It is unknown if LV dilation in NCCM with DCM is secondary to advanced NCCM or represents hypertrabeculation that may occur in a distinct subgroup of DCM patients. In this study 70% of the patients with NCCM with DCM had heart-failure, indicating that in NCCM, like in other cardiac diseases, progressive heart-failure may lead to LV dilatation. On the other hand a normal LV function in approximately 30% of the patients with NCCM and DCM could not explain the LV dilatation. Similarly, the novel DCM diagnostic criteria include DCM without LV dysfunction(173). Our results suggest that mutations in the tail of *MYH7* and in *TTN* may predispose to LV dilatation, with or without LV dysfunction and in some cases with RV dysfunction. Most important is that there was no apparent difference

in outcome for DCM, with or without hypertrabeculation(174). Similarly, in a smaller number of cases, the nosology of concomitant NCCM with HCM remains part of the poorly understood spectrum of hypertrabeculation. In families with a mutation, relatives with DCM and HCM, all had the familial mutation, and therefor belong together with NCCM to a wider cardiomyopathy spectrum. The mechanism of hypertrabeculation needs to be explored by focusing on the role of additional genetic defects or non-genetic factors.

*Risk for relatives*

Family screening showed that relatives had less severe cardiac features than the index patients among all subtypes of NCCM, which can be explained by early detection through screening that allows early treatment and prevention of severe complications. Risk for finding a cardiomyopathy in relatives was higher in families from index cases with a mutation, than for families without a mutation. Nevertheless, cardiologic family screening is recommended for all cases, because family screening may also identify asymptomatic relatives with a cardiomyopathy in families without evidence for genetic disease(8). The fact that familial NCCM occurs in families with and without a mutation, showed that not all genetic causes for NCCM have been identified. Although we cannot exclude unkown genetic defects in cases without a mutation, it is more likely that non-genetic causes with a low genetic risk are involved than unkown genetic causes, because the relatives had low risk for cardiomyopathy.

*Genes*

NCCM phenotypes were related to genetic causes. The *MYH7* gene was a major genetic cause for NCCM. The location of the mutations in *MYH7* could predict cardiac phenotypes. An explanation for the association between mutations in the tail and NCCM with DCM could be that mutations in the tail domain may interfere with the binding site for *TTN*, and thus may have a similar effect as *TTN* mutations, which are important causes of DCM and also predict DCM without NCCM in relatives. Although mutations in the head of *MYH7* were previously associated with HCM our study did not endorse that *MYH7* head mutations was related to NCCM with HCM(52). Our results endorse the previously reported association of concomitant Ebstein anomaly and NCCM with *MYH7* mutations. Similarly *MYBPC3*, a major cause for HCM, was observed in families with NCCM with HCM and increased risk for HCM without hyertrabiculation in relatives.

*Study Limitations*

Not all participating NCCM index cases had NGS DNA testing using the latest genetic cardio-panel, indicating the possibility of underreporting of genetic causes. Another cause of underreporting of familial disease might be that cardiomyopathy phenotypes in families may have been missed because not all relatives participated. Given the retrospective design of the study, clinical data of index cases and relatives may be missing. Furthermore, age-dependent penetrance may play a role rendering more relatives affected in the future.

**Conclusions**

NCCM phenotypes of index patients and the genetic defect may predict the cardiomyopathy phenotype and the severity of the disease in relatives. The strongest familial segregation of NCCM phenotypes was observed for isolated NCCM. NCCM with DCM was associated with *MYH7* tail domain and *TTN* mutations, with worse outcome and with DCM without NCCM in relatives. NCCM with HCM was related to *MYBPC3,* and HCM without NCCM in relatives.



**Perspectives**

*Competency in knowledge:*

In familial NCCM, the genetic cause and the distinct NCCM phenotype, of isolated NCCM, NCCM with DCM, or NCCM with HCM, of index cases may help predict risk for relatives.

*Translational outlook:*

The etiologic and genetic heterogeneity of NCCM demands stratification for genetic risk to distinguish families with a high genetic burden, and high risk for relatives. Families of patients where NCCM may be caused by non-genetic causes or by (yet unknown) genetic causes with small effects, (like genetic modifiers), may have low risk for relatives. Ultimately designing a risk model to predict risk for relatives with genetic and clinical data of index case may be achieved by collecting data from large family screening studies.

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## Online supplement

**Table S1: (likely) pathogenic mutations.**

| Gene | NM-Number | Nucleotide change | Amino change |
|---|---|---|---|
| ACTC1 | NM_0051594.4 | c.811A>G | p.(Met271Val) |
| ACTN2 | NM_001103.2 | c.574C>T | p.(Arg192*) |
| ACTN2 | NM_001103.2 | c.586delG | p.(Asp196fs) |
| DSP | NM_004415.2 | c.3084+1G>A | p.(?) |
| DSP | NM_004415.2 | c.6687delA | p.(Arg2229fs) |
| LMNA | NM_170707.3 | c.1608+5G>C | p.(?) |
| MIB1 | NM_020774.2 | c.2589_2590dupTG | p.(Glu864fs) |
| MIB1 | NM_020774.2 | c.2701C>T | p.(Arg901*) |
| MIB1 | NM_020774.2 | c.2827G>T | p.Val943Phe |
| MYBPC3 | NM_000256.3 | c.1831G>A | p.(Glu611Lys) |
| MYBPC3 | NM_000256.3 | c.2373dupG | p.(Trp792fs) |
| MYBPC3 | NM_000256.3 | c.2827C>T | p.(Arg943*) |
| MYBPC3 | NM_000256.3 | c.442G>A | p.(Gly148Arg) |
| MYBPC3 | NM_000256.3 | c.897delG | p.(Lys301fs) |
| MYBPC3 | NM_000256.3 | c.932C>A | p.(Ser311*) |
| MYH7 | NM_000257.2 | c.1633G>A | p.(Asp545Asn) |
| MYH7 | NM_000257.2 | c.2678C>T | p.(Ala893Val) |
| MYH7 | NM_000257.2 | c.2713T>C | p.(Cys905Arg) |
| MYH7 | NM_000257.2 | c.2863G>A | p.(Asp955Asn) |
| MYH7 | NM_000257.2 | c.415G>T | p.(Val139Leu) |
| MYH7 | NM_000257.2 | c.495G>A | p.(Met165Ile) |
| MYH7 | NM_000257.2 | c.547G>A | p.(Gly183Arg) |
| MYH7 | NM_000257.2 | c.5754C>G | p.(Asn1918Lys) |
| MYH7 | NM_000257.2 | c.5773C>G | p.(Arg1925Gly) |
| MYH7 | NM_000257.2 | c.689T>C | p.(Phe230Ser) |
| MYH7 | NM_000257.2 | c.715_717delGAC | p.(Asp239del) |
| MYH7 | NM_000257.2 | c.732+1G>A | p.(?) |
| MYH7 | NM_000257.2 | c.798T>A | p.(Tyr266*) |
| MYH7 | NM_000257.2 | c.847T>G | p.(Tyr283Asp) |
| MYH7 | NM_000257.2 | c.902T>A | p.(Leu301Gln) |
| PLN | NM_002667.3 | c.25C>T | p.(Arg9Cys) |
| PLN | NM_002667.3 | c.40_42delAGA | p.(Arg14del) |
| PRDM16 | NM_022114.3 | c.2848C>T | p.(Arg950*) |

| | | | |
|---|---|---|---|
| PRDM16 | NM_022114.3 | c.56delA | p.(Asn19fs) |
| RBM20 | NM_001134363.1 | c.1900C>T | p.(Arg634Trp) |
| RYR2 | NM_001035.2 | c.169-198_273+823del | p.(?) |
| SCN5A | NM_198056.2 | c.4299+1delG | p.(?) |
| TAZ | NM_000116.3 | c.646G>A | p.(Gly216Arg) |
| TNNT2 | NM_001001430.1 | c.629_631delAGA | p.(Lys210del) |
| TPM1 | NM_000366.5 | c.250G>A | p.(Asp84Asn) |
| TTN | NM_001267550.1 | c.104421_104425del5 | p.(Arg34807fs) |
| TTN | NM_001267550.1 | c.4459G>T | p.(Glu1487*) |
| TTN | NM_001267550.1 | c.4583G>A | p.(Trp1528*) |
| TTN | NM_001267550.1 | c.48313-1G>A | p.(?) |
| TTN | NM_001267550.1 | c.49917delC | p.(Ser16640fs) |
| TTN | NM_001267550.1 | c.53656_53663del8 | p.(Pro17886*) |
| TTN | NM_001267550.1 | c.68484delG | p.(Lys22828fs) |
| TTN | NM_001267550.1 | c.74666dupA | p.(Tyr24889*) |
| TTN | NM_001267550.1 | c.77254_77255delAA | p.(Lys25752fs) |
| TTN | NM_001267550.1 | c.7967delG | p.(Gly2656fs) |
| TTN | NM_001267550.1 | c.80514delA | p.(Val26839fs) |
| TTN | NM_001267550.1 | c.82309_82312dupGGTA | p.(Asn27438fs) |
| TTN | NM_001267550.1 | c.89165delC | p.(Pro29722fs) |
| TTN | NM_001267550.1 | c.92683C>T | p.(Arg30895*) |

**Table S2: The correlation between clinical features and cardiac phenotype of NCCM index cases and the cardiac phenotype of affected relatives.**

| | Cardiomyopathy 107/466 | | | | Isolated NCCM 41/107 | | | | NCCM with DCM 31/107 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Index case | n=107 | OR | 95%CI | P-value | n=41 | OR | 95%CI | P-value | n=31 | OR | 95%CI | P-value |
| Under 18 (97) | 25 | 1.16 | 0.69-1.94 | 0.575 | 7 | 0.54 | 0.20-1.43 | 0.214 | 4 | 0.38 | 0.12-1.22 | 0.104 |
| Isolated NCCM (164) | 43 | 1.33 | 0.85-2.07 | 0.210 | 28 | 7.32 | 3.06-17.55 | <0.001 | 10 | 0.62 | 0.26-1.50 | 0.287 |
| NCCM with DCM (266) | 54 | 0.71 | 0.46-1.09 | 0.117 | 13 | 0.28 | 0.12-0.65 | 0.003 | 21 | 2.74 | 1.14-6.59 | 0.025 |
| NCCM with HCM (36) | 10 | 1.29 | 0.61-2.77 | 0.506 | 0 | 0 | 0 | 0.999 | 0 | 0 | 0 | 0.999 |
| Asymptomatic (93) | 33 | 2.17 | 1.33-3.56 | 0.002 | 9 | 0.48 | 0.20-1.18 | 0.108 | 4 | 0.24 | 0.08-0.74 | 0.013 |
| LV systolic dysfunction (285) | 56 | 0.66 | 0.42-1.01 | 0.057 | 19 | 0.65 | 0.30-1.43 | 0.289 | 20 | 1.97 | 0.83-4.67 | 0.124 |
| RV systolic dysfunction (103) | 18 | 0.68 | 0.39-1.19 | 0.172 | 6 | 0.76 | 0.26-2.21 | 0.610 | 8 | 2.26 | 0.80-6.42 | 0.126 |
| MACE (143) | 26 | 0.67 | 0.41-1.09 | 0.107 | 10 | 0.99 | 0.40-2.45 | 0.979 | 7 | 0.86 | 0.32-2.31 | 0.765 |
| Mutation (276) | 80 | 2.38 | 1.47-3.85 | <0.001 | 27 | 0.47 | 0.20-1.15 | 0.098 | 25 | 1.59 | 0.57-4.43 | 0.374 |
| *MYH7* (114) | 44 | 2.95 | 1.85-4.70 | <0.001 | 22 | 2.26 | 1.02-5.04 | 0.046 | 19 | 3.17 | 1.33-7.54 | 0.009 |
| *MYH7* head (69) | 29 | 2.99 | 1.75-5.11 | <0.001 | 21 | 7.48 | 2.86-19.55 | <0.001 | 8 | 0.89 | 0.35-2.31 | 0.818 |
| *MYH7* tail (45) | 15 | 1.85 | 0.95-3.58 | 0.069 | 1 | 0.09 | 0.01-0.72 | 0.023 | 11 | 9.76 | 2.81-33.98 | <0.001 |
| *TTN* (44) | 8 | 0.75 | 0.34-1.66 | 0.477 | 1 | 0.21 | 0.03-1.75 | 0.148 | 2 | 0.79 | 0.15-4.16 | 0.793 |
| *MYBPC3* (45) | 14 | 1.49 | 0.77-2.89 | 0.242 | 1 | 0.10 | 0.01-0.80 | 0.030 | 0 | 0 | 0 | 0.998 |

**Table S2 continued: The correlation between clinical features and cardiac phenotype of NCCM index cases and the cardiac phenotype of affected relatives.**

| | NCCM with HCM 1/107 | | DCM 19/107 | | | | HCM 15/107 | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Index case | n=1 | OR | n=19 | OR | 95%CI | P-value | n=15 | OR | 95%CI | P-value |
| Under 18 (97) | 0 | NA | 2 | 0.35 | 0.075-1.656 | 0.187 | 12 | 24.00 | 5.95-96.82 | <0.001 |
| Isolated NCCM (164) | 0 | NA | 4 | 0.34 | 0.103-1.091 | 0.069 | 1 | 0.09 | 0.01-0.67 | 0.020 |
| NCCM with DCM (266) | 0 | NA | 15 | 4.71 | 1.447-15.339 | 0.010 | 5 | 0.44 | 0.14-1.38 | 0.160 |
| NCCM with HCM (36) | 1 | NA | 0 | 0 | 0 | 0.999 | 9 | 136.50 | 14.75-1263.13 | <0.001 |
| Asymptomatic (93) | 0 | NA | 11 | 4.71 | 1.628-13.654 | 0.004 | 9 | 4.19 | 1.35-13.01 | 0.013 |
| LV systolic dysfunction (285) | 0 | NA | 10 | 1.14 | 0.412-3.163 | 0.799 | 6 | 0.55 | 0.18-1.66 | 0.287 |
| RV systolic dysfunction (103) | 1 | NA | 4 | 1.51 | 0.433-5.268 | 0.518 | 0 | 0 | 0 | 0.998 |
| MACE (143) | 0 | NA | 4 | 0.86 | 0.255-2.879 | 0.803 | 5 | 1.67 | 0.51-5.42 | 0.396 |
| Mutation (276) | 0 | NA | 15 | 1.33 | 0.399-4.410 | 0.644 | 13 | 2.43 | 0.51-11.52 | 0.265 |
| *MYH7* (114) | 0 | NA | 3 | 0.23 | 0.062-0.848 | 0.027 | 0 | 0 | 0 | 0.997 |
| *MYH7 head* (69) | 0 | NA | 0 | 0 | 0 | 0.998 | 0 | 0 | 0 | 0.998 |
| *MYH7 tail* (45) | 0 | NA | 3 | 1.27 | 0.318-5.040 | 0.737 | 0 | 0 | 0 | 0.998 |
| *TTN* (44) | 0 | NA | 5 | 10.90 | 2.323-51.127 | 0.002 | 0 | 0 | 0 | 0.999 |
| *MYBPC3* (45) | 0 | NA | 0 | 0 | 0 | 0.999 | 13 | 585.00 | 49.49-6915.38 | <0.001 |

# Chapter 4

## Diagnostic CMR Imaging Criteria in Noncompaction Cardiomyopathy and the Yield of Genetic Testing

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*Submitted*

**Abstract**

Hypertrabeculation is fairly common in healthy study populations. Genetic testing plays an important role distinguishing genetic from non-genetic noncompaction cardiomyopathy patients. A retrospective studyof 62 noncompaction cardiomyopathy patients, of whom 53% had a (likely) pathogenic variant ((L)PV) showed that correlation between different CMR diagnostic criteria varied from moderate to very strong. In multivariate regression analysis independent positive predictors for having a (L)PV were familial cardiomyopathy, trabecular mass, and meeting Petersen criteria in ≥2 long axis views, while left bundle branch block and hypertension were negative predictors. These predictors may help guiding referral for genetic diagnostics, cascade screening.

## Introduction

Noncompaction is characterized by excessive trabeculations of the left ventricle (LV) (165), and includes patients with a genetic cardiomyopathy (CMP) and patients with secondary, pathologic remodeling of the LV. In addition, physiologic hypertrabeculation occurs in people without CMP (175). In the patients with genetic NCCM identification of a (likely) pathogenic variant ((L)PV) allows to perform genetic cascade family screening, identify accurately relatives with the genetic risk for having a CMP. In this way, early (subclinical) detection may help to reduce morbidity and mortality of cardiomyopathies. In the non-genetic NCCM patients (without a (L)PV or a family history) a low risk for relatives is expected .

For the diagnosis of noncompaction many different, partially overlapping, echocardiographic and cardiac magnetic resonance (CMR) imaging criteria have been proposed (21, 139, 176-179). On echocardiography the Jenni criteria (end-systolic short axis noncompacted (NC)/compacted (C) ratio >2) and on CMR the Petersen criteria (end-diastolic long-axis diastolic NC/C ratio >2.3) are used most frequently in clinic practice (20). Population surveys suggested that up to 40% of thepopulation may have signs of hypertrabeculation meeting diagnostic criteria for NCCM. Since there is a wide range of noncompaction in patients with (genetic) NCCMis may be complicated to distinguish NCCM from hypertrabeculation in healthy people. In this perspective, recognizing cases with a high risk of having a genetic cardiomyopathy, before doing a genetic test may help to identify the patients and their families who would benefit most from referral for genetic counseling and DNA testing.

In NCCM like in hypertrophic CMP, younger age at presentation and family history of CMP, are associated with having a (L)PV (180, 181). In hypertrophic CMP also morphological characteristics (i.e. maximum wall thickness of the LV and reverse curve morphology of the septum) may help distinguish genetic from sporadic disease (182). For NCCM it has not been established if cardiac morphology of NCCM is related to genetic cause. The aim of the current study was to assess the association between clinical and morphological characteristics, and find which of the different proposed diagnostic CMR criteria allows distinguishing NCCM patients with high risk of having a mutation.

## Methods

*Study population*

This retrospective study consisted of 62 adult NCCM patients diagnosed by echocardiography using Jenni criteria (21), who also had CMR and were referred to the clinical genetics department in the Erasmus Medical Center between 2006 and 2017. Due to the retrospective study design the Medical Research Involving Human Subjects Act did not apply for this study. Clinical data were retrieved from the medical records of the patient, including age, gender, body surface area (BSA), ECG and echocardiography. The date of CMR was defined as baseline. Familial CMP was defined as having at least one first-degree relative with a non-ischemic CMP. Information on the occurrence of adverse cardiac events at follow-up was collected from the medical records. Heart failure that required hospitalization was defined as hospitalization for new-onset or worsening signs of chronic heart failure.. Ventricular arrhythmias included sustained ventricular tachycardia, ventricular fibrillation, or appropriate ICD shock.

*DNA variant classification*

Ascertainment of family history, genetic counseling, and DNA testing were performed at the clinical genetics department . DNA was isolated from peripheral blood with informed consent of the patients. DNA next generation panels for testing CMP genes were gradually introduced starting with 8 genes in 2006 to 52 CMP genes in 2017. Of the 46 index patients, 35 (76%) has been tested with next generation sequencing panel of ≥42 cardiomyopathy genes. In 3 (10%) of the patients without a (L)PV less than 42 cardiomyopathy genes were tested. Assessment of the pathogenic effect of genetic variants was performed with the use of Alamut Interactive Biosoftware (Rouen, France). The classification system of variants was adapted from the proposed sequence variation classification of the American College of Medical Genetics (55).Variants classified as likely pathogenic (class IV) or pathogenic (class V) were included in this study.

*CMR protocol*

CMR imaging was performed on clinical 1.5T (n=46) and 3T (n=16) MRI systems (Signa Excite, n=27; Discovery MR750, n=16; Discovery MR450, n=12; Signa HDxt, n=6; Signa Genesis, n=1, all GE Healthcare, Milwaukee, WI, USA). Functional imaging was performed using a segmented, balanced steady-state free precession cine imaging with breath holding. Cine images were performed with slice thickness 8mm and interslice gap of 2mm. Late gadolinium enhancement (LGE) images were acquired 10–15 min after the administration of 0.1-0.2mmol/kg of gadolinium-based contrast agent using an inversion-recovery gradient-echo pulse sequence

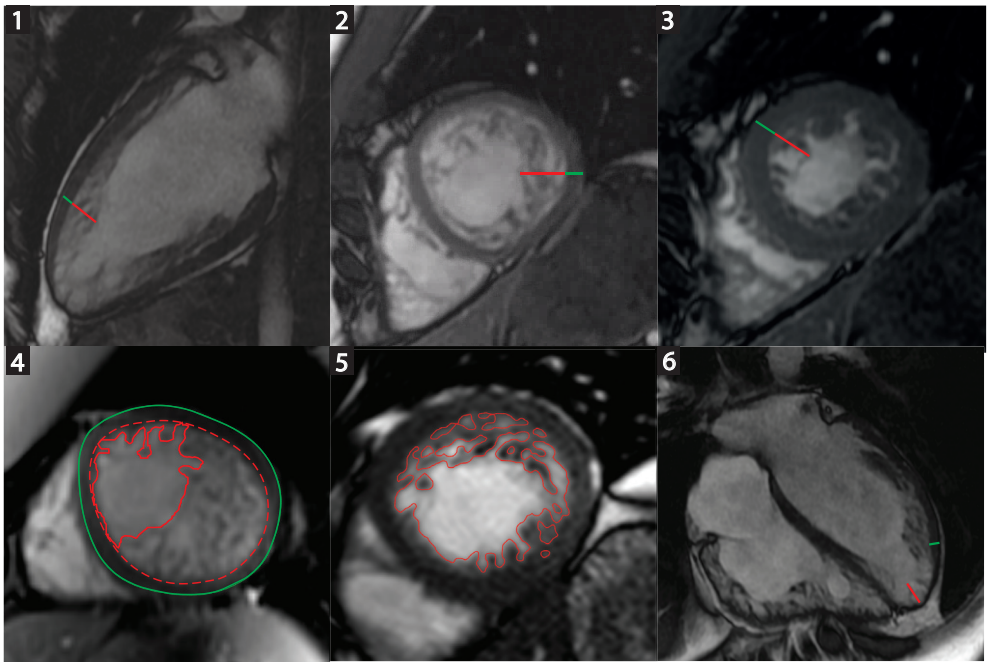
with continued adjustment of inversion time to null normal myocardium. Cine and LGE images were acquired in standard long-axis views and short axis orientations covering the entire LV.

*CMR analysis*

All CMR images were evaluated blinded to the results of DNA testing. On the cine short-axis stack endo- and epicardial contours were manually traced in end-diastole and end-systole according to Society for Cardiovascular Magnetic Resonance guidelines on CMR image post-processing (183), including papillary muscles and excluding trabeculations, to determine LV end-diastolic volume, end-systolic volume, ejection fraction (EF) and mass. Volumes and mass were corrected for BSA calculated using the formula of Haycock (184). Maximum thickness of the septal and inferolateral wall in the end-diastole were measured.

For global longitudinal strain (GLS) measurements two-dimensional feature tracking CMR analyses were performed. GLS was assessed by manually drawing endocardial borders, without inclusion of papillary muscles and trabeculations, in both end-diastolic and end-systolic phases at the long axis 2, 3 and 4 chamber (CH) views. Subsequently, endocardial borders were automatically tracked during the entire cardiac cycle. Visual assessment of the endocardial feature tracking was performed, in case of inaccurate tracking the endocardial contours were adjusted. Counterclockwise rotation was assigned a positive value and clockwise rotation a negative value. Absolute twist was the sum of the basal and apical rotation, neglecting the clockwise or counterclockwise direction (185).

The following diagnostic CMR criteria described in the literature as markers for NCCM were measured and summarized in figure 1: 1. Petersen criteria in long-axis (139), 2. Petersen criteria in short-axis (139), 3. Stacey criteria in short-axis (176), 4. Jacquier criteria using LV mass (177), 5. Captur criteria using fractal dimension (FD) (178) and 6. Choi criteria (179).




| CMR criteria | Measurement | Cut off for positive criteria |
|---|---|---|
| 1. Petersen long axis (4) | Ratio of NC/C layer measured during end-diastole in all 3 long-axis views (2, 3 and 4 chamber) | Ratio >2.3 in any long-axis view |
| 2. Petersen short axis (4) | Ratio of NC/C layer measured during end-diastole in short axis | Ratio >2.3 in any segment |
| 3. Stacey (5) | Ratio of NC/C layer measured during end-systole in short axis | Ratio ≥2.0 in any segment |
| 4. Jacquier (6) | [LV mass with trabeculations - LV mass without trabeculations]/ [LV mass without trabeculations] x 100 measured in end-diastole | >20% |
| 5. Captur (7) | Fractal dimension measured in short axis slices of the apical third of the left ventricle | >1.30 in any slice |
| 6. Choi (8) | Ratio of the thickness of apical trabeculation to that of C myocardium in the apical lateral segments (apex/C ratio) measured during end-diastole in all 3 long-axis views | Ratio >3.15 in any long-axis view |

**Figure 1: CMR imaging criteria in noncompaction cardiomyopathy .**
Petersen criteria in long-axis NC/C ratio assessed by evaluating long-axis 2, 3, and 4CH cine images for a distinct 2-layered appearance of a NC endocardial layer versus a C endocardial layer during end-diastole. The maximal NC layer and the according C wall thickness were measured in the same segment. The LV true apex and papillary muscles were excluded from the measurements. A NC/C ratio of >2.3 in any segment during end-diastole was the diagnostic requirement for the Petersen long-axis criteria (139).

*Petersen criteria in long-axis* NC/C ratio assessed by evaluating long-axis 2, 3, and 4CH cine images for a distinct 2-layered appearance of a NC endocardial layer versus a C endocardial layer during end-diastole. The maximal NC layer and the according C wall thickness were measured in the same segment. The LV true apex and papillary muscles were excluded from the measurements. A NC/C ratio of >2.3 in any segment during end-diastole was the diagnostic requirement for the Petersen long-axis criteria (139). *Petersen criteria and Stacey criteria in short-axis* The LV was divided according to the 17 segments model of the LV by the American Heart association using

short-axis stack cine images (186). In 16 segments the maximum NC and the according C layer was measured. Segment 17 (the apex) and the papillary muscles were excluded from the measurements. The diagnostic criteria used for short-axis end-diastole were a NC/C ratio of >2.3 in end-diastole for Petersen criteria (139) and a NC/C ratio of ≥2.0 in end-systole for Stacey criteria (176). *Jacquier criteria using LV mass* After measuring LV end-diastolic mass (including papillary muscles), a second measurement was performed where we adjusted the endocardial tracings to include also the trabeculations. The percentage of trabeculation was derived by the following equation: [LV mass with trabeculations - LV mass without trabeculations]/[LV mass without trabeculations] x100. A percentage of >20% end-diastolic LV trabeculated mass of total LV mass was diagnostic for the Jacquier criteria (177). Also the higher cut-off of >35% proposed by Choi et al. was used (179). *Captur criteria using fractal dimension* We recorded maximal FD for the apical third of the LV excluding the most apical short-axis slice because of partial voluming. A maximum FD of ≥1.30 for the apical third was considered diagnostic for the Captur criteria (178). Eleven patients were not eligible for analysis because automated contour detection and FD assessment failed. This was mainly due to low contrast between blood and myocardium/trabeculations (1.5T 8 (17%) and 3.0T 3 (19%)). *Choi criteria* We measured the ratios of the thickness of apical trabeculation to that of C myocardium in the apical lateral segments (apex/C ratio) in all 3 long axis views. A ratio of >3.15 was diagnostic for the Choi criteria (179). We also scored the presence of an apical type NCCM, which was defined by Choi et al. as involving >3 segments meeting a NC/C ratio in diastole of >2.3 in the apical level (segment 13 through 17) (179). *Number of criteria* To evaluate how many diagnostic criteria were fulfilled per patient we included the sum of fulfilled criteria. We included: 1. the Petersen long axis, 2. Petersen short axis, 3. Stacey short axis, 4. Jacquier with the cut-off by Choi et al. and 5. Choi criteria. Choi's cut-off (>35% trabeculae) for the Jacquier criteria was used, because this cut-off has higher sensitivity (179). The Captur criteria were not included because the FD could not be measured in all patients. Also this criterion was not significant in the univariate regression analysis.

For CMR imaging analysis post processing ReportCARD 4.0 (GE Healthcare, Milwaukee, WI, USA) and Medis software were used (QMass software version 8.1 and Qstrain software version 2.0, Medis, Leiden, The Netherlands). To calculate the FD we used software that was made available by Captur et al. (187) on OsiriX lite (version 8.5, Pixmeo SARL, Geneva, Swiss).

*Volunteers*

Because normal values of GLS, absolute twist, and FD are not yet well established and may vary depending on the software, these parameters were measured in 18 adult healthy volunteers (age 32 ± 7 years, 33% male). These volunteers were previously described by van der Zwaan et al. and were scanned on Signa Excite 1.5T scanner (188). In 1 volunteer the FD could not be correctly assessed. The GLS was -24 ± 3%, absolute twist 12.7 ± 7.0°, and maximum FD of the apical third was 1.30 ± 0.08. Using the cut off ≥1.30, 8 (47%) fulfilled the criteria of Captur.

*Statistical analysis*

Statistical analysis was performed with SPSS statistical software, version 25.0 (SPSS Inc., Chicago, IL). Continuous variables were compared by Mann-Whitney U test because small samples are often not normally distributed. Categorical data were compared with Pearson Chi-Squared or with Fishers exact test when appropriate. Odds ratios (OR) with 95% confidence interval (CI) for having a (L) PV were calculated using binary logistic regression. To identify independent predictors of a positive genetic test, variables were tested in a multivariate logistic regression analysis using forward stepwise selection. Variables were entered if the p-value was less than 0.10 and removed if the p-value was more than 0.10. We used receiver operating characteristics (ROC) curves to determine the area under the curve (AUC) of the different diagnostic criteria and the multivariate logistic regression model.

## Results

**Table 1: Baseline characteristics of NCCM patients.**

| | Total (n=62) | (L)PV (n=33) | No (L)PV (n=29) | p-value |
|---|---|---|---|---|
| Male | 35 (56%) | 18 (55%) | 17 (59%) | 0.75 |
| Caucasian | 54 (87%) | 31 (94%) | 23 (79%) | 0.13 |
| Body surface area, m2 | 2.0 (0.3) | 1.9 (0.3) | 2.0 (0.3) | 0.52 |
| Age at CMR, years | 39 (16) | 38 (15) | 40 (16) | 0.71 |
| Familial cardiomyopathy | 35 (56%) | 23 (70%) | 12 (41%) | 0.03 |
| Diagnosed by family screening | 17 (27%) | 11 (33%) | 6 (21%) | 0.27 |
| Diabetes Mellitus | 2 (3%) | 1 (3%) | 1 (3%) | 0.93 |
| Hypertension | 9 (15%) | 2 (6%) | 7 (24%) | 0.02 |
| Coronary artery disease | 1 (2%) | 0 (0%) | 1 (3%) | 0.47 |
| Left bundle branch block | 10 (16%) | 2 (6%) | 8 (28%) | 0.04 |
| **CMR parameters** | | | | |
| LV EDV index, ml/m² | 117 (32) | 123 (34) | 111 (29) | 0.25 |
| Dilated LV* | 43 (69%) | 26 (79%) | 17 (59%) | 0.09 |
| LV ESV index, ml/m² | 70 (30) | 75 (33) | 63 (25) | 0.10 |
| LV EF, % | 42 (11) | 41 (12) | 44 (9) | 0.32 |
| LV EF <40% | 19 (31%) | 11 (33%) | 8 (28%) | 0.78 |
| LV mass index, gr/m2 | 54 (14) | 53 (12) | 55 (16) | 0.71 |
| Global longitudinal strain, % | -18.0 (4.4) | -17.3 (4.4) | -18.9 (4.4) | 0.09 |
| LV absolute twist (deg) | 10.5 (5.9) | 10.1 (6.0) | 11.1 (5.7) | 0.42 |
| Maximal septal wall thickness, mm | 6.7 (1.7) | 6.4 (1.6) | 7.1 (1.7) | 0.11 |
| Septal hypertrophy (≥13mm) | 0 (0) | 0 (0) | 0 (0) | |
| Maximal inferolateral wall thickness, mm | 6.1 (1.5) | 5.8 (1.4) | 6.5 (1.6) | 0.19 |
| Presence of LGE† | 12 (23%) | 7 (26%) | 5 (20%) | 0.79 |

Data is presented as number (percentage) or mean (standard deviation).
CMR: cardiac magnetic resonance imaging, EDV: end-diastolic volume, EF: ejection fraction, ESV: end-systolic volume index, LGE: late gadolinium enhancement, LV: left ventricular.
* LV EDVindex for males >105 ml/m², females >96 ml/m²
† LGE was performed in 27 patients with a (L)PV and 25 without a (L)PV patients.

### *Baseline characteristics*

The study population consisted of 62 patients from 45 families and was categorized into two groups: 33 patients with a (L)PV and 29 without a (L)PV (table 1). Of the 33 patients with a (L)PV 14 (42%) had a (L)PV in *MYH7* (figure 2). Two (3%) patients had two (L)PVs (online table 1). In 22 (49%) of the families a (L)PV was identified. Seventeen (27%) patients were relatives and 11 of them had a (L)PV. Familial CMP was documented in 35 (56%) patients, which was more common in patients with a (L)PV (n=23, 70%), than in the patients without a (L)PV (n=12, 41%; p=0.03). Hypertension was more common in patients without a (L)PV (with a (L)PV n=2 (6%), without a (L)PV n=7 (24%); p=0.02). The LV was dilated in 43 (69%) patients and in 19 (31%) LV-EF was <40%. There were no differences in LV-EF, GLS, LV mass, LV wall thickness and LV absolute twist between the patients with and without a (L) PV. During a median follow-up of 4 years (interquartile range 1 - 7 years) 19 (31%) of the patients had a major adverse cardiac event consisting of heart failure requiring

hospitalization, transient ischemic attack, stroke, ventricular arrhythmia, heart transplant or cardiac death. There were no differences in outcome between the two groups (online table 2).

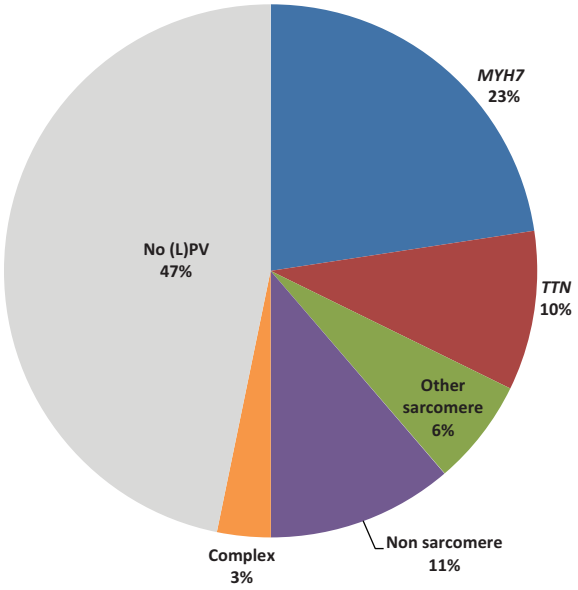



**Figure 2: Genetics of 62 NCCM patients.**
In 53% patient of the study population a (L)PV was identified, with highest prevalence of MYH7 (L) PVs. Other sarcomere genes: 2 MYBPC3, 2 TPM1; Non sarcomere genes: 1 LMNA, 2 PLN, 1 DSP, 1 MIB1, 1 PRDM16, 1 RYR2; Complex 1 TTN and MIB1, 1 ACTN2 and SCN5A.

*Diagnostic criteria on CMR*

An overview of the diagnostic CMR criteria and the corresponding parameters are shown in table 2. Only one patient did not meet any of the NCCM criteria on CMR. All but one (2%) patient fulfilled the diagnostic criteria proposed by Petersen in long axis and criteria by Jacquier. In contrast, the Stacey's short axis criteria were fulfilled in less than half of the cases. Figure 3 shows the distribution of the noncompacted segments meeting diagnostic criteria in the American Heart association 17 segment model in short axis view in diastole (Petersen) and systole (Stacey). The apical and mid-lateral segments were the dominant locations for meeting Petersen and/or Stacey criteria. More segments fulfilled the short axis Petersen than the Stacey criteria (meeting Petersen criteria 23% (232/992 segments); meeting Stacey criteria 8% (80/992 segments); $p<0.01$). In online table 3 the correlation of all CMR criteria are shown. Correlation between criteria varied from moderate (47%) to very strong (97%). Of the criteria, Petersen long-axis with

Jacquier had the highest agreement (97%), while Stacey with Jacquier had the lowest (47%).

**Table 2: Diagnostic CMR imaging criteria in NCCM patients.**

| | Total (n=62) | (L)PV (n=33) | No (L)PV (n=29) | p-value |
|---|---|---|---|---|
| **Petersen long axis end-diastole** | | | | |
| **Meeting criteria (ratio >2.3)** | 61 (98%) | 33 (100%) | 28 (97%) | **0.47** |
| Maximum NC/C ratio | 3.6 (0.9) | 3.8 (1.0) | 3.3 (0.8) | **0.02** |
| Number of long axis views with ratio >2.3 | | | | |
| 0 or 1 | 19 (31%) | 5 (15%) | 14 (48%) | **0.01** |
| 2 or 3 | 43 (35%) | 28 (85%) | 15 (52%) | |
| Maximum NC, mm | 14.2 (4.1) | 15.2 (4.2) | 13.0 (3.8) | **0.04** |
| Maximum C, mm | 4.1 (0.9) | 4.1 (1.0) | 4.0 (0.8) | **0.93** |
| **Petersen short axis end-diastole** | | | | |
| **Meeting criteria (ratio >2.3 in ≥1 segment)** | 52 (84%) | 29 (88%) | 23 (79%) | **0.36** |
| Maximum NC/C ratio | 3.9 (1.5) | 4.2 (1.6) | 3.6 (1.4) | 0.07 |
| Mean number of segments meeting criteria | 3.7 (2.3) | 3.9 (2.4) | 3.3 (2.2) | 0.41 |
| Maximum NC, mm | 17.0 (4.4) | 17.5 (4.7) | 16.5 (4.0) | 0.51 |
| Maximum C, mm | 7.5 (1.9) | 7.4 (2.1) | 7.5 (1.7) | 0.48 |
| **Stacey short axis end-systole** | | | | |
| **Meeting criteria (ratio ≥2.0 in ≥1 segment)** | 30 (48%) | 17 (52%) | 13 (45%) | **0.60** |
| Maximum NC/C ratio | 2.2 (0.8) | 2.3 (0.8) | 2.1 (0.7) | 0.28 |
| Mean number of segments meeting criteria | 1.3 (1.9) | 1.6 (2.1) | 0.9 (1.5) | 0.31 |
| Maximum NC, mm | 14.2 (3.6) | 14.7 (3.8) | 13.7 (3.3) | 0.50 |
| Maximum C, mm | 10.8 (2.5) | 10.4 (2.9) | 11.1 (1.9) | 0.06 |
| **Jacquier** | | | | |
| **Meeting criteria (>20%)** | 61 (98%) | 33 (100%) | 28 (97%) | **0.47** |
| Meeting criteria with Choi cut off (>35%) | 39 (63%) | 24 (73%) | 15 (52%) | 0.09 |
| Trabecular mass, (±SD) | 39 (9) | 42 (10) | 36 (7) | <0.01 |
| **Captur*** | | | | |
| **Maximum fractal dimension apical third ≥1.30** | 48 (94%) | 26 (93%) | 22 (96%) | **0.67** |
| Maximum fractal dimension apical third | 1.43 (0.09) | 1.43 (0.09) | 1.43 (0.09) | 0.91 |
| **Choi criteria** | | | | |
| **Meeting criteria (ratio >3.15)** | 35 (57%) | 21 (66%) | 14 (48%) | **0.22** |
| Choi ratio | 3.4 (1.9) | 3.8 (1.6) | 3.0 (2.2) | 0.15 |
| Apical type | 22 (36%) | 14 (42%) | 8 (28%) | 0.22 |
| **Number of fulfilled criteria†** | | | | |
| **Average number of criteria (0-5)** | 3.5 (1.1) | 3.8 (1.1) | 3.2 (1.1) | 0.06 |
| ≥4 criteria | 31 (50%) | 21 (64%) | 10 (34%) | 0.02 |

Data is presented as number (percentage) or mean (standard deviation). C: compacted, NC: noncompacted, * In 11 patients Cardiac Magnetic Resonance (CMR) imaging was not eligible for analysis

† Sum of meeting the following 5 criteria: Petersen long axis, Petersen short axis, Stacy short axis, Jacquier with Choi's cut-of, and Choi criteria.

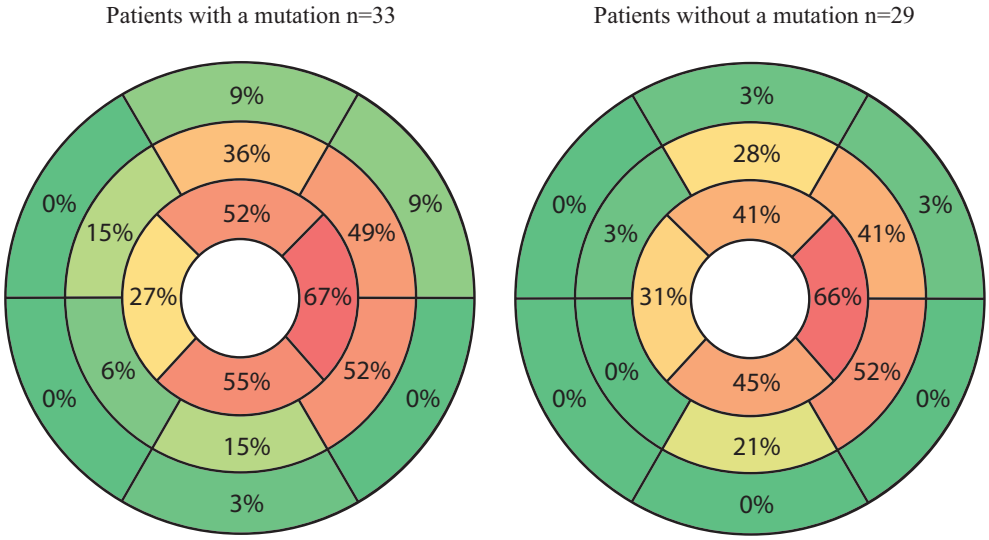



**Figure 3a: Segments meeting Petersen (diastole) short axis criteria.**

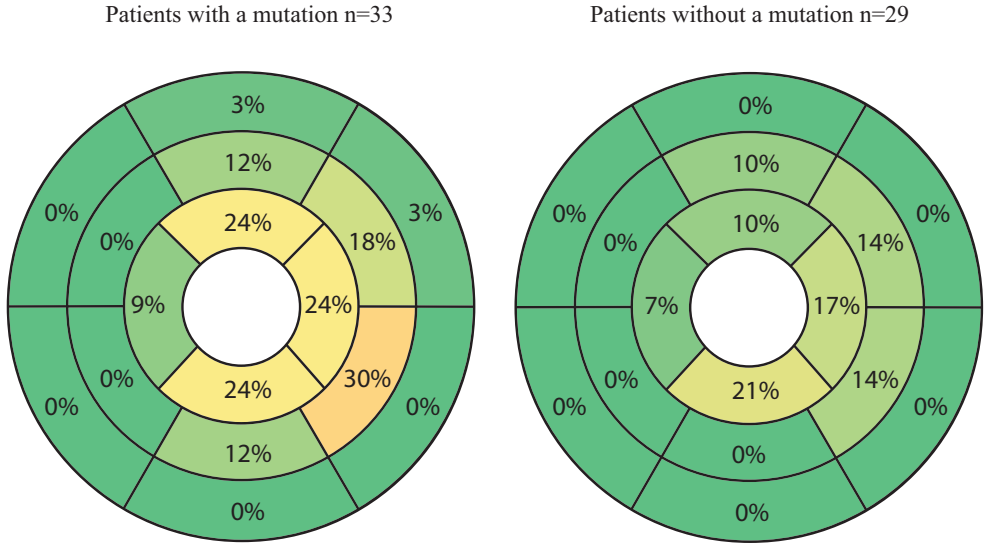



**Figure 3b: Segments meeting Stacey (systole) short axis criteria.**
Greener shades indicate low frequency of LV segment meeting diagnostic criteria to red shades indicating high frequency of LV segment meeting diagnostic criteria. The apical and mid-lateral segments were the dominant locations for meeting Petersen and/or Stacey criteria. More segments fulfilled the short axis Petersen than the Stacey criteria (Meeting Petersen criteria 23% (232/992 segments); meeting Stacey criteria 8% (80/992 segments); p<0.01).

*(L)PV status and CMR criteria*

On average the NCCM patients in this cohort met 3.5 of 5 CMR diagnostic criteria (table 2). Patients with a (L)PV had more extensive trabeculations; a higher maximum NC/C ratio measured in any long axis (p=0.02), more often fulfilling Petersen criteria in ≥2 long axis views per patient (p=0.01), and higher percentage of trabecular mass (p<0.01). Patients with a (L)PV were more often meeting at least 4 CMR diagnostic criteria than patients without a (L)PV (with a (L)PV 64%, without a (L)PV 34%; p=0.02). In figure 4, ROC analysis illustrate the association between different diagnostic CMR parameters and NCCM with a (L)PV.

In multivariate binary logistic regression analysis including both CMR and non-CMR parameters, independent positive predictors for having a (L)PV were familial CMP (OR: 10.1, 95%CI: 1.9-54.5), percentage increase in trabecular mass (OR: 1.2, 95%CI: 1.0-1.3) and meeting Petersen criteria in ≥2 long axis views (OR: 7.9, 95%CI: 1.5-42.5), while left bundle branch block (OR: 0.03, 95%CI: 0.0-0.8) and hypertension (OR: 0.03, 95%CI: 0.0-0.4) were negative predictors (table 3). The ROC-curve of this multivariate model had an AUC of 0.89 (95%CI 0.82-0.97) (central illustration). In patients with familial disease without a (L)PV, a genetic cause can be expected. Excluding these patients from the group without a (L)PV led to similar results, except for hypertension, which was not a significant predictor anymore (Online table 4).

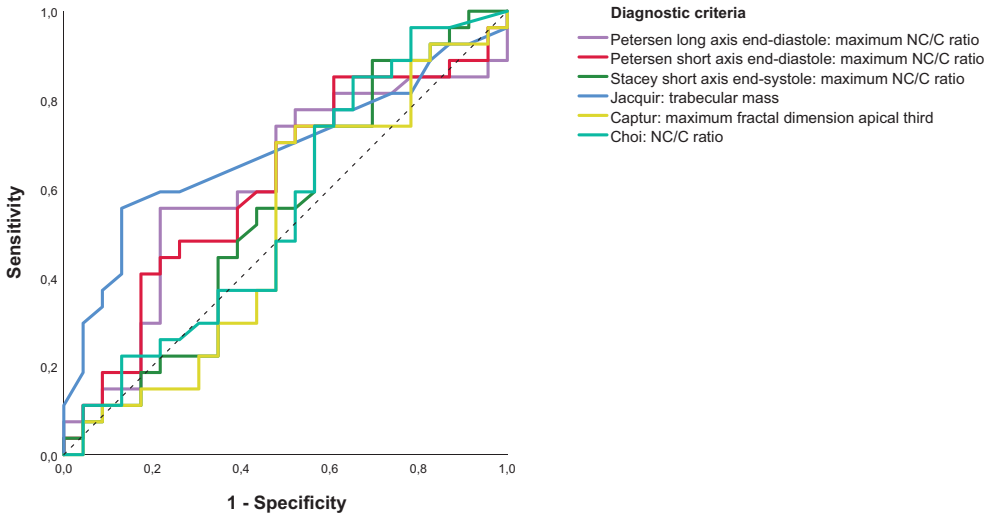
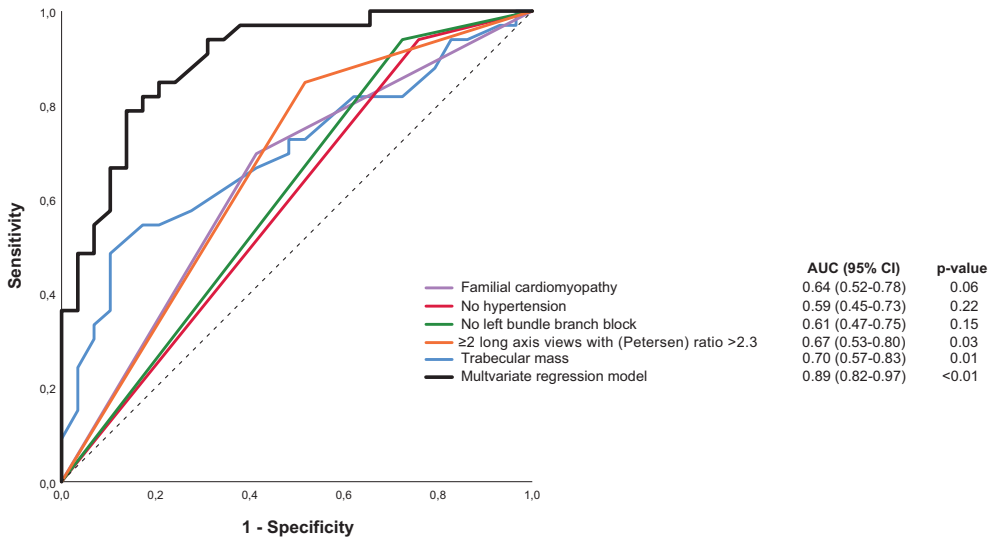



**Figure 4: ROC curve analysis of association for different diagnostic CMR criteria with finding a (L)PV.**

Only trabecular mass was a good predictor using diagnostic criteria for finding a (L)PV with an area under the curve of 0.70 (95% CI 0.57-0.83). C: compacted, CI: confidence interval, NC: noncompacted, ROC: Receiver-operator characteristic.




**Central illustration: Receiver-operator characteristic (ROC) curve analysis in noncompaction cardiomyopathy for prediction of finding a mutation.**
Noncompaction cardiomyopathy (NCCM) was genetic in half of the study population. Genetics may play an important role distinguishing patients from people with physiologic hypertrabeculations. In multivariate regression analysis independent positive predictors for a positive genetic test were familial cardiomyopathy, trabecular mass, and meeting Petersen criteria in ≥2 long axis views, while left bundle branch block and hypertension were negative predictors. The ROC-curve of this multivariate model had an area under the curve of 0.89 and provided a good model for finding a (likely) pathogenic variant in NCCM.
AUC: area under the curve, CI: confidence interval.

### *Different (L)PVs*

The results of the different diagnostic criteria were similar for patients with single (L)PVs , *MYH7* n=14, *TTN* n=6, other sarcomere n=4 (2 *MYBPC3* and 2 *TPM1*), non-sarcomere n=7 (1 *LMNA*, 2 *PLN*, 1 *DSP*, 1 *MIB1*, 1 *PRDM16* and *RYR2*) and complex genotypes n=2 (patients with two (L)PVs: 1 *TTN* and *MIB1*; 1 *ACTN2* and *SCN5A*) (Online table 5). Around two third of the patients with a *MYH7, TTN* or a non-sarcomere (L)PV fulfilled more than 4 diagnostic criteria, compared to 34% of the patients without a (L)PV. Patients with a complex genotype fulfilled almost all the different diagnostic criteria. The patient category with other sarcomere (L)PVs (2 MYBPC3, 2 TPM1) fulfilled the least diagnostic criteria on CMR.

**Table 3: Univariate and multivariate binary logistic regression for the prediction of a positive genetic test in NCCM patients.**

| Variable | Univariate | | Multivariate | |
|---|---|---|---|---|
| | OR (95% CI) | p-value | OR (95% CI) | p-value |
| **Male** | 0.85 (0.31-2.32) | 0.75 | | |
| Age at CMR , years | 0.99 (0.96-1.02) | 0.62 | | |
| Familial cardiomyopathy | 3.26 (1.14-9.29) | 0.03 | 10.08 (1.87-54.53) | **0.01** |
| Hypertension | 0.20 (0.04-1.07) | 0.06 | 0.03 (0.00-0.44) | **0.01** |
| Left bundle branch block | 0.17 (0.03-0.88) | 0.03 | 0.03 (0.00-0.75) | **0.03** |
| LV end-diastolic volume index, ml/m2 | 1.01 (1.00-1.03) | 0.15 | | |
| LV ejection fraction, % | 0.96 (0.92-1.02) | 0.18 | | |
| Reduced LV ejection fraction (<50%) | 2.13 (0.61-7.46) | 0.24 | | |
| Global longitudinal strain, % | 0.91 (0.80-1.03) | 0.15 | | |
| LV absolute twist, deg | 0.97 (0.89-1.06) | 0.49 | | |
| **CMR imaging criteria** | | | | |
| Petersen long axis end-diastole | NA | | | |
| **Maximum NC/C ratio** | 1.79 (0.98-3.28) | 0.06 | | |
| **≥2 long axis views with ratio >2.3** | 5.23 (1.58-17.32) | 0.01 | 7.85 (1.45-42.46) | 0.02 |
| Petersen short axis end-diastole | 1.89 (0.48-7.51) | 0.37 | | |
| **Maximum NC/C ratio** | 1.33 (0.93-1.90) | 0.11 | | |
| Stacey short axis end-systole | 1.31 (0.48-3.56) | 0.60 | | |
| **Maximum NC/C ratio** | 1.44 (0.72-2.91) | 0.31 | | |
| Jacquier end-diastolic (Choi >35%) | 2.49 (0.87-7.16) | 0.09 | | |
| **Trabecular mass, %** | 1.10 (1.03-1.18) | 0.01 | 1.16 (1.04-1.30) | 0.01 |
| Captur fractal dimension | 0.59 (0.05-6.96) | 0.68 | | |
| **Maximum fractal dimension apical third** | 0.53 (0.00-303.11) | 0.84 | | |
| Choi criteria | 2.05 (0.73-5.73) | 0.17 | | |
| **NC/C ratio** | 1.25 (0.95-1.65) | 0.11 | | |
| Number of fulfilled criteria* | | | | |
| **Number of criteria** | 1.59 (0.98-2.58) | 0.06 | | |
| **≥4 criteria** | 3.33 (1.17-9.44) | 0.02 | | |

C: compacted, CI: confidence interval, LV: left ventricle, CMR: cardiac magnetic resonance imaging, NC: noncompacted, OR: odds ratio

* Sum of meeting the following 5 criteria: Petersen long axis, Petersen short axis, Stacy short axis, Jacquier with Choi's cut-of, Choi criteria.

## Discussion

This study included cardiomyopathy patients fulfilling the echocardiographic Jenni criteria for NCCM to investigate the association between clinical and CMR characteristics and if CMR features allowed to distinguish which patients had high risk of having a mutation. Approximately half of the patients had a (L)PV. In patients with and without a (L)PV the correlation between the different CMR criteria ranged from moderate to very strong. We presented a model with the combination of clinical as well as CMR parameters that could predict finding a mutation with an AUC of 0.89. Patients with a (L)PV had more often familial cardiomyopathy and more extensive trabeculations. However, the common used cut offs used for the CMR criteria were not associated with finding a (L)PV. Patients with hypertension or left bundle branch block were less likely to have a (L)PV. We also showed that important factors for cardiomyopathy like LV systolic dysfunction and LV dilatation did not predict finding a (L)PV.

*Genetic NCCM patients*

Identification of the causative (L)PV is of importance. Firstly, it facilitates cascade family screening. This allows identifying accurately relatives at risk of having a cardiomyopathy and also helps to reassure relatives, who do not have the familial (L)PV. Secondly, it may help to distinguish patients with a genetic CMP from the frequently observed healthy cases with physiologic hypertrabeculation (16, 17). Thirdly, it was shown that the prognosis of patients with a (L)PV, can be predicted by the LV systolic function in contrast to cases without a (L)PV (180). In conclusion, identification of patients who are most likely to have a genetic defect may help in the clinic to know which patient will benefit most from referral for genetic testing and may save costs and clinical resources.

*Secondary causes for hypertrabeculation*

The genetic yield in index patients in this study (49%) was higher than in previous studies (32 to 38%) (8, 155). An explanation may be that in this cohort of selected patients of a tertiary referral center, a high prevalence of LV systolic dysfunction was noted. In the literature having a reduced LV-EF is associated with finding a (L)PV in NCCM (147, 185). Another explanation in this cohort may be that no significant difference in age at presentation was observed between patients with or without a (L)PV. Previous studies showed that patients without a (L)PV are older (8, 189). When no (L)PV is identified, noncompaction may be attributed to non-genetic, secondary causes for hypertrabeculation or still unknown genetic (L)PVs. In this study left bundle branch block and hypertension were negatively associated with having a (L)PV. Other studies also found a higher prevalence of hypertension

in patients without a (L)PV (8, 9). These characteristics may influence loading conditions leading to cardiac remodeling into the noncompaction phenotype. Previous studies showed that pregnancy, athletes and race might be secondary causes for noncompaction (26, 40, 61, 152). In this study no athletes or pregnant women were included. We did not found race to be a predictor of having a (L)PV however the majority in our population was Caucasian and the sample size was small. Our results need to be validated in larger cohorts and possibly other factors like age or secondary causes may play a role in distinguishing genetic patients.

*Diagnostic criteria*

In NCCM gold standard for the diagnostic criteria are still missing. In this study different diagnostic criteria were fulfilled in patients meeting Jenni criteria on echocardiography with the lowest yield of Stacey short axis criteria of 48% and highest yield of the Petersen long axis-criteria were fulfilled in 98%. However, in large population studies 15% up to 40% of the people fulfilled the Petersen long axis criteria for noncompaction on CMR (16, 17). Despite this low specificity of some of the criteria, poor agreement between criteria was noted in this study. For a more accurate diagnosis the criteria may need to change from imaging criteria solely to a diagnostic model in which imaging criteria are combined with clinical characteristics, genetics, and functional features. Identifying the causative (L)PV in individuals with hypertrabeculation may help to confirm a diagnosis of NCCM, since people without a cardiomyopathy are expected not to carry a (L)PV. Following this analogy identification of characteristics of patients who are most likely to have a (L)PV may also help in the clinic, to distinguish patients from physiologic trabeculations. A composed diagnostic tool including the morphological and functional criteria may help to more accurately distinguish cases with physiologic hypertrabeculation from (asymptomatic) patients with a genetic CMP requiring lifelong follow-up.

*Study limitations*

This study may have some limitations. Firstly, the group of patients without a (L) PV included patients with familial CMP. In these familial patients novel genetic causes are expected, which may have led to misclassification of these patients. However, additional analysis showed that excluding patients with familial CMP in the group with no (L)PV gave similar results. Secondly, a selection bias could have been introduced since the study only included patients presenting in a tertiary referral center and only patients with a CMR and genetic testing were included. Other limitations may be due to the retrospective design of the study. Over time different MRI scanners were used, and newer scanners have better image quality,

making it easier to distinguish trabeculae from other structures or blood. This was also an important reason why fractal analysis could not be performed in all patients. Furthermore, not all participating NCCM index cases had extensive next-generation DNA sequencing cardio-panel testing, which may have led to underreporting of genetic causes.

## Conclusion

NCCM patients with a (L)PV can be distinguished from patients without a (L) PV using clinical and morphologic features. Important predictors of having a (L)PV were familial CMP, no left bundle branch block, no hypertension, higher trabecular mass and meeting Petersen criteria in ≥2 long axis views. These predictors may help guiding genetic diagnostics, cascade screening and clinical treatment strategies.

## Perspectives

*Competency in medical knowledge*
Genetics in NCCM may help assessing prognosis, guide family screening and predict risks for relatives. This study shows NCCM patients with a (likely) pathogenic variant can be distinguished by having familial cardiomyopathy, no left bundle branch block, no hypertension, higher trabecular mass and meeting Petersen criteria in ≥2 long axis views.
*Translational Outlook*
Frequently patients can be diagnosed with noncompaction according to diagnostic criteria on CMR. This makes it difficult to distinguish patients with a genetic cardiomyopathy from patients with physiologic hypertrabeculation. Predictors for finding a (likely) pathogenic variant can help the clinician to decide to refer patients for genetic testing.

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# Chapter 5

## Systematic review of genotype- phenotype correlations in noncompaction cardiomyopathy

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**Abstract**

**Background:** A genetic cause can be identified in 30% of noncompaction cardiomyopathy patients (NCCM) with clinical features ranging from asymptomatic cardiomyopathy to heart failure with major adverse cardiac events (MACE).

**Methods and Results:** To investigate genotype-phenotype correlations, the genotypes and clinical features of genetic NCCM patients were collected from the literature. We compared age at diagnosis, cardiac features and risk for MACE according to mode of inheritance and molecular effects for defects in the most common sarcomere genes and NCCM subtypes. Geno- and phenotypes of 561 NCCM patients from 172 studies showed increased risk in children for congenital heart defects ($p<0.001$) and MACE ($p<0.001$). In adult NCCM patients the main causes were single missense mutations in sarcomere genes. Children had more frequently an X-linked or mitochondrial inherited defect ($p=0.001$) or chromosomal anomaly's ($p<0.001$). MYH7 was involved in 48% of the sarcomere gene mutations. MYH7 and ACTC1 mutations had lower risk for MACE than MYBPC3 and TTN ($p=0.001$). The NCCM/DCM cardiac phenotype was the most frequent subtype (56%; $p=0.022$) and was associated with an increased risk for MACE and high risk for left ventricular (LV) systolic dysfunction (<0.001). In multivariate binary logistic regression analysis MYBPC3, TTN, arrhythmia -, non-sarcomere non arrhythmia cardiomyopathy – and x-linked genes were genetic predictors for MACE.

**Conclusions:** Sarcomere gene mutations were the most common cause in adult patients with lower risk of MACE. Children had multi-systemic disorders with severe outcome, suggesting that the diagnostic and clinical approaches should be adjusted to age at presentation. The observed genotype-phenotype correlations endorsed that DNA diagnostics for NCCM is important for clinical management and counselling of patients.

## Introduction

Noncompaction cardiomyopathy (NCCM) is a rare cardiomyopathy characterised by excessive trabeculation of the left ventricle (21), and is also known as left ventricular hypertrabeculation/ noncompaction (LVHT/LVNC). Diagnostic criteria are based on cardiac imaging with echocardiography or cardiac magnetic resonance imaging (CMR) requiring at least a twofold increase of ratio of endocardial hypertrabeculation (21, 25, 190). Genetics plays an important role in NCCM in at least half the case; 30% of the index patients have a mutation or chromosome defect. In addition, unknown genetic causes are expected in in 20% of the index patients with familial disease, who do not have a mutation (8). In cardiomyopathy patients, who do not have familial disease or a mutation, the cause of NCCM may involve unknown low penetrance genetic causes and/or cardiac stress induced mechanism (11, 175). In addition physiologic hypertrabeculation occurs in people without a cardiomyopathy.

The most prevalent genetic causes for NCCM are defects in the same sarcomere genes that are frequent causes for hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM); *MYH7*, *MYBPC3* and *TTN* (8, 191), and are autosomal dominantly inherited with reduced penetrance. Less frequent are cases of NCCM inherited as X-linked or mitochondrial traits, or chromosome defects, which are, usually associated with complex congenital malformations in children (6, 192, 193).



Establishing genotype-phenotype correlations is important for genetic counselling and clinical management of NCCM. Previous studies suggested that the genetic defect, left ventricular systolic function and age at diagnosis were important predictors of cardiac features and risk for major adverse cardiac (8, 194). Since NCCM is a relatively rare, genetically heterogonous cardiomyopathy, large studies are needed to establish a correlation between genetic defects and clinical outcome. By performing a systematic review of the NCCM literature for reports of NCCM patients with documented genotype and phenotypes, we could combine and analyse the results of smaller studies to determine the genetic spectrum of NCCM and establish if genetic causes could help predict high risk profiles for major adverse cardiac events in NCCM.

## Methods

### *Data Availability*

The data of this study is extracted from publications, which were available online. The authors declare that all supporting data are available within the article and its

online supplementary files. Since data was obtained from publications, no consent from an institutional review committee was required.

*Data collection*

The collection of data included publications from 1999 to march 2018. We selected English publications. Inclusion criteria were that the complete results of DNA-testing or cytogenetic analysis and the cardiologic data of each individual patient were available. Only NCCM patients fulfilling the current diagnostic criteria were included in the study. Patients presumed to be reported more than once by different studies (based on mutation specifics: same amino acid change and base change in the same gene; sex; age, also with respect to date of publication; and corresponding authors) were included once. A detailed description of the search terms used for this study, the collection of studies and inclusion of patient data is presented in online methods and online figure 1.

Specifics of the genetic defects of each patient and information on family histories of cardiomyopathies (NCCM, HCM or DCM) or sudden death below age of 50 years in relatives were collected. The included cardiologic data consisted of ECG, the applied diagnostic criteria used to diagnose NCCM, the modality of diagnosis (echocardiography, cardiac magnetic resonance imaging (CMR), computed tomography (CT), and the cardiac symptoms. Information on the occurrence of congenital heart disease (CHD) and neuromuscular disease in NCCM patients was recorded. Patients were classified as having left ventricular dilatation when this was reported. In case this information was missing the occurrence of left ventricular (LV) dilatation was determined from presented LV measurements; patients were classified with LV dilation when left ventricular end diastolic diameter (LVEDD) was ≥60mm in males or ≥54mm in females, or left ventricular end diastolic diameter index (LVEDDi) was ≥31mm in males or ≥32mm in females (195). For children, dimensions of the left ventricle of <2 SDs from the reference range were classified as dilated (145). Patients were classified as having left ventricular systolic dysfunction if reported, or if fractional shortening (FS) was <25% in males or <27% in females, or if LV ejection fraction was <55% in males and females. We recorded the occurrence and the age of a patient suffering sudden cardiac death (SCD), heart transplantation, having a left ventricular assist device, stroke, or heart failure requiring hospital admission.

*NCCM subtypes*

NCCM patients were classified into the four NCCM subtypes (when LV diameters were known): Isolated NCCM, NCCM/HCM, NCCM/DCM and NCCM/HCM/

DCM. Patients were classified as isolated NCCM if when they had normal LV dimensions without LV hypertrophy. NCCM patients were categorized into NCCM/HCM if they also had LV wall hypertrophy of ≥13mm or the diagnosis HCM was reported. A NCCM patient was classified as NCCM/DCM if the patient had LV dilatation. The NCCM patient was classified as NCCM/HCM/DCM if the patient had LV dilation and LV wall hypertrophy.

*Genetic causes*

In total 80 genes were reported as the genetic causes for NCCM, of which 14 were excluded from this review, because there was not sufficient evidence to support that these genes could be the cause of a cardiomyopathy. The genetic defects were grouped according to the molecular function of the gene, into sarcomere- or arrhythmia cardiomyopathy genes, non-sarcomere and non-arrhythmia cardiomyopathy genes. Genes involved in cardiac development, mitochondrial genes, X-linked inherited disorders and chromosome defects were also analysed separately. Figure 1 presents an overview of the genetic causes: the sarcomere genes were *ACTC1, ACTN2, DES, LDB3, MYBPC3, MYH7, MYL2, NEBL, OBSCN, TNNC1, TNNI3, TNNT2, TPM1* and *TTN*. The arrhythmia genes were 10 genes associated with ion channels and transport: *ABCC9, ANK2, CACNA2D1, CASQ2, HCN4, KCNE3, KCNH2, KCNQ1, RYR2* and *SCN5A*. The group of cardiomyopathy genes, which were not sarcomere or arrhythmia genes: *DMPK, DSP, DTNA, FKTN, HFE, JUP, LMNA, PKP2, PLEC, PLN, PRDM16, RBM20* and *SGCD*. Eight genes were previously associated with developmental defect of the heart: *MIB1, MIB2, NKX2.5, NOTCH1, NSD1, PTPN11, TBX20* and *TBX5*. X –linked inherited NCCM included the *DMD, FHL1, GLA, LAMP2, RPS6KA3* and *TAZ* genes. Mitochondrial dysfunction as the cause of NCCM was considered when a mutations occurred in a gene affecting mitochondrial functioning, including the nuclear genes with a mitochondrial function or the mitochondrial DNA genes: *HADHB, HMGCL, MIPEP, MLYCD, MT-ATP6, MT-CO1, MT-CO3, MTFMT, MT-ND1, MT-ND2, SDHA, SDHD, TMEM70* and *VARS2*. Some additional genes were only reported in patients with complex genotypes, including: *BB2, BMPR1A, CSRP3, EMD, ITGA7, MT-TL1, MYH6, MYH7B, MYLK2, MYPN, PLEKHM2, RANGRF,* and *SH3PXD2B*. The current analysis did not include single cases of genetic causes for which there was little evidence for involvement in cardiomyopathy (*ARFGEF2, CYP2C9*2, GARS, LMX1B, MBL2, MMACHC, MSH6, NONO, PKD1, PKD2, PMP22, POMT2, SMC1A* and *YWHAE*).

We compared the clinical features in patients according to the genetic causes, i.e. in patients with sarcomere gene mutations, in patients with mutations in cardiomyopathy genes (i.e. sarcomere, arrhythmia, and other cardiomyopathy

genes). In patients with non- autosomal dominant inherited NCCM, the phenotypes of patients were compared according to the characteristics of the genetic cause (complex genotypes, X-linked, mitochondrial defects, and chromosome defects).

*Statistical analysis*

Categorical data were analysed with Pearson's chi-squared test or Fisher exact test. For comparison of medians the independent samples Kruskal-Wallis test was used. Odds ratios (OR) with 95% confidence interval (CI) for MACE were calculated using binary logistic regression. To identify independent predictors for MACE, variables were tested in a multivariate logistic regression analysis using the enter method. Age, sex and other parameters with a p-value <0.05 were stepwise entered in to the multivariate model. Multiple imputation for the entered predictors was used to handle missing data in the multivariate regression models. Statistical analysis was performed with SPSS statistical software, version 21.0 (SPSS Inc., Chicago, IL).

## Results

**Table 1: Cardiac features of 541 genetic NCCM patients*.**

| | Children (<18 yrs) n=244 | Adults n=297 | Total n=541 | p-value |
|---|---|---|---|---|
| NCCM index patients | 195/244 (80%) | 201/297 (68%) | 396/541 (73%) | 0.001 |
| Male (%) | 122/238 (51%) | 154/297 (52%) | 276/535 (52%) | ns |
| Median age at diagnosis in years (IQR) | 0 (0-10) | 41 (30-53) | 23 (1-43) | NA |
| Congenital heart disease | 45/244 (18%) | 13/297 (4%) | 58/541 (11%) | <0.001 |
| Ebstein anomaly | 11/45 (24%) | 10/13 (77%) | 21/58 (36%) | <0.001 |
| Atrial septal defect | 17/45 (38%) | 7/13 (54%) | 24/58 (41%) | ns |
| Ventricular septal defect | 20/45 (44%) | 2/13 (15%) | 22/58 (38%) | ns |
| Patent ductus arteriosus | 11/45 (24%) | 1/13 (8%) | 12/58 (21%) | ns |
| Patent foramen ovale | 4/45 (9%) | 1/13 (8%) | 5/58 (9%) | ns |
| Hypoplastic left heart | 2/45 (4%) | 0/13 (0%) | 2/58 (3%) | ns |
| Aortic coarctation | 2/45 (4%) | 0/13 (0%) | 2/58 (3%) | ns |
| Heart failure | 115/244 (47%) | 153/297 (52%) | 268/541 (50%) | ns |
| Left ventricular dilatation | 101/150 (67%) | 105/170 (62%) | 206/326 (64%) | ns |
| Left ventricular systolic dysfunction | 123/179 (69%) | 131/233 (56%) | 254/412 (62%) | 0.010 |
| Pacemaker | 12/244 (5%) | 14/297 (5%) | 26/541 (5%) | ns |
| ICD | 19/244 (8%) | 63/297 (21%) | 82/541 (15%) | <0.001 |
| Major adverse cardiac events | 94/244 (39%) | 55/297 (18%) | 149/541 (28%) | <0.001 |
| Stroke | 2/244 (1%) | 6/297 (2%) | 8/541 (1%) | ns |
| Heart failure requiring hospital admission | 34/244 (14%) | 6/297 (2%) | 40/541 (8%) | <0.001 |
| Left ventricular assist device | 6/244 (3%) | 2/297 (1%) | 8/541 (1%) | ns |
| Heart transplantation | 25/244 (10%) | 5/297 (2%) | 30/541 (5%) | <0.001 |
| Sustained VT/VF or appropriate shock | 30/244 (12%) | 23/297 (8%) | 53/541 (10%) | ns |
| Cardiac death | 35/244 (14%) | 10/297 (3%) | 45/541 (9%) | <0.001 |
| Neuromuscular symptoms | 35/60 (58%) | 15/83 (18%) | 50/143 (35%) | <0.001 |

ICD implantable cardiac device; IQR interquartile range; LV left ventricular; ns not significant; NA Not applicable; NCCM noncompaction cardiomyopathy
* age at diagnosis was missing for 20 cases.

### *Study population*

The literature searches yielded 1978 publications reporting NCCM patients. After removing duplicates from the different searches 990 papers remained of which 172 fulfilled the inclusion criteria. In total 561 patients were included in this review (online figure 1). Age at presentation was reported in 541/561, and sex was reported in 554 cases. Among the NCCM patients 415 (72%) were index cases (the first patient diagnosed with NCCM in a family) and 159 (28%) were affected family members (table 1). The study population consisted of 244 children, diagnosed before the age of 18 years and 297 adults. The diagnosis was based on echocardiography in 402 NCCM patients, cardiac magnetic resonance imaging (CMR) in 66, 104 had an echocardiography and CMR and 2 patients were diagnosed at autopsy.

**Table 2: Genetics of 396 NCCM index patients.**

| | Children* | Adults n=297 | Total n=541 | p-value |
|---|---|---|---|---|
| **Type of mutation** | | | | <0.001 |
| Missense mutation | 98 (50%) | 119 (59%) | 217 (55%) | |
| Other mutations† | 56 (29%) | 63 (31%) | 119 (30%) | |
| Complex genotype** | 14 (7%) | 15 (7%) | 29 (7%) | |
| Chromosome defect | 27 (14%) | 4 (2%) | 31 (8%) | |
| **Mode of inheritance*** ** | | | | 0.001 |
| Autosomal dominant | 105 (75%) | 162 (89%) | 267 (83%) | |
| X-Linked | 26 (19%) | 10 (5%) | 36 (11%) | |
| Mitochondrial | 9 (6%) | 10 (5%) | 19 (6%) | |
| Familial cardiomyopathy | 60 (31%) | 109 (54%) | 169 (43%) | <0.001 |
| NCCM in family | 38 (20%) | 68 (34%) | 106 (27%) | 0.001 |
| HCM / DCM in family | 28 (14%) | 49 (24%) | 77 (19%) | 0.012 |
| SCD before age 50 yrs in family | 34 (17%) | 44 (22%) | 78 (20%) | ns |

DCM dilated cardiomyopathy; HCM hypertrophic cardiomyopathy; SCD sudden cardiac death
* presentation before age 18 years, ** at least two mutations, † nonsense- or frameshift mutations, small deletions or insertions *** Chromosome defects and complex genotypes excluded.

### *Genetics of NCCM*

In total 369 unique genetic defects in 66 genes were included. The majority of genetic causes were single mutations (85%) of which autosomal dominantly inherited missense mutation were the most frequent (55%; table 2). In 7% of the patients an X-linked inherited genetic defect was reported, mostly in *TAZ* (n=31, 6%). In 32 (6%) of the patients a chromosome defect was reported, of whom 28 (88%) were diagnosed in childhood (table 2, 3). Detailed descriptions of the reported mutations are presented in the online supplement table 1. More than 50 percent (292/651) of the genetic defects were reported in sarcomere genes, most prevalently in *MYH7* (n=142, 25%; figure 1, table 4, 5). Mutations in arrhythmia genes were observed in 11% of the patients, in particular in *HCN4* (n=22, 4%)(table 4). Details of the complex genotypes are presented in the online supplement table 2. The complete list of reported chromosome defects is presented in the online supplement table 3, showing the 1p36 locus was the most frequently reported, in 13 patients, and 22q11 defects in three patients.

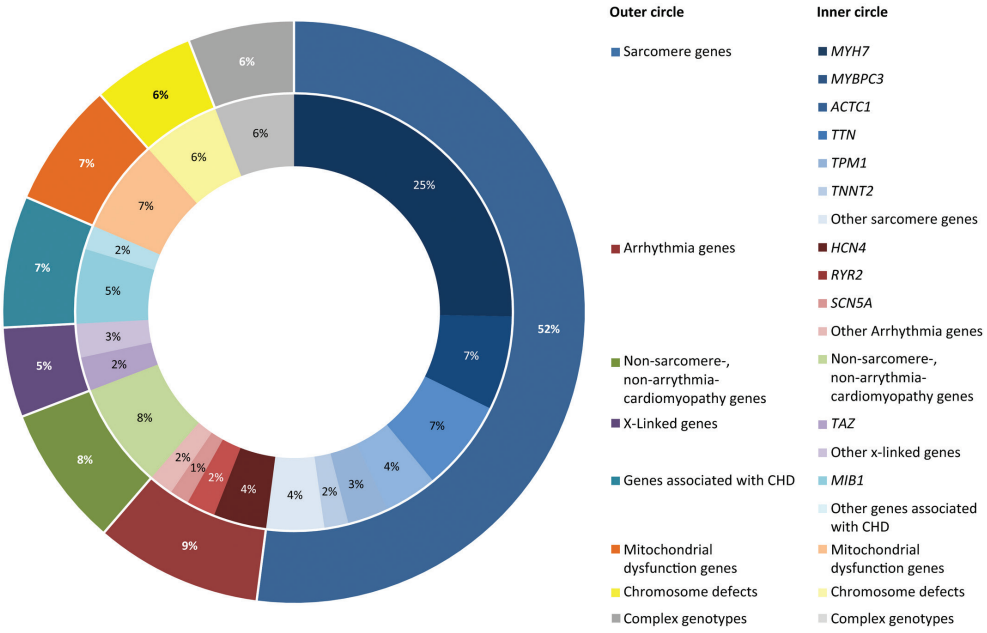



**Figure 1: Genetic noncompaction cardiomyopathy.**
Other sarcomere genes: ACTN2, DES, LDB3, MYL2, NEBL, OBSCN, TNNC1 and TNNI3.
Other arrhythmia genes: ABCC9, ANK2, CACNA2D1, CASQ2, KCNE3, KCNH2 and KCNQ1
Non-sarcomere, non-arrhythmia-cardiomyopathy genes: DMPK, DSP, DTNA, FKTN, HFE, JUP, LMNA, PKP2, PLEC, PLN, PRDM16, RBM20 and SGCD
Other X-linked genes: DMD, FHL1, GLA, LAMP2 and RPS6KA3
Other genes associated with CHD: MIB2, NKX2.5, NOTCH1, NSD1, PTPN11, TBX20 and TBX5.
Mitochondrial-functioning: HADHB, HMGCL, MIPEP, MLYCD, MT-ATP6, MT-CO1, MT-CO3, MTFMT, MT-ND1, MT-ND2, SDHA, SDHD, TMEM70 and VARS2.
Chromosome defect: see online table 3
Complex genotypes: see online table 2.

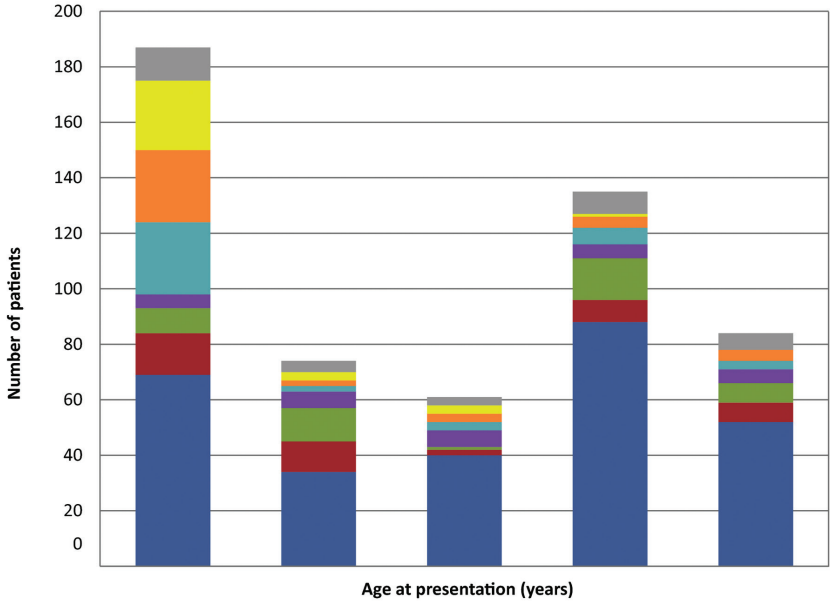
**Table 3: Genotype- phenotype correlations in 561 genetic NCCM patients.**

| | Cardiomyopathy genes* | Complex Genotype** | X-linked | Mitochondrial | Chromosome defects |
|---|---|---|---|---|---|
| | n=416 (74%) | n=33 (6%) | n=41 (7%) | n=39 (7%) | n=32 (6%) |
| Male | 203/411 (49%) | 18/32 (44%) | 35/41 (83%) | 22/38 (58%) | 7/32 (22%) |
| Median age at diagnosis in years (IQR) | 29 (10-45) | 23 (0-45) | 0 (0-29) | 0 (0-27) | 0 (0-9) |
| Congenital heart disease | 38/416 (9%) | 2/33 (1%) | 0/41 (0%) | 6/39 (15%) | 13/32 (41%) |
| Neuromuscular symptoms | 12/90 (13%) | 4/9 (44%) | 14/16 (88%) | 9/16 (56%) | 11/12 (92%) |
| LV dilatation | 142/245 (58%) | 14/16 (88%) | 24/27 (89%) | 17/18 (94%) | 10/15 (67%) |
| LV systolic dysfunction | 177/307 (43%) | 19/28 (68%) | 27/36 (75%) | 21/29 (72%) | 11/21 (52%) |
| Major adverse cardiac events† | 97/416(23%) | 10/33 (30%) | 21/41 (51%) | 10/39 (26%) | 11/32 (34%) |
| Stroke | 6/416 (1%) | 2/33 (6%) | 0/41 (0%) | 0/39 (0%) | 0/32 (0%) |
| Heart failure requiring hospital admission | 18/416 (4%) | 2/33 (6%) | 9/41 (22%) | 3/39 (8%) | 8/32 (25%) |
| Left ventricular assist device | 6/4316 (1%) | 0/33 (0%) | 1/41 (2%) | 1/39 (3%) | 0/32 (0%) |
| Heart transplantation | 21/416 (5%) | 2/33 (6%) | 4/41 (10%) | 2/39 (5%) | 1/32 (3%) |
| Sustained VT/VF or appropriate shock | 38/416 (10%) | 5/33 (15%) | 5/41 (12%) | 2/39 (5%) | 3/32 (9%) |
| Cardiac death | 23/416 (6%) | 1/33 (3%) | 9/41 (22%) | 7/39 (18%) | 5/32 (16%) |

IQR interquartile range; LV left ventricular; NCCM noncompaction cardiomyopathy
*cardiomyopathy genes: sarcomere-, arrhythmia-, non-sarcomere non-arrhythmia cardiomyopathy genes or a NCCM gene associated with congenital heart disease
** Patients with multiple mutations
† Major adverse cardiac events was composed of stroke, heart failure requiring hospital admission, left ventricular assist device, heart transplantation, sustained VT/VF or appropriate shock and cardiac death.

### *Children and adult NCCM patients*

Overall genetic NCCM in children was associated with more severe features, CHD (p<0.001), LV systolic dysfunction (p=0.010), MACE (p<0.001), and neuromuscular signs (p<0.001; table 1). The NCCM index cases diagnosed in childhood were more likely to have a chromosomal anomaly (p<0.001), an X-linked inherited disorder (p=0.001; table 2) or mitochondrial defect. In 52% of the patients diagnosed before the age of 10 years there was an autosomal dominant inheritance, 37% had a sarcomere gene defects, 8% had an arrhythmia gene, 5% non-sarcomere non-arrhythmia cardiomyopathy genes and 3% a gene associated with CHD. In contrast, 84% of the patients above 20 years had an autosomal dominant inheritance of which 64% in a sarcomere gene (p<0.001; figure 2). Familial cardiomyopathy was reported more frequently in families of adult - than paediatric index patients (adults 54% vs children 31%, p<0.001; table 2). In 93 (57%) families all affected relatives were reported to have NCCM. Sudden cardiac death (SCD <50y) in relatives was reported in 78 (20%) of the families (table 2).




| | 0-10 (n=187) | 11-20 (n=74) | 21-30 (n=61) | 31-50 (n=135) | 51-90 (n=84) |
|---|---|---|---|---|---|
| Complex | 12 | 4 | 3 | 8 | 6 |
| Chromosomal | 25 | 3 | 3 | 1 | 0 |
| Mitochondrial | 26 | 2 | 3 | 4 | 4 |
| X-Linked | 26 | 2 | 3 | 6 | 3 |
| Genes associated with CHD | 5 | 6 | 6 | 5 | 5 |
| Non-sarcomere-, non-arrhythmia- cardiac | 9 | 12 | 1 | 15 | 7 |
| Arrhythmia | 15 | 11 | 2 | 8 | 7 |
| Sarcomere | 69 | 34 | 40 | 88 | 52 |

**Figure 2: Genetic causes by age at diagnosis in 541 NCCM patients.**

*Genotype-phenotype correlations across the 5 groups of genetic causes*

Comparing the occurrence of cardiac features, risk for MACE, concomitant CHD and neuromuscular signs in the five groups of genetic causes depicted in table 3 showed that LV dilatation was most prominent in (94%) patients with a mitochondrial defect, and risk for MACE was highest in patients with X-linked inherited NCCM. Patients (23%) with a mutation in a cardiomyopathy gene had lowest risk for MACE ($p<0.001$). CHD occurred in 41% of the cases with a chromosome defect and in 9% of the patients with a mutation in a cardiomyopathy gene ($p<0.001$). No CHD were reported in patients with X-linked NCCM. Neuromuscular disease was more frequent in patients with X-linked NCCM and patients with a chromosome defect and was rarely reported in patients with a cardiomyopathy gene mutation ($p<0.001$).

**Table 4: Genotype- phenotype correlations in 416 NCCM patients with a mutation in a single cardiomyopathy gene.**

| | Sarcomere gene | Arrhythmia genes | Non-sarcomere non-arrhythmia cardiomyopathy genes | Genes associated with CHD | |
|---|---|---|---|---|---|
| | n =292 (70%) | n =52 (12%) | n=44 (11%) | n=28 (7%) | p-value |
| Male | 147/290 (51%) | 21/52 (40%) | 19/41 (46%) | 16/28 (57%) | ns |
| Median age at diagnosis in years (IQR) | 30 (13-44) | 15 (4-43) | 28 (13-46) | 23 (13-47) | ns |
| Congenital heart disease | 27/292 (9%) | 1/65 (2%) | 4/44 (9%) | 6/28 (21%) | - |
| Neuromuscular symptoms | 6/72 (8%) | 0/4 (0%) | 6/12 (50%) | 0/2 (0%) | - |
| LV dilatation | 112/199 (56%) | 3/6 (50%) | 18/22 (82%) | 8/18 (44%) | - |
| LV systolic dysfunction | 136/211 (65%) | 9/35 (26%) | 23/35 (66%) | 9/26 (35%) | <0.001 |
| Major adverse cardiac events† | 57/292 (20%) | 16/52 (31%) | 18/44 (41%) | 6/28 (21%) | 0.009 |
| Stroke | 6/292 (2%) | 0/52 (0%) | 0/44 (0%) | 0/28 (0%) | - |
| Heart failure requiring hospital admission | 15/292 (5%) | 0/52 (0%) | 3/44 (7%) | 0/28 (0%) | - |
| Left ventricular assist device | 6/292 (2%) | 0/52 (0%) | 0/44 (0%) | 0/28 (0%) | - |
| Heart transplantation | 13/292 (5%) | 0/52 (0%) | 6/44 (14%) | 2/28 (7%) | - |
| Sustained VT/VF or appropriate shock | 15/292 (5%) | 12/52 (23%) | 9/44 (21%) | 2/28 (7%) | - |
| Cardiac death | 16/292 (6%) | 2/52 (4%) | 5/44 (11%) | 0/28 (0%) | - |

IQR interquartile range; LV left ventricular; MACE major adverse cardiac event; NCCM noncompaction cardiomyopathy; ns not significant

† Major adverse cardiac events was composed of stroke, heart failure requiring hospital admission, left ventricular assist device, heart transplantation, sustained VT/VF or appropriate shock and cardiac death.

*Genotype-phenotype correlation: cardiomyopathy genes*

Altogether 74% of the patients had a mutation in a cardiomyopathy gene, i.e. in a sarcomere -, arrhythmia -, non-sarcomere non-arrhythmia cardiomyopathy -, or a CHD gene (table 4). LV systolic dysfunction was more frequent in patients with a mutation in a sarcomere gene, or in a non-sarcomere, non-arrhythmia cardiomyopathy gene mutation than in those with a mutation in an arrhythmia gene or a gene associated with CHD ($p<0.001$). In this group of patients defects in non-sarcomere, non-arrhythmia cardiomyopathy genes were associated with an increased risk for MACE ($p=0.009$).

**Table 5: Genotype- phenotype correlations in 292 NCCM patients with a mutation in a sarcomere gene.**

| | MYH7 | ACTC1 | MYBPC3 | TTN | Other sarcomere genes* | |
|---|---|---|---|---|---|---|
| **Total number of patients with a mutation in a sarcomere gene** | n=142 (48%) | n=38 (13%) | n=39 (13%) | n=22 (8%) | n=51 (17%) | p-value |
| Male | 71/141 (50%) | 20/38 (53%) | 17/39 (44%) | 15/21 (68%) | 24/50 (48%) | ns |
| Median age at diagnosis in years (IQR) | 30 (10-42) | 26 (13-48) | 28 (11-46) | 43 (29-54) | 30 (8-44) | 0.043 |
| Congenital heart disease | 16/142 (11%) | 6/38 (16%) | 3/39 (8%) | 1/22 (5%) | 1/51 (2%) | - |
| Neuromuscular disease | 4/32 (13%) | 0/1 (0%) | 1/13 (8%) | 1/19 (5%) | 0/7 (0%) | - |
| LV dilatation | 49/80 (61%) | 7/36 (19%) | 17/24 (71%) | 15/21 (71%) | 24/38 (63%) | <0.001 |
| LV systolic dysfunction | 68/106 (64%) | 8/14 (57%) | 18/27 (67%) | 16/21 (76%) | 26/43 (61%) | ns |
| Major adverse cardiac events | 19/142 (13%) | 4/38 (10%) | 16/39 (41%) | 7/22 (32%) | 11/51 (22%) | 0.001 |
| Stroke | 4/142 (3%) | 0/38 (0%) | 1/39 (3%) | 1/22 (4%) | 0/51 (0%) | - |
| Heart failure requiring hospital admission | 6/142 (4%) | 1/38 (3%) | 3/39 (8%) | 1/22 (5%) | 4/51 (8%) | - |
| Left ventricular assist device | 0/142 (0%) | 0/38 (0%) | 4/39 (10%) | 1/22 (5%) | 1/51 (2%) | - |
| Heart transplantation | 4/142 (3%) | 1/38 (3%) | 2/39 (5%) | 2/22 (9%) | 4/51 (8%) | - |
| Sustained VT/VF or appropriate shock | 4/142 (3%) | 2/38 (5%) | 6/39 (15%) | 1/22 (5%) | 2/51 (4%) | - |
| Cardiac death | 3/142 (2%) | 0/38 (0%) | 9/39 (23%) | 2/22 (9%) | 2/51 (4%) | - |

IQR interquartile range; LV left ventricular; MACE major adverse cardiac event; NCCM noncompaction cardiomyopathy; ns not significant

* *ACTN2, DES, LDB3, MYH7B, MYL2, NEBL, OBSCN, TNNC1, TNNC1, TNNI3, TNNT2* and *TPM1*.

*Genotype-phenotype correlations: sarcomere genes*

In 52% (n=292) NCCM was caused by a sarcomere gene mutation, in particular in *MYH7, MYBPC3, ACTC1,* and *TTN,* representing about 43% of the published genetic causes (figure 1). Other sarcomere mutations were reported in the 11 genes: *ACTN2, DES, LDB3, MYL2, NEBL, OBSCN, TNNC1, TNNI3, TNNT2* and *TPM1*. The *TTN* mutations were reported in adult patients. Mutation in *ACTC1* had less LV dilatation (p<0.001; table 5). Among the patients with a sarcomere gene defect, the *MYBPC3* mutations were associated with increased risk for MACE. Patients with a mutation in *MYH7* or *ACTC1* had the lowest risk of MACE of all patients with a sarcomere gene defect (p=0.001).

**Table 6: NCCM subtypes in 349 patients*.**

| | Isolated NCCM n=95 (27%) | NCCM/HCM n=47 (13%) | NCCM/DCM n=195 (56%) | p-value** | NCCM/HCM/ DCM n=12 (3%) |
|---|---|---|---|---|---|
| **Adult** | 54/95 (57%) | 25/45 (58%) | 104/194 (54%) | 0.798 | 1/12 (8%) |
| Cardiomyopathy gene | 84/95 (88%) | 37/47 (80%) | 139/195 (76%) | <0.001 | 3/12 (30%) |
| • Sarcomere | 72/95 (76%) | 30/47 (81%) | 110/195 (79%) | 0.006 | 2/12 (67%) |
| • Arrhythmia | 3/95 (3%) | 3/47 (8%) | 3/195 (2%) | - | 0/12 (0%) |
| • Non-sarcomere-, non-arrhythmia- cardiac | 4/95 (4%) | 0/47 (0%) | 17/195 (12%) | - | 1/12 (33%) |
| • Genes associated with CHD | 5/95 (5%) | 4/47 (11%) | 9/195 (6%) | - | 0/12 (0%) |
| X-Linked | 3/95 (3%) | 3/47 (7%) | 19/195 (10%) | - | 5/12 (50%) |
| Mitochondrial | 1/95 (1%) | 3/47 (7%) | 15/195 (8%) | - | 2/12 (20%) |
| Chromosomal | 5/95 (5%) | 3/47 (7%) | 10/195 (5%) | - | 0/12 (0%) |
| Complex genotype | 2/95 (2%) | 1/47 (2%) | 12/195 (6%) | - | 2/12 (17%) |
| Left ventricular systolic dysfunction | 42/86 (49%) | 4/18 (22%) | 148/185 (80%) | <0.001 | 11/12 (92%) |
| Major adverse cardiac events | 21/95 (22%) | 7/47 (15%) | 66/195 (34%) | <0.001 | 9/12 (75%) |
| Stroke | 1/95 (1%) | 1/47 (2%) | 3/195 (2%) | - | 1/12 (8%) |
| Heart failure requiring hospital admission | 6/95 (6%) | 3/47 (6%) | 16/195 (8%) | - | 5/12 (42%) |
| Left ventricular assist device | 0/95 (0%) | 0/47 (0%) | 3/195 (2%) | - | 1/12 (8%) |
| Heart transplantation | 4/95 (4%) | 0/47 (0%) | 15/195 (8%) | - | 4/12 (33%) |
| Sustained VT/VF or appropriate shock | 3/95 (3%) | 4/47 (9%) | 23/195 (12%) | - | 2/12 (17%) |
| Cardiac death | 5/95 (5%) | 2/47 (4%) | 28/195 (14%) | - | 3/12 (25%) |

*225 patients could not be classified because of missing data
** Comparing isolated NCCN, NCCM/HCM and NCCM/DCM.

*Noncompaction cardiomyopathy subtypes*

The NCCM subtypes were isolated NCCM (n=95), NCCM with HCM (n=47), NCCM with DCM (n=195) and NCCM with HCM and DCM (n=12) (table 6). In 225 patients there were insufficient data to classify the patients to the NCCM subtypes, and these patients were excluded from this analysis. The NCCM/HCM and NCCM/DCM cardiac phenotypes were mostly reported in patients with defects in sarcomere genes (p=0.006). Patients with mutations in the tail of *MYH7* (starting at c.2524) were more likely to have the NCCM/DCM subtype (83%), than patients with *MYH7* mutations in the head of the gene (42%). The NCCM/DCM had more often LV systolic dysfunction (p<0.001). The patients with NCCM/HCM had the least often LV systolic dysfunction and had the lowest risk for MACE (p<0.001). The NCCM/HCM/DCM phenotype occurred mostly in children (p=0.014) with mutations in other than cardiomyopathy genes and was associated with severe outcome (p<0.001).

**Table 7: Univariate and multivariate binary logistic regression for the prediction of major adverse cardiac events in genetic NCCM patients.**

| Variable | univariate | | multivariate Model 5* | |
|---|---|---|---|---|
| | OR (95% CI) | p-value | OR (95% CI) | p-value |
| Male | 0.87 (0.60-1.28) | 0.486 | 0.69 (0.41-1.15) | 0.154 |
| Age at diagnosis in years | 0.98 (0.97-0.99) | <0.001 | 0.99 (0.97-1.00) | 0.010 |
| Congenital heart disease | 1.24 (0.69-2.24) | 0.469 | | |
| Neuromuscular symptoms | 1.92 (0.92-3.99) | 0.082 | | |
| LV dilatation | 2.52 (1.45-4.37) | 0.001 | 1.39 (0.19-10.23) | 0.716 |
| LV systolic dysfunction | 3.65 (2.24-5.94) | <0.001 | 2.71 (1.19-6.15) | 0.021 |
| Isolated NCCM | 1 | - | 1 | - |
| NCCM/HCM | 0.62 (0.24-1.58) | 0.312 | 0.56 (0.07-4.65) | 0.560 |
| NCCM/DCM | 1.80 (1.02-3.18) | 0.042 | 0.87 (0.10-7.36) | 0.883 |
| NCCM/HCM/DCM | 10.57 (2.62-42.60) | 0.001 | 1.66 (0.04-62.08) | 0.742 |
| *MYH7* | 1 | - | 1 | - |
| *ACTC1* | 0.76 (0.24-2.39) | 0.641 | 1.05 (0.29-3.82) | 0.936 |
| *MYBPC3* | 4.50 (2.02-10.03) | 0.000 | 4.73 (1.98-11.26) | 0.000 |
| *TTN* | 3.02 (1.09-8.37) | 0.033 | 3.45 (1.17-10.22) | 0.025 |
| Other sarcomere genes | 1.78 (0.78-4.06) | 0.170 | 1.76 (0.75-4.15) | 0.195 |
| Arrhythmia genes | 2.88 (1.34-6.16) | 0.007 | 3.94 (1.71-9.08) | 0.001 |
| Non-sarcomere-, non-arrhythmia- cardiomyopathy | 4.48 (2.07-9.69) | 0.000 | 4.56 (2.00-10.42) | 0.000 |
| Genes associated with CHD | 1.77 (0.63-4.92) | 0.277 | 2.52 (0.83-7.63) | 0.101 |
| X-linked | 6.80 (3.12-14.83) | 0.000 | 5.65 (2.32-13.81) | 0.000 |
| Mitochondrial | 2.23 (0.94-5.31) | 0.069 | 1.67 (0.66-4.24) | 0.284 |
| Chromosome defect | 3.39 (1.41-8.13) | 0.006 | 2.41 (0.89-6.54) | 0.083 |
| Complex genotype | 2.81 (1.16 -6.83) | 0.022 | 2.58 (1.00-6.68) | 0.051 |

*Model 5 included: sex, age, LV dilatation, LV systolic function and NCCM subtype.
CHD congenital heart disease; DCM dilated cardiomyopathy, HCM hypertrophic cardiomyopathy; LV left ventricular; NCCM noncompaction cardiomyopathy.



*Patient characteristics, genetics and outcome*

Univariate binary logistic regression analysis including both phenotype and genetic parameters predictors for MACE are presented in table 7. Sex was missing in 1%, age at diagnosis in 4%, LV systolic function in 38%, NCCM subtype in 38% and LV dilatation in 43%. In online table 4 we show stepwise multivariate regression. In the final model age (OR 0.99, 95%CI 0.97-1.00) LV systolic function (OR 2.71, 95%CI 1.19-6.15) and a number of genes were associated with MACE. These genes include *MYBPC3* (OR 4.73, 95%CI 1.98-11.26), *TTN* (OR 3.45 95%CI 1.17-10.22), arrhythmia genes (OR 3.94 95%CI 1.71-9.08), non-sarcomere non-arrhythmia cardiomyopathy genes (OR 4.56, 95%CI 2.00-10.42) and X-linked genes (OR 5.65, 95%CI 2.32-13.81) (table 7). Complex genotypes and chromosome defects did not remain as significant predictors for MACE after correction.

## Discussion

A large number of reported NCCM cases were reviewed to investigate if genetic cause could predict the clinical risk profile for age at diagnosis, NCCM subtype, clinical features, risk for MACE, and LV systolic dysfunction. The collected data concerned only reported genetic NCCM patients (i.e. excluded about one third of the mutation carriers, who are expected to be non-penetrant carriers (without cardiomyopathy) who were not reported) (194, 196). Of the reported patients, children were more likely to have syndromic forms of NCCM with complex genotypes, X-linked inherited conditions, mitochondrial and chromosomal defects. These forms of NCCM had high risk for severe outcome, with congenital heart defects and neuromuscular symptoms. In contrast, patients diagnosed in adulthood had significant less severe outcome associated with autosomal dominantly inherited mutations in predominantly sarcomere genes. The correlation between genetic causes, outcome and age at diagnose suggests that different diagnostic approaches and clinical care may be needed for children, for instance including neurological examinations. Although the application of large panels of cardiomyopathy genes, allowed identification of mutations in 80 genes, in case regular NGS DNA testing is inconclusive a genetic cause cannot be excluded and additional mitochondrial gene - and chromosome analysis are important subsequent diagnostics steps, in particular when the NCCM is diagnosed in a child.

### *Genotype-phenotype correlations*

Mutations in genes affecting sarcomere function represented more than half of the genetic causes of genetic NCCM, in particular in adults. Mutations in *MYH7* were most prevalent, followed by mutations in *ACTC1, MYBPC3* and *TTN* as described previously (8, 155). Patients with a mutation in the sarcomere genes had lowest risk for MACE compared to patients with other genetic causes for NCCM. Comparing the clinical features of patients with different sarcomere gene mutation showed major differences for risk of MACE; patients with a mutation in *MYBPC3 and TTN* had a much higher risk for MACE than the patients with a *MYH7* mutation. This endorses the importance of DNA testing in NCCM, because the DNA diagnosis may help to predict outcome. Secondly these results show that further large studies of NCCM patients with mutations are needed to confirm and refine the identified genotype-phenotype correlations for improved patient tailored clinical management. Extensive phenotyping of NCCM may be the next step for finding more precise genotype-phenotype correlations. Recently, NCCM has been classified into subtypes based on concurrent occurrence of dilated and/or hypertrophic LV (165). This subdivision improves prediction of genetic defect and helps to predict LV systolic dysfunction and risk for MACE (194, 196). This review

shows that isolated NCCM was associated with defects in cardiomyopathy genes.

*Family history*

In genetic diseases a high prevalence of familial disease is expected. In this review familial cardiomyopathy was observed in 43% of genetic NCCM. The explanation for this low rate of familial cardiomyopathy may be that patients without familial disease had a de novo mutation or large chromosome defects. Another explanation might be that family histories were differentially ascertained across studies, or that patients may not have been aware that relatives had a cardiomyopathy or relatives were diagnosed after the completion of the study. In addition the underreporting of familial disease may be explained because relatives may be asymptomatic carriers of a cardiomyopathy mutation. Previously we observed that 48% of affected relatives who were diagnosed by family screening were asymptomatic (194). In NCCM 37% of relatives who are carriers of a mutation in a cardiomyopathy gene are asymptomatic and approximately 30% of the carriers have no signs of a cardiomyopathy (non-penetrance), which is similar to the rate of asymptomatic or unaffected carriers in families with HCM (194, 197). Familial cardiomyopathy was reported more frequent in adult index patients, although it is usually easier to attain genetic and/or cardiologic testing of the parents of young cases than of patients diagnosed in adulthood. An explanation for the discrepancy of familial disease could be that X-linked NCCM and chromosomal abnormalities were more frequent in children, and detection of patients in families with X-linked inheritance depends on the number of male relatives of the mother of the patient, and therefore it may be more difficult to detect affected relatives than in families with autosomal dominant inherited forms.

*Noncompaction phenotype*

NCCM is associated with a large number mutations in a large number of genes, leading to different defective molecular functions in cardiomyocytes. This raises the question if these defects have a common final effect on the myocardium or that different pathogenic mechanisms are involved. For now the observed differences in clinical features, NCCM phenotype and risk profiles suggest the latter. Future expansion of genetic testing with whole genome sequencing may identify regulatory influences on gene expression explaining the phenotype. In particular because many genetic defects occur also in HCM and DCM. It is expected that also the newly developed models using induced pluripotent stem cells differentiated into cardiomyocytes can be used to investigate genetic and epigenetic modifiers explaining cardiomyopathy phenotypes.

## Limitations

The majority of the included studies were case-reports or small case-series. Therefore the included patients were expected to have been subject to referral, selection and publication bias. We cannot exclude overrepresentation in reporting of rare genetic causes like for instance mutations in *TAZ* gene in children. This review focussed only on the genetically confirmed NCCM patients, which are approximately one third of all NCCM (8). The design of this study precludes identifying differences in prognosis between known genetic and other causes for NCCM.

## Conclusions

The most frequent causes of genetic NCCM are mutations in sarcomere genes, with a relatively low risk of adverse events, occurring mostly in adults. In general, age at diagnosis and cardiac outcome were related to specific genetic causes. Rare X-linked and chromosome defects were more frequent among children with severe outcome. These observations endorsed to adjust molecular diagnostics and clinical approaches to age at presentation. The observed genotype-phenotype correlations show that DNA diagnostics for NCCM is important. Identifying the genetic cause for NCCM allows risk stratification and may help clinical management and counselling of patients and their relatives.

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# Chapter 6

## *FLNC* missense variants in familial noncompaction cardiomyopathy

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*Missense FLNC Mutations associated with familial noncompaction Cardiomyopathy and Congenital Heart Defects, Cardiogenetics, 9(1).*

**Abstract**

The majority of familial noncompaction cardiomyopathy (NCCM) is explained by pathogenic variants in the same sarcomeric genes that associated with hypertrophic (HCM) and dilated (DCM) cardiomyopathy. Pathogenic variants in the filamin C gene (*FLNC*) have been linked to HCM and DCM. We expand the spectrum of *FLNC* related cardiomyopathies by presenting two families with likely pathogenic *FLNC* variants showing familial segregation of NCCM and concurrent coarctation of the aorta and/or mitral valve abnormalities.

## Introduction

Noncompaction cardiomyopathy (NCCM) is characterized by excessive trabeculation of the left ventricle (LV) with a noncompacted to compacted ratio of more than 2 according to current echocardiographic criteria, or 2.3 on CMR(3, 25). Approximately 10% of patients diagnosed with NCCM have concurrent congenital heart defects (CHD)(22, 165).

In 30-40% of cases diagnosed with NCCM a pathogenic variant can be identified. Around 80% of these pathogenic variants involve the same sarcomere genes, that are the major causes for hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM), in particular *MYH7, MYBPC3* and *TTN* (54, 147). Filamin C (*FLNC)* plays a central role in muscle functioning by maintaining the structural integrity of the muscle fibers. Pathogenic variants in *FLNC* were found to be associated with a wide spectrum of myopathies ranging from cardiomyopathies to distal skeletal myopathies. Truncating *FLNC* variants were previously associated with dilated cardiomyopathy (198-200) and missense variants were identified in familial HCM and restrictive cardiomyopathy (15, 20). *FLNC* has not been associated with NCCM or CHD before.

We present two Dutch families where familial NCCM with CHD were linked to rare *FLNC* missense variants. These observations suggest that the spectrum of clinical manifestations of *FLNC* variants include familial NCCM with CHD.



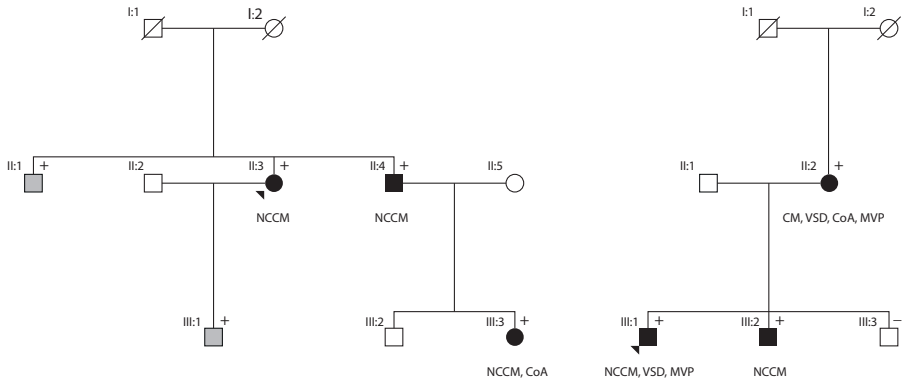


**Figure 1: Pedigrees of families A and B.**
Pedigrees of NCCM families with missense FLNC variants. Black filled symbols are affected family members. Gray filled symbols are family members with hypertrabeculation not meeting NCCM criteria. The + sign indicates family members carrying a FLNC likely pathogenic variant (p.(Arg2133Cys) in family A and p.(Pro2393Ser) in family B. CM, cardiomyopathy; CoA, coarctation of the aorta; MVP, mitral valve prolapse; NCCM, non-compaction cardiomyopathy; VSD, ventricular septal defect.

## Case reports

*Family A*

In this family (figure 1A), a 52-year-old woman (II:3) was first diagnosed with NCCM when she underwent cardiologic examination for a suspected perimyocarditis. Echocardiography showed pericardial effusion and normal LV dimensions without LV dysfunction. The LV walls showed hypertrabeculation with end-systolic non-compacted/compacted (NC/C) ratio >2. Electrocardiographically, inferolateral repolarization abnormalities were observed. Cardiac magnetic resonance imaging (CMR) confirmed the diagnosis of NCCM with diastolic NC/C ratio >2.3 in the LV inferoseptal wall. She had elevated CK levels of 1234 U/L [ref <200U/l]. No signs for neuromuscular disease were detected at neurologic examination. After seven years of follow-up, she remained cardiologically asymptomatic (NYHA class I).

Family screening revealed NCCM in two relatives. A niece (III:3), was diagnosed with NCCM at age 21 and had surgery at age seven for coarctation of the aorta (CoA). She fulfilled both the echocardiography- and CMR diagnostic criteria for NCCM and had excessive long chordae of the anterior mitral valve leaflet (figure 2A and 2B). The noncompaction had not been recognized in the past on echocardiography. Cardiologic screening of an asymptomatic brother (II:4) at age 54 years showed that he also had NCCM; with a NC/C ratio of 2.3 on echocardiography and a ratio of 2.9 on CMR. No previous cardiac imaging had been performed. He had elevated CK-levels of 265 U/L without neuromuscular signs. Ten years after the diagnosis NCCM he had an episode of atrial fibrillation that required electric cardioversion. CMR from the brother (II:1) and the son (III:1) of the proband were performed at age 57 and 15 years, respectively, showing borderline NC/C ratios of respectively 2.1 and 2.2 on MRI, i.e. just below the diagnostic criteria. Proband III-2 did not participate in the family screening.

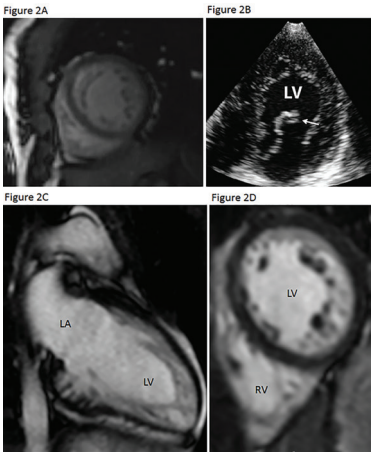


**Figure 2: Imaging of two NCCM patients.**
**Family A. patient III.3 2A:** Short axis of the left ventricle on cardiac magnetic resonance (CMR) showing the prominent trabeculae and intertrabecular recesses in NCCM. **2B.** Echocardiogram of the LV of patient (III.3) with NCCM and excessive long chordae of the anterior mitral valve leaflet (arrow). **Family B. patient III.1 2C:** Two-chamber long-axis of the left ventricle on CMR. **2D.** Short axis view of the left ventricle on CMR showing the prominent trabeculae and intertrabecular recesses in NCCM.

*Family B*

In family B (figure 1B) the diagnosis NCCM in a 17-year-old boy (III:1) was made by echocardiography. He was referred because of multiple unexplained episodes of syncope. He also had a ventricular septal defect (VSD) and a mild mitral valve prolapse (MVP). CMR revealed partial LV noncompaction from the apex to midventricular region with an NC/C ratio of 3.0 (figure 2C and 2D). An implantable cardioverter-defibrillator (ICD) was implanted. After 9 years of follow-up, the LV function remained normal without ICD shocks. CK-levels were elevated (419-1188 U/l) in the absence of neuromuscular signs

His mother (II:2) was under cardiologic surveillance because she had a VSD, MVP, CoA and a bicuspid aortic valve. The CMR showed that she complied for the diagnostic criteria for NCCM with a NC/C ratio of 2.4. She had diastolic LV dysfunction, with preserved LV systolic function and underwent multiple cardiac ablations for atrial fibrillation. At age 44 years she experienced severe bradycardia, which necessitated cardiac resuscitation, resulting from combined flecainide and metoprolol treatment. A pacemaker was implanted. Her highest CK-level was 1174 U/l. The proband's brother (III:2) also suffered multiple episodes of syncope and was diagnosed with NCCM at age 19, with a NC/C ratio of 3.1 on CMR. His highest CK-level was 294U/l. Proband III-3 was screened cardiologically and had no signs of NCCM on echocardiography. No DNA analysis was performed.

*Genetic testing*

Diagnostic DNA NGS targeted testing of a panel of 54 cardiomyopathy genes, that did not include *FLNC*, as presented in online supplement 1, did not reveal a genetic causes for NCCM in the two index cases. Also single-nucleotide polymorphism-array DNA testing showed no structural DNA changes. Subsequently, whole exome sequencing was performed in the NCCM patients II:1, III:1 and III:3 from family A and III:1, III:2, and II:2 from family B. Patients II-2 from family B was included because we suspected that a causative mutation may underlie a spectrum of cardiac phenotypes. Written informed consent was obtained from all participating family members. The investigation conforms to the principles outlined in the Declaration of Helsinki. Variants were annotated using ANNOVAR(201) and filtered using an in-house developed pipeline. Only variants segregating within each family, affecting exons or splice sites, with a population frequency below 0.01 in ExAC, NFE, GoNL were kept. For in silico prediction of the effect of nonsynonymous variants we used align GVGD (202), SIFT (203) and Polyphen (204) and ensemble scores LR and Radial SVM (205). We selected variants who were predicted to be damaging by 3 of 5 prediction programs. Segregating synonymous variants and variants predicted to be tolerated were excluded.

In Family A, two candidate genes remained after filtering, *MYH4* and *FLNC*, of which only the last was previously associated with cardiomyopathy. A variant in **FLNC** (c.6397C>T, p.(Arg2133Cys), NM_001458.4, confirmed by sanger sequencing) segregated with the cardiac phenotype of NCCM in the three NCCM patients and in the two relatives with borderline NCCM features. A variant in the same location (p.(Arg2133His)) was previously reported in a family with HCM and classified as probably pathogenic (206). In family B, a novel *FLNC* variant (c.7177C>T, p.(Pro2393Ser)) was identified. The two *FLNC* variants were absent in the Genome Aggregation Database (http://gnomad.broadinstitute.org), affect highly conserved amino acids, and were predicted to be deleterious by multiple *in silico* prediction programs. No *FLNC* variants were found in thirteen unrelated NCCM patients without a CHD and without a pathogenic variant in 48 cardiomyopathy genes.

*Histology*

Right ventricular endomyocardial biopsy (RVEMB) samples from the proband of family B (III:1) were stained with hematoxylin and eosin as well as Masson's trichrome. To visualize protein aggregation and autophagic activity in cardiomyocytes, immunohistochemistry for microtubule-associated protein 1A/1B-light chain 3 (LC3) was performed, as described previously (207). Light microscopic analysis showed nonspecific cardiomyopathic changes of myocyte hypertrophy and increased interstitial fibrosis (Online Figure 3.A). The RV did not show an excessively thickened endocardial layer or hypertrabeculation, or intracellular aggregates or autophagic activity (Online Figure 3.B).

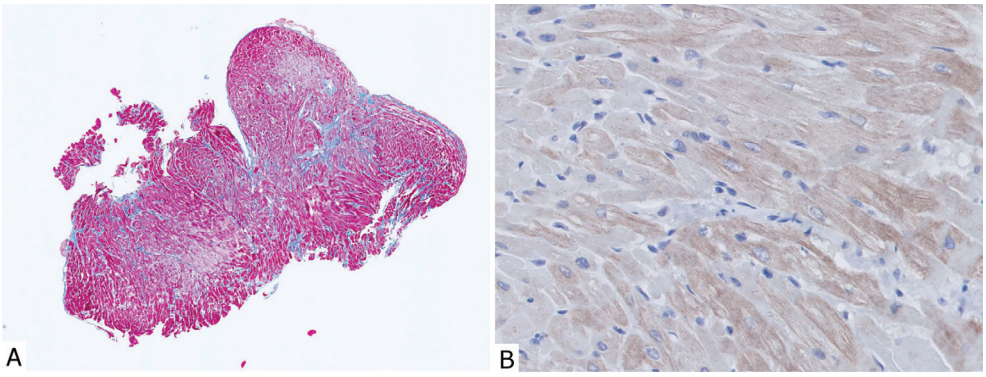


**Figure 3: Myocardial tissue staining of FLNC likely pathogenic variant carrier (Family B. III:1).** Myocardial Tissue Analysis of RVEMB samples from FLNC likely pathogenic variant carrier (Family B. III:1). **3A.** Masson's trichrome staining, original magnification x20, shows mild fibrosis. **3B.** Immunohistochemical staining (LC3), original magnification x200, does not show protein aggregates with autophagic activity in cardiomyocytes. Faint LC3 background cytoplasmic staining can be observed.

## Discussion

This is the first report linking *FLNC* to NCCM in two families with rare *FLNC* variants. In these two families the cardiac phenotype included NCCM, NCCM with concurrent CoA, NCCM with concurrent VSD and MPV and also a NCCM patient with a complex CHD consisting of VSD, MVP, CoA and bicuspid aortic valve, and myocardial dysfunction. These observations suggest that missense *FLNC* variants may cause familial NCCM with or without one or more structural heart defects. Missense *FLNC* variants in the N and C terminal domains of *FLNC* have been associated with hypertrophic- and restrictive cardiomyopathy, causing sarcomeric aggregates containing *FLNC* leading to sarcomere dysfunction (206). Other *FLNC* domains were associated with myofibrillar myopathy, showing similar intracellular aggregates in skeletal muscles (208, 209). A large study of inherited cardiovascular disease patients showed that truncating *FLNC* variants were associated predominantly with an overlapping phenotype of severe dilated- and arrhythmogenic cardiomyopathies (199). The cardiomyopathy phenotypes associated with *FLNC* variants have not included NCCM so far, however signs of LV hypertrabeculation not fulfilling NCCM diagnosis in some carriers of a truncating *FLNC* variants have been reported (199). FLNC has not been linked to CHD, sofar, to the best of our knowledge.

Aortic coarctation in NCCM patients seems rare with an estimated prevalence of less than 1% (22). We report two families with familial NCCM and a likely pathogenic *FLNC* variant in which CoA occurred; in one family one patient had NCCM with concomitant CoA. In the other family a NCCM patient with the familial *FLNC* variant had complex congenital cardiac defects including a CoA. It remains to be elucidated how *FLNC* and other genes can cause a cardiomyopathy with or without a CHD, and skeletal myopathy in other patients. It may be that *FLNC* resembles *MYH7* in that aspect. Because among all the known genes associated with cardiomyopathies as well as skeletal myopathies, *MYH7* variants occurs the most frequent in NCCM, and is also linked to Ebstein anomaly with and without NCCM, HCM, DCM, or Laing distal myopathy (23, 210). Suggesting that variants affecting distinct domains of sarcomeric proteins may define the spectrum of cardiac and skeletal muscle phenotypes.

Pathogenic *FLNC* variants are expected to disrupt the structure of the sarcomeric protein, leading to the formation of protein aggregates resulting in an impairment of the sarcomere function (206, 211). One of the identified variants in this study, *FLNC* p.(Arg2133Cys), may have a similar effect as the reported *FLNC* variant p.(Arg2133His) at the same location with another amino-acid substitution, that was shown to have

disrupting actin aggregates in cardiac tissue (206). We found elevated CK levels in two of the three NCCM patients in both affected families. Elevated CK levels were also noted in a previous study regarding *FLNC* variants with myopathy but also in patients with only a cardiac phenotype of HCM (206). For the novel *FLNC* variant p.(Pro2393Ser), we observed fibrosis in the RV myocardium samples, indicating a damaging effect of the variant on the cardiac muscle. Fibrosis was also observed in previous reports with *FLNC* mutations (199, 206). The cardiac fibrosis observed in the patients with the *FLNC* variant suggests that similar pro-fibrotic mechanisms may be involved as observed in *MYBPC3* cardiomyopathy (212). Similarly, to the original report no signs of intracellular aggregates or autophagic activity in the RV of the patient or were noted in patients with a *FLNC* related cardiomyopathy (199, 206). The RV of this patient did not show evidence for hypertrabeculation morphologically or on imaging. However this does not exclude an effect of the *FLNC* variant on the left ventricle, since RV hypertrabeculation in NCCM is rarely reported, and the NCCM presents predominantly with a LV phenotype.

As previous studies showed, genetics plays an important role in approximately half of the NCCM cases (8). The genetics of NCCM are complex and affect mostly genes associated with myopathies including sarcomere or mitochondrial dysfunctioning. In this perspective *FLNC* fits into the genetically heterogeneous background of NCCM. Further studies are needed to assess the exact role and mechanisms of *FLNC* in NCCM, aortic coarctation and mitral valve abnormalities.

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# Epilogue

## Discussion

*Genetics of NCCM*

Our goal was to find out what proportion of NCCM may have a genetic cause. We identified a (likely) pathogenic variant ((L)PV) in 32% of the patients (8). With a higher prevalence in children (45%) than in adult patients (30%). In 16% of the patients familial disease was present, without an identified genetic cause. These findings suggest that approximately half of the cases can be considered genetic. The prevalence of pathogenic variants found in our studies is supported by other NCCM cohorts studies in which 35% to 38% of NCCM patients had a pathogenic variant (9, 10). One study showed a somewhat lower (23%) prevalence of genetic causes in children (<21 years) (213). This could be explained by the fact that a smaller number of genes were tested and also by differences in patient ascertainment. The conclusion is that for now overall approximately one third of NCCM patients have a pathogenic variant.

Cases with a mutation as well as familial cases, even if still a mutation has to be identified, can be classified as genetic. Our study, with data on systematic screening of first-degree relatives, as recommended in the Netherlands, suggest that 37% of NCCM cases is familial. Of the familial cases 44% (16% of the total NCCM population) had familial disease without a mutation. It needs to be explained that the healthcare system in the Netherlands allows performing extensive cardiologic family screening, also in families without a mutation (15). In other countries, other regulations for screening relatives might apply and this may explain that few studies addressed the issue of frequency of familial NCCM. Also, in Erasmus Medical Center, a tertiary referral center for cardiomyopathies, screening of relatives is offered in a special designed multidisciplinary out-patient clinic, and the results of cardiologic exams of the family members are available together with the results of DNA testing. In situations when cardiologic family screening is not available for all at risk relatives, familial NCCM may go unnoticed leading to underreporting of familial disease. This may explain why the prevalence of familial disease in previous studies were lower than in our study (37%) ranging from 13% to 26% (9, 10, 147, 213). The information that a high percentage of familial cases did not have a mutation in a known cardiomyopathy gene (44%), as observed in our study could not be extracted from other studies.

There are several explanations for not finding the genetic cause in families with familial cardiomyopathy. For instance, there may be a yet unknown genetic cause, that may be identified in the near future when whole exome or genome sequencing are used for diagnostic purposes. With future broad application of

whole genome sequencing, we will not only look for exonic genetic defects, but also for defects in intronic DNA regions regulating the expression of known and unknown cardiomyopathy genes. Mutations in intronic regions may cause, just like truncating exonic mutations, haplo-insufficiency. Secondly, to date only monogenetic causes for NCCM are identified. With the increasing insight into the effects of DNA variants in the future with the wider use of novel DNA sequencing techniques, modifying genes and even complex genetic burdens associated with NCCM may be identified. These novel genetic analyses may help to identify complex genetic causes, consisting of multiple – in themselves- non penetrant variants that together may act as modifiers or as a susceptibility with additive pathogenic effects. We should also consider that familial occurrence of secondary causes may occur in NCCM in multiple relatives. For instance, hereditary hypertension in multiple relatives may provoke remodeling of the left ventricle into the NCCM phenotype with or without additional genetic susceptibility or genetic modifiers. In conclusion, if we assume that a genetic cause can be found in all familial patients. The proportion of genetic patients is between one third and half of the NCCM patients. In the other half hypertrabeculation may be provoked by environmental factors, an underlying cause leading to altered loading conditions or an interaction between environmental factors or other causes and genetic susceptibility for noncompaction.

Since NCCM is clearly heterogonous, we wanted to know if the clinical feature and outcome could be linked to the underlying causes. Genetic NCCM involves more than 66 genes and other genetic causes (24). Most of the identified genetic defects cause isolated sarcomeric damage, some defect also cause congenital heart disease and have other multi systemic manifestations (24). In patients with isolated NCCM *MYH7, TTN* and *MYBPC3* are the most prevalently affected genes (9, 10, 15, 147). Patients with congenital heart disease may have different genetic causes. For instance NCCM patients with Ebstein anomaly often have a mutation in the *MYH7* gene or -as we showed- NCCM patients with an aortic coarctation may have a defect in the *FLNC* gene (23, 214). Patients with neuromuscular manifestations are more likely to have defects in X-linked *TAZ*-gene, defects in mitochondrial genes or genes predisposing to skeletal myopathies (215). The age at presentation may play an important role in predicting the genetic cause. Patients presenting at younger age have more often extra cardiac manifestation, associated with complex defects in sarcomere genes, or have other rare genetic causes that are seldom observed in adult patients (24). The severity of the disease may be linked to specific genetic causes, like in other cardiomyopathies. For instance, adult HCM patients with truncating *MYBPC3* mutations were found to have a more severe disease than

patients with missense mutations (216, 217). In time, it is expected that by sharing information on specific variants and clinical features between cardiogenetic centers all over the world, more insights in genotype-phenotype associations emerge, explaining the specific features of the spectrum of cardiomyopathies.

The overlap of major genetic cause indicates that the NCCM, HCM and DCM phenotypes represent a cardiomyopathy spectrum. Sarcomere gene mutations are the main causes of NCCM as well as of other cardiomyopathies (i.e. HCM or DCM) (218, 219). Different cardiac phenotypes could to some degree be explained by the location of the mutation, and the function of the affected domain. These observations do not explain how the exact same genetic defect (i.e. (L)PV)) can lead to different cardiomyopathy phenotypes even within families. Deep phenotyping of NCCM (i.e. ascertainment of concomitant signs of HCM or DCM in NCCM patients) did show overlapping characteristics of other cardiomyopathies in NCCM patients and their relatives (15). Our study showed that the location of the mutations in *MYH7* could predict cardiac phenotype in NCCM (15). Patient with mutations in the tail were more likely to have NCCM with a dilated ventricle, the main characteristic of DCM. An explanation may be that mutations in the tail domain may interfere with the binding site for *TTN*, and thus may have a similar effect as *TTN* mutations, which are important causes of DCM (220). Also, NCCM patients with *TTN* mutations, had a higher prevalence of left ventricular dilatation and had more relatives with DCM with and without NCCM. A molecular study strengthens the observation that mutations in the head and tail domain may lead to different phenotypes by showing differences in interaction with actin (221). NCCM patients with a pathogenic variant in *MYBPC3*, the most frequent cause of HCM- but less frequent in NCCM-, were more likely to have signs of left ventricular hypertrophy and have relatives with HCM without NCCM (15). Analyses of the contractile function showed that type of mutation (missense vs. truncating mutations) in same gene could lead to marked differences in contractility of heart tissue (222). Other explanations for the difference in phenotypes may include individual differences in genetic modifiers, epi-genetic factors and/or different pathway activation.

*Consequences of identifying a disease causing mutation*

Since a large part of NCCM is caused by genetic defects, genetic testing plays an important role in NCCM. Finding the causative gene in genetic diseases has multiple advantages. Most importantly, identifying the genetic cause may facilitate family screening. Patients have been shown to benefit from early diagnosis and this has been proven to be cost effective in HCM (223, 224). A molecular diagnosis helps to identify accurately which relatives are at risk and/or also reassures family

members in whom the genetic defect can be excluded. DNA testing may also help to distinguish genetic from sporadic patients, with low risk for relatives. This of course has major implications for family screening strategies. Eventually, leading for low risk families, to adjust follow up frequencies of adult relatives with normal cardiologic exams, once there is sufficient evidence. We are now, at the beginning of finding genotype-phenotype correlations in patients with a mutation, and larger studies are needed to validate and expand these novel identified correlations. Still an important aspect of genetic testing are possible socio-economic consequences of being a mutation carrier. We also have to remain aware of and offer help with the psychological impact of a positive test result and the burden of needing lifelong cardiologic follow-up, for a patients who may or may not ever exhibit symptoms (126).

Also in this perspective, prediction of prognosis for relatives who were identified by family screening to be carriers of a mutation is of great importance, and this had not been extensively studied yet. We found that relatives with a mutation and the noncompaction phenotype had lower risk for cardiac symptoms and cardiac events compared to index cases (15). Probably because relatives were diagnosed by screening, in an earlier disease stage with less symptoms. More than one third of the relatives with a positive genetic test had a normal cardiac function and cardiac phenotype, indication a high percentage of non-penetrance. However, these patients cannot be excluded from cardiac follow-up since age related penetrance is a well-known feature in genetic cardiomyopathies, and they still may develop a cardiomyopathy later in life (133, 225). In HCM cohort studies 6-14% of relatives with a mutation without cardiomyopathy at initial screening developed HCM later on (133, 197, 226). For NCCM, it has not yet been established if relatives who do not have cardiac phenotype at initial screening, may develop a cardiomyopathy later on. Information on the risk of having a cardiomyopathy and the risk for severe cardiac complications is needed to counsel and design follow-up schedules for relatives at risk of familial NCCM with and without a mutation. This also applies to relatives of apparently sporadic cases (without a family history and mutation), because we cannot rule out that these patients carry a genetic risk for cardiomyopathy. Since these patients may have a mutation in an unknown cardiomyopathy gene, or that they have a more complex genetic etiology, carrying smaller but still elevated risk for cardiomyopathy for relatives. We observed that asymptomatic relatives with a phenotype had a low risk for cardiac events. And this may hold also for relatives without a cardiomyopathy phenotype (15). However, longer follow-up studies are needed to assess accurately the risks for relatives carrying a mutation with and without a cardiomyopathy phenotype.

*Etiology*

Noncompaction cardiomyopathy (NCCM) refers to an imaging-based description of the trabecular morphology of the left ventricular wall. The myocardial phenotype results from an incompletely understood pathophysiological mechanisms. A discussion about the etiology of the phenotype has been going on for a number of years (11-14). The theories are mainly based on circumstantial evidence on possible underlying mechanisms involved in the formation of noncompaction or hypertrabeculation. The most heard view is that NCCM should be considered a congenital heart defect (227).

The highly trabeculated left ventricle of NCCM patients resembles the heart of all vertebrate animals, including humans, during early embryogenesis (227). In the early embryo the heart is a loose interwoven mesh of muscle fibers. This mesh, looking like a trabecular network –or sponge-, provides the heart muscle with oxygen and nutrients from luminal blood. In week 4-6 of gestation the heart starts to form coronary arteries, the noncompaction is no longer needed and the myocardium solidifies. The compaction of the heart begins at the base of the heart to the apex and from epicard to endocard (227). According to the congenital heart defect theory the compaction process fails to complete in a NCCM patient and NCCM would thus be regarded as an embryological deformation. This would explain why NCCM patients predominantly have hypertrabeculation at the apex of the heart. It is hypothesized that this unfinished myocardium could lead to heart failure and arrhythmias. More unfinished myocardium would lead to worse outcome and also explains prenatally diagnosed NCCM and early presentation in childhood (124). In NCCM, patients diagnosed in childhood often have a worse prognosis than adults (24).

However, there are also arguments against this theory, based on opposite observations. To begin with, it is not sure whether the hypertrabeculation in noncompaction cardiomyopathy has an embryological origin. In a histology study only a miniscule subset of the postnatal trabeculations expressed ANF/NPPA (228). The absence of ANF/NPPA, the hallmark of embryonic cardiac trabeculations before the compaction stage, makes it unlikely that trabeculation observed in adults, are remnants of an arrest in embryologic development (228). Also age-related penetrance of NCCM is observed, like in other cardiomyopathies, for instance dilated cardiomyopathy (DCM) or hypertrophic cardiomyopathy (HCM). In fact, the majority of patients is diagnosed in adulthood, not in childhood (217). Another argument against the hypothesis of a prenatal arrest in cardiac development was presented by a study showing transient increased trabeculations in women during

pregnancy, in as much as 25% of pregnancies, which resolved post-partum in the majority of the women (152). It is unlikely that a congenital heart defect appears and resolves in adulthood. Moreover as the disrupted embryogenesis hypothesis would be true one would expect that disturbance of developmental genes play an important role in the pathogenesis. Most frequently defects in sarcomere genes were identified, defects in developmental genes are only scarcely reported, especially in adult patients (24). When defects in developmental genes were identified, patients had a higher incidence of congenital heart disease (24). We believe that defects in these genes cause congenital heart defects and secondary to the heart defect loading conditions of the LV may alter, leading to hypertrabeculation. This would also explain the high incidence of congenital heart disease in NCCM (22).

Another question is whether hypertrabeculation leads to cardiomyopathy. Cold-blooded animals, unlike humans, have highly trabeculated hearts. The degree of trabeculation was not related to the cardiac performance of animal species (229). Some large cold-blooded animals, showing characteristic hypertrabeculated ventricles, have blood pressures in range of the human blood pressure and same stroke work per gram ventricular heart tissue (230, 231). In humans, higher incidence hypertrabeculation is observed in healthy cohorts, especially in individuals of Hispanic or African origin (17, 29, 31, 40). Loading conditions (volume/pressure load) play an important role in remodeling of the left ventricular myocardium. Transient increased loading conditions may explain the appearance of trabeculations in athletes and in women during pregnancy (26, 61, 152). Which also makes a congenital arrest in heart development less likely. Increased incidences of hypertrabeculation in other conditions with increased loading conditions of the heart are also known, including hematologic diseases, chronic renal failure and hypertension (8, 9, 40, 232-234). In this scenario, NCCM may be caused by different mechanisms that activate the same remodeling pathway leading to the noncompaction phenotype.

A common pathway leading to noncompaction may also be an explanation for the many different genes associated with NCCM. This relatively rare cardiomyopathy, has a large spectrum of genetic causes, like HCM or DCM (218, 219). To date genetic defects in as much as 66 different genes, involving different cellular functions, explain approximately one third of the NCCM, approximately half the familial cases (8, 24). The majority of genetic variants associated with NCCM in humans occurs in genes encoding for the sarcomeric protein, but also genetic defects encoding for mitochondrial genes, affecting cellular energy management or in genes involved in calcium handling regulating the sarcomere contraction force,

have were related to NCCM. These genetic defects have a damaging effect on the cardiomyocyte functioning. How these damaged cardiomyocytes may respond to loading conditions and how this may lead to common remodeling pathway activation has still to be unraveled *(figure 1)*.

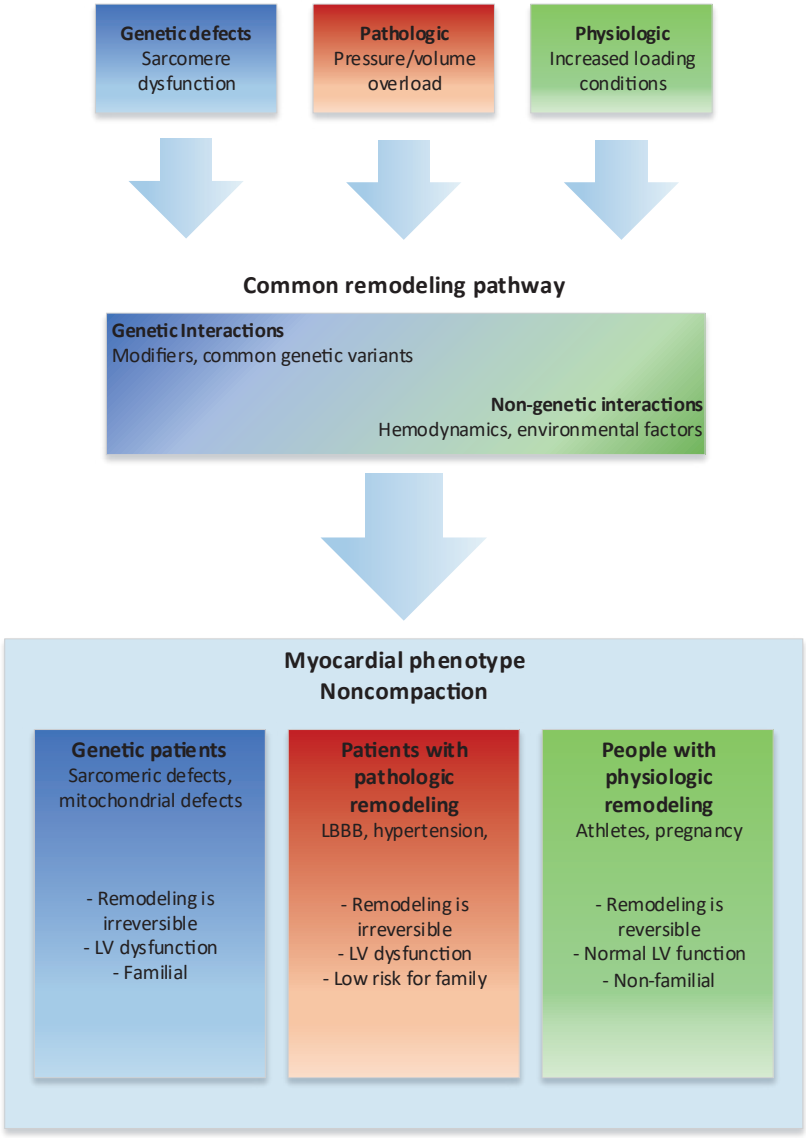


**Figure 1: Spectrum of cases with the myocardial phenotype noncompaction.**
The myocardial phenotype noncompaction can have different causes, which activate common pathway(s) leading to the same noncompacted phenotype. (LBBB: left bundle branch block, LV: left ventricular).

Our data indicates that NCCM may have different etiologies leading to the same myocardial phenotype. As demonstrated in *figure 1* three main group can be distinguished. Two of the three groups involve cardiomyopathy patients which require medical follow-up and one group includes healthy cases. In the genetic group of patients heredity plays an important role. As our study showed in around one third of the patients a genetic defect can be identified (8). In 37% of the patients family members with a cardiomyopathy can be observed (8). Since the genetic defects identified in NCCM patients overlap with other cardiomyopathies also patients with other genetic cardiomyopathies can be observed within families, making NCCM a part of the spectrum of genetic cardiomyopathies (15). It is likely that genetic modifiers and epigenetics determine the myocardial phenotype in these patients.

In the group of patients with pathologic remodeling NCCM is associated with other medical traits, which influence loading conditions and cardiac remodeling, activating similar genetic modifiers leading to same pathway activation requiring the formation of the hypertrabeculation phenotype. In these patients the hypertrabeculation may be a sign of myocardial distress. In the sporadic cases found in our study a higher incidence of hypertension was observed (8). This would indicate that hypertension is a secondary cause for NCCM, which is may also be a cause for left ventricular hypertrophy (235). This may also indicate that different genetic modifiers in patients lead to different myocardial phenotypes. In our sporadic patients also a higher incidence of left bundle branch blocks was observed (8). Left bundle branch blocks may lead to increased leading loading conditions of the left ventricle, which may lead to left ventricular remodeling (153). In these way congenital heart defects, which are frequently identified, may also lead to the NCCM phenotype (22).

The last group, the group of people with physiologic remodeling, are the healthy cases in which benign factors may lead to a phenocopy of the hypertrabeculated left ventricle. These cases include cases from Hispanic and African descend, but also athletes and women who are pregnant (26, 40, 61, 152). These cases are probably not at risk for cardiac events. In the clinic it may be important to distinguish the different groups. In the hereditary group family screening is important for early identification of patients at risk for serious cardiac events. The group with pathologic remodeling may also needs medical surveillance, while the last group do not need follow-up and can be discharged from medical follow-up. Concluding that still genetic modifiers leading to NCCM need to be identified, to confirm this theory.

*Mouse models for NCCM*

In the past decade different mice models have been developed to study NCCM. These mouse models have shown that some genes were related to a NCCM phenotype in mice hearts. A good example is a mice model for the *TAZ*-gene, associated with Barth syndrome, (236). *TAZ*-gene knock down mice showed hypertrabeculation at early gestation (237). Other genetic defects leading to NCCM in humans and in mice includes gene mutations in *MIB1*, *YWHAE*, *NKX 2-5* and *PTPN11* (81, 238-242). These genes are very rare identified causes in NCCM patient cohorts, regulating a variety of cellular mechanisms like cell differentiation. None of these genes were primarily associated with sarcomere or cardiomyocyte dysfunction. Of the current mouse models with the more common identified causes for NCCM, i.e. defects in *MYH7* and *MYBPC3*, none showed noncompaction. A mice model of a *TNNT2* mutation was established using the same mutation as in a family with NCCM (243). *TNNT2* encodes for the protein cardiac troponin T which is located in the thin filament of the sarcomere and is a rare sarcomeric cause for NCCM. The mice with the *TNNT2* defect showed reduction of left ventricular systolic function, but no marked hypertrabeculation. Concluding that some mice models exhibit NCCM, including a model of the *TAZ*-gene, but an animal model for NCCM with a sarcomere gene mutation is still missing.

Some of the mice studies were searching for common pathways for the noncompaction phenotype. The Notch pathway, was found to be involved in the process leading to noncompaction (81, 244-247). The Notch pathway is highly conserved among species and has a key role in cellular proliferation and differentiation. Defects in the *MIB1* gene are a good example how disturbed Notch signaling can cause NCCM. Ubiquitination of *JAG1* by *MIB1* in the myocardium allows Notch1 activation in the endocardium. *NOTCH1*-dependent chamber maturation leads to compacted ventricular myocardium in the adult mouse. In mice with a *MIB1* loss of function allele, *NOTCH1* activity is impaired and compact myocardium remains abnormally proliferative. This disruption in compaction results in the NCCM phenotype. The involvement of *NOTCH1* in NCCM seems to be more complicated. Some reports showed a down-regulation of the Notch pathway (81, 244, 246), while others found that up-regulation of this pathway lead to hypertrabeculation (245, 247). A possibility might be that other pathways are more important for the hypertrabeculation phenotype. Other pathways not directly linked to the Notch pathway are proposed, like the non-canonical Wnt pathway (191, 248, 249). This pathway is among others involved in cellular polarization. The knock-down mice-models had normal cardiomyocyte proliferation, however cardiomyocyte myofibrillogenesis and cardiomyocyte polarization were altered.

Also, in *Fkbp1a*-deficient mice, which up-regulated the Notch pathway, have shown defects in cardiomyocyte myofibrillogenesis and polarity (247). This raises the possibility of a common pathogenic pathway for hypertrabeculation (250).

The data collected in our studies and the presented mouse models show that NCCM is a very heterogeneous syndrome. The idea of a common pathway does also apply to other heterogeneous cardiomyopathies, like HCM. The hallmarks of HCM; hypertrophy, fibrosis, and myofilament disarray were linked to hyper-contractility of the cardiomyocyte (251). Different mechanisms may underlie this pathogenic common hypercontractility including: changes in actin-activated ATPase (252, 253), an increase in the number of functionally accessible heads in the sarcomere for interaction with actin (254) or increased myofilament $Ca^{2+}$ sensitivity (255). Interestingly, defects in *MYBPC3* are directly linked to the above described mechanisms, which is the most common cause for HCM. In NCCM these associations of a common pathway, with the more common causes in patients cohorts have not been identified. The hallmark of HCM, interstitial fibrosis, occurs also frequently in NCCM and may be related to a chronic pressure overload (48). In this perspective the myocardial reaction on increased loading conditions may explain a proportion of NCCM. This may also partly explain the identified overlap in cardiac phenotype and genotype of HCM and NCCM patients. How pathways are activated by damaged sarcomeres or loading conditions, how these pathways work and lead to the noncompaction phenotype is still unknown and remains to be elucidated.

*Future perspectives*

After more than two decades we are still at the beginning of defining and understanding NCCM. The morphological expression of hypertrabeculation of the left ventricle is associated with defects in (mainly) the sarcomere genes. The application of whole exome or genome sequencing of NCCM patients in the near future will reveal novel genetic causes and genetic interactions with modifiers, some of which may explain remodeling into different cardiomyopathy phenotypes within families. This will also lead to a surplus of variants of unknown clinical significance (VUS). A better understanding of these variants is needed. Therefor a functional model needs to be constructed for testing the pathogenicity of these variants. This may be facilitated by worldwide sharing of data or by functional analysis of the variants using clustered regularly interspaced short palindromic repeats (CRISPR) gene editing in cell or animal studies. Functional evidence for pathogenicity of a variant will improve classification of variants. This will make the variants of unknown significance a group for additional testing, rather than a big question mark.

*Risk stratification models*

It is important to develop risk stratification models for risk prediction of susceptibility for heart failure or life-threatening arrhythmias in NCCM. Large international, multicenter prospective registries are crucial to clarify genotype-phenotype correlations in NCCM. Mutation associated risks will help in making clinical decisions, like if an ICD needs to be implanted.

Not only estimating risk for patients, but also for their relatives is imperative. For now, the risk for relatives of having inherited a familial genetic defect is mostly 50%, since the most common genetic causes are inherited as autosomal dominant traits (rare X-linked and mitochondrial causes are mostly encountered in severely affected children). However, for families of patients, non-penetrance in a relatively large part of relatives, results in a much lower actual risk for cardiac symptoms and events. Risk prediction for relatives with the morphological phenotype without symptoms or even for the relatives carrying the genotype without a phenotypical expression seems low. The risk for relatives of non-familial index patient in which no mutation is identified is low, but remains elevated, since genetic susceptibilities cannot be excluded. Improved risk classification for this group may lead to improved guidelines for family screening. In the distant future with a more complete picture of disease-causing genetic defects and mechanisms for NCCM, genetic predisposition may be estimated in all NCCM patients. This will allow to make a distinction between high and low genetic risks for NCCM and provide accurate risk prediction for relatives of the patients.

The specificity of the current diagnostic criteria is not satisfactory. The extension of normal trabeculation has to be established and this will help to improve specificity of the diagnostic criteria. Also, distinguishing people with physiologic excessive trabeculation, from patients with NCCM, who are at risk for cardiac events, would be an important step in risk stratification of the hypertrabeculation spectrum. For this reason, we believe that novel diagnostic criteria for NCCM leading to improved accuracy of the NCCM diagnosis, should include; 1) imaging criteria to identify trabeculation; 2) cardiac functioning parameters like left ventricular function (including left ventricular volumes, ejection fraction and strain analysis) could help in separating the benign trabeculation from cardiomyopathy; 3) genetic status to incorporate patients, who are at risk to develop cardiac dysfunction in the future and for risk assessment of their relatives; 4) histological criteria are needed, to confirm the diagnosis and to support these diagnostic criteria in NCCM patients. To achieve this, we have to aim at performing large

population studies and create international registries. On the basis of such registries international committees may reach a consensus on improved diagnostic criteria.

In conclusion, genetic causes currently explain a large proportion of NCCM. Improved diagnostic criteria, incorporating genetics, cardiac function and histology, may help to distinguish NCCM patients from the benign cases. The majority of genetic causes involve cardiomyocyte sarcomere functioning, but also genetic defects involving mitochondrial function, affecting cellular energy management or in genes involved in calcium handling regulating the sarcomere contraction force, have been identified. These genetic defects may have a damaging effect on the cardiomyocyte functioning in common. How these damaged cardiomyocytes may lead to hypertrabeculation is yet unclear. Likewise, it is not known why left ventricular remodeling occurs in the cases, who do not have a genetic cause, which is approximately half of the NCCM population. The role of the sarcomere, of loading conditions and mitochondrial functioning in activating similar remodeling pathways in these different groups of patients remains challenging.

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## Summary

Noncompaction cardiomyopathy (NCCM) is a rare disease. NCCM is characterized by hypertrabeculation of myocardium the left ventricle. Recent improvements in cardiac imaging techniques led to an increasing in detection and showed that NCCM occurs less frequently than hypertrophic (HCM) and dilated cardiomyopathy (DCM). The noncompaction phenotype is hypothesized to be a morphologic expression of different pathophysiologic mechanisms in patients with a cardiomyopathy. Interestingly signs of hypertrabeculation may be observed in healthy population without signs of cardiomyopathy, for instance in athletes or in women during pregnancy. In these cases cardiac overload might be related to localized hypertrabeculation of the left ventricle.

In patients diagnosed with NCCM the clinical symptoms range from mild to severe outcomes. Heritability plays an important role in NCCM. In around one third of NCCM patients a genetic defect can be identified. In an additional 15% there is familial disease without a known genetic cause. In around 50% of the NCCM patients there is no evidence for a genetic disease; no mutation in a cardiomyopathy gene or familial disease. These cases may have yet unknown (complex) genetic cause(s) carrying small risk for relatives. Alternatively, NCCM may be attributed to non-genetic, secondary causes for hypertrabeculation.

Finding a genetic cause for NCCM is of importance for patients and their families, enabling to predict genetic risk and perform family cascade screening. This allows accurate identification of relatives which have an increased risk of developing a cardiomyopathy and it also offers the possibility to reassure relatives who do not carry the genetic risk variant. It is important to know if specific causes are related to outcome. In this thesis we investigated if the genotype is of additional value for clinical management. More precisely whether specific genetic defects could predict the clinical features and the outcome (i.e. the risk of severe cardiac complications) in NCCM.

**Chapter 1.** This thesis starts with a chapter that was published in a monography on NCCM. It presents practical guidelines for the genetic diagnosis of NCCM for clinical geneticists and cardiologists with an overview of the genetic causes for NCCM, a description of the routine of genetic diagnostics, genetic counseling, DNA testing and the current view on family screening for NCCM. The chapter illustrates the importance of integrating genetic diagnostics to clinical management of NCCM patients by conveying appropriate information to patients and their families, in order to make early diagnosis and timely treatment accessible for the

families of all NCCM patients

**Chapter 2.** This chapter presents the largest genetic NCCM study performed worldwide to date. The retrospective multicenter study was performed in collaboration with the four major cardiogenetic centers in the Netherlands (EMC, UMCG, UMCU and AMC) to establish 1) spectrum of genetic causes for NCCM and 2) correlations between genetics, clinical features, and outcomes, in 327 unrelated children and adults with NCCM. In this study we identified differences in genetic causes between children and adults. Children had more often a genetic cause, had more complex genetic causes with multi-systemic features and severe outcome. In contrast NCCM in adults was mostly linked to missense mutations in sarcomere genes. In about half the cases, mostly diagnosed in adulthood, there was no evidence for a genetic cause. These cases did not have familial disease and DNA testing did not result in the identification of a disease causing variant. The main findings of this study were that NCCM is a heterogeneous condition and genetic stratification has an important role in clinical care. NCCM was due to an inherited cause in half of the cases; in 32% a mutation could be identified and in 15% there was familial cardiomyopathy without a known mutation. Mutations in *MYH7, MYBPC3* and *TTN* were the most common (71%). Adults were more likely to have sporadic NCCM (no disease causing variants nor affected family members). The risk of having a reduced left ventricular systolic dysfunction was higher for genetic patients, with highest risk for patients with multiple mutations and *TTN* mutations. In children, high risk for major cardiac events was related to having a mutation. In adults risk for major cardiac events was related to left ventricular systolic dysfunction in mutation carriers. This was not observed in sporadic cases. Patients with *MYH7* mutations had low risk for major adverse cardiovascular events (MACE). Our results showed that DNA diagnostics and family history carry information that may help to distinguish genetic from non-genetic NCCM. Moreover, it complement cardiologic management and, prediction of outcome and subsequently guide management of patients with follow-up tailored to genetic status and associated risk. Hence, it showed the importance of genetic testing in NCCM.

**Chapter 3.** The third chapter of the thesis focused on family screening in NCCM. In this chapter we looked for a way of predicting the severity and cardiac morphology in relatives of NCCM patients. In this chapter we investigated the results of family screening for NCCM in the families that are followed at the cardiogenetic clinic at the Erasmus Medical Center. We focused on familial NCCM and present the outcome of – cardiac examinations and (when appropriate) genetic family testing

of the families of 114 NCCM index patients. We were particularly interested if the specific NCCM phenotypes, NCCM with DCM, NCCM with HCM, and isolated NCCM (without HCM or DCM) segregated within families, and if these specific NCCM phenotypes were linked to distinct genetic defects and were related to outcome. Cardiac family screening revealed that 109 (23%) of 472-screened relatives from 58 (51%) families had a cardiomyopathy; 81 relatives from 39 families (of 54 index patients) with a mutation and 28 relatives from 19 families (of 60 index patients) without a mutation. A third of the relatives with an inherited mutation did not have a cardiomyopathy (non-penetrance), which is important information for the counseling of relatives. The relatives who were diagnosed with NCCM were less likely to have left ventricular systolic dysfunction or major adverse cardiac events than the index cases. NCCM with DCM occurred in 56% of all NCCM patients and was associated LV systolic dysfunction, mutations in the tail of *MYH7* and 38% was asymptomatic. Isolated NCCM (43%) was associated with milder course, mutations in the head of *MYH7,* and 42% were asymptomatic. NCCM with HCM was associated with *MYBPC3* mutations. Familial segregation of distinct NCCM phenotypes was observed in 47% of the affected relatives; 55% isolated NCCM, 34% NCCM with DCM and 33% NCCM and HCM. Thirty-six relatives from 24 families had HCM or DCM without hypertrabeculation. The conclusion was that the distinct NCCM phenotype and related risk for relatives might be predicted by the phenotype and genotype of the index cases. These insights can be applied to personalize clinical management and family screening of NCCM.

**Chapter 4.** In this chapter we investigated if cardiac magnetic resonance imaging (CMR) could help to distinguish which NCCM patients have a mutation. As the previous studies have indicated, it is important to distinguish genetic NCCM with high risk for relatives, from patients with sporadic NCCM and low risk for relatives. Since NCCM is frequently diagnosed on CMR, we were interested if CMR could help to identify which patients were most likely to have a genetic defect. For this purpose we evaluated 62 NCCM patients referred for CMR who had genetic testing at the Erasmus Medical Center between 2005 and 2017. Thirty-three (53%) patients had a mutation. In all patients previously proposed CMR diagnostic modalities were ascertained (i.e. criteria according to Petersen (short- and long-axis), Stacey, Jacquier, Captur and Choi). Correlation between different CMR criteria varied from moderate to very strong. On average the NCCM patients met four of the different diagnostic modalities. Genetic patients met more diagnostic modalities than patients without a mutation. In multivariate binary logistic regression analysis with CMR and non-CMR parameters, independent positive predictors for a genetic defect were familial cardiomyopathy, trabecular mass, and meeting

Petersen criteria in ≥2 out of 3 long axis views, while left bundle branch block and hypertension were negative predictors. The ROC-curve of this multivariate model had a good area under the curve of 0.89. Concluding that CMR criteria and patient characteristics may help to predict if a patient has a genetic defect. In this way CMR interpretations may contribute in guiding cardiologists to refer patients for genetic testing.

**Chapter 5.** Since NCCM is a relatively rare, genetically heterogeneous cardiomyopathy, there are few studies that are large enough to have sufficient statistical power to establish a correlation between the genetic defects and the clinical features. By performing a systematic review of the NCCM literature for reports of NCCM patients with documented genotype and phenotypes, we could combine and analyze the results of smaller studies to determine the genetic spectrum of NCCM and establish if genetic causes could help predict high-risk profiles for major adverse cardiac events (MACE) in NCCM. For this purpose we have conducted a systematic search of the literature regarding genetics and clinical features of NCCM. The literature search for patients diagnosed with noncompaction cardiomyopathy yielded 1978 papers. After removing duplicates from the different searches 990 reports remained of which 172 fulfilled our inclusion criteria. In this way, clinical and genetic data of 561 patients were included. In total 244 (43%) children were included showing a high prevalence of congenital heart disease and increased risk for MACE compared to adults. Children had more frequently an X-linked or mitochondrial inherited defect or chromosomal anomalies. In adult NCCM patients the main causes were single missense mutations in sarcomere genes. Overall, *MYH7* was involved in 48% of the sarcomere gene mutations. *MYH7* and *ACTC1* mutations had lower risk for MACE, than *MYBPC3* and *TTN*. In this analysis, the combination of NCCM and DCM was the most frequent observed among the different NCCM phenotypes – as described in the previous chapter, with more than half of the cases showing these overlapping cardiac features. The NCCM-DCM phenotype was associated with an increased risk for MACE and high risk for left ventricular systolic dysfunction. The literature review showed that genetic defects were related to age at presentation, concomitant congenital heart defects and cardiac outcome. These observations endorsed the conclusions from our study presented in Chapter 2 of this thesis, that DNA diagnostics for NCCM are important for clinical management and counseling.

**Chapter 6.** In nearly half of the families with familial NCCM sequencing of cardiomyopathy gene panels consisting of approximately 50 genes cannot identify a mutation. In the last chapter we present a novel NCCM gene in two families.

To identify the genetic cause for familial NCCM whole exome sequencing was performed in two families, one from the University Medical Center Groningen and one from our center and. In these two families a variant in the filamin C gene (*FLNC)* segregated with the NCCM phenotype. Also in both families there was a patient with coarctation of the aorta and mitral valve abnormalities. Based on these results we present *FLNC* as a novel NCCM gene. Pathogenic variants in *FLNC* have been linked previously to HCM and DCM. We expand the spectrum of *FLNC* related cardiomyopathies with familial segregation of NCCM and concurrent coarctation of the aorta, mitral valve abnormalities.

**Conclusions**

Distinguishing which NCCM patients and their relatives have a high genetic risk, has important implications for both the patients and their relatives in health management and cardiac surveillance. Genetic patients can be distinguished by using DNA testing, family history and clinical markers. We showed that for prediction of adverse events in patients, information on genotype and phenotyping the left ventricle is important. The distinct cardiac phenotypes: isolated NCCM, NCCM with DCM and NCCM with HCM were related to genotypes, outcome and segregated within families. These studies contributed to our understanding of the etiology of NCCM and in the relation between the cause of the disease and the clinical features. We hope that patients and their families may benefit from these insights and that our study results may contribute to the development of new and improved diagnostic criteria, and guidelines for NCCM.

## Nederlandse samenvatting

Noncompactie cardiomyopathie (NCCM), ook wel LVNC (linkerventrikel non-compactie cardiomyopathie) genoemd, is een ziekte van de hartspier. De diagnose NCCM is gebaseerd op het belangrijkste kenmerk van de ziekte, de hypertrabecularisatie van de linkerhartkamer (ventrikel). Hypertrabecularisatie zijn bijzonder grove spiervezelbundels, waartussen zich holtes vormen, die bij NCCM patiënten meer herkenbaar zijn **(figuur 1)**. Verbeteringen van de beeldvormingstechnieken van het hart hebben ertoe geleid dat NCCM steeds vaker herkend wordt. Ook is het duidelijk geworden dat deze cardiomyopathie minder vaak voorkomt dan andere cardiomyopathieën zoals hypertrofische (HCM) en dilaterende cardiomyopathie (DCM). Opvallend is dat hypertrabecularisatie van de linkerventrikel ook kan optreden bij personen die geen cardiomyopathie hebben, bijvoorbeeld bij sporters of bij vrouwen tijdens de zwangerschap. De veronderstelling is dat in deze gevallen een cardiale overbelasting de oorzaak zou zijn.

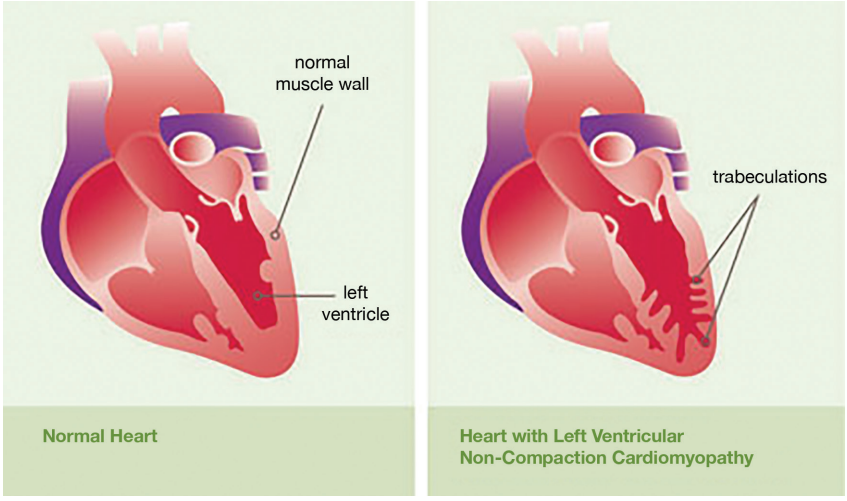


**Figure 1: Schematische weergaven van NCCM.**
Een schematische weergave van het normale hart (links) en een hart met noncompaction cardiomyopathie (rechts). Het hart met noncompaction cardiomyopathie heeft hypertrabecularisatie van het hartspierweefsel van de linkerventrikel. De afbeelding is overgenomen van: https://www.cincinnatichildrens.org/service/c/cardiomyopathy/types/left-ventricular-non-compaction-cardiomyopathy.

De klinische symptomen (het fenotype) bij cardiomyopathie patiënten met NCCM kunnen van persoon tot person sterk verschillen, variërend van mild tot ernstig hartfalen (5). Erfelijkheid speelt een belangrijke rol; ongeveer één derde van de NCCM patiënten heeft een (waarschijnlijk) ziekte veroorzakende gen variant (10). Een deel van deze varianten, voornamelijk die in sarcomeer genen, komen ook vaak voor bij patiënten met HCM of DCM. In NCCM komen daarnaast ook zeldzamere genetische defecten voor die leiden tot syndromale beelden, waarbij meerdere orgaansystemen zijn aangedaan (54). In ongeveer een derde van de patiënten komt NCCM bij meerdere mensen in de familie voor (familiaire NCCM). Bij ongeveer de helft van de familiare patiënten kan een oorzakelijk gendefect worden aangetoond. In de andere helft van de patiënten, kan ondanks het familiair voorkomen van de ziekte, de genetische oorzaak niet worden aangetoond.

Het vinden van de genetische oorzaak is belangrijk voor NCCM patiënten en hun familieleden. Als een genetische oorzaak is gevonden kan met DNA-onderzoek op betrouwbare wijze worden vastgesteld welke familieleden een verhoogd risico op een cardiomyopathie hebben. Indien het genetische defect wordt geïdentificeerd in familieleden kunnen ze poliklinisch door de cardioloog worden gecontroleerd, om in een vroeg stadium symptomen van de ziekte te kunnen behandelen en eventuele ernstige complicaties te voorkomen. Indien bij een familielid van een patiënt met genetisch defect met DNA-onderzoek de erfelijke aanleg wordt uitgesloten, kan het betreffende familielid worden gerustgesteld met het feit dat er geen verhoogde kans is voor het ontwikkelen van een cardiomyopathie. Tevens is er dan geen verhoogd risico voor zijn of haar nageslacht. Echter bij ongeveer twee derde van de NCCM patiënten kan geen ziekte veroorzakende genetisch defect worden aangetoond en kan bij familieleden de ziekte niet worden aangetoond of worden uitgesloten met een DNA-onderzoek.

Ook is het voor de behandelaars en de patiënten en hun familie van belang om de kans op ernstige symptomen te weten. Om die reden was de correlatie tussen de genetische oorzaak (het genotype) en de ernst van de ziekte (het risico op levensbedreigende cardiale ritmestoornissen en hartfalen), het fenotype, de focus van dit promotieonderzoek. Inzichten in het verband tussen genotype en fenotype kunnen belangrijk zijn voor het bepalen van risico's van symptomen en complicaties. Op die manier zou een gepersonaliseerde behandeling mogelijk zijn en de counseling van de patiënt en zijn of haar familieleden aangepast kunnen worden aan het genotype.

**Hoofdstuk 1.** Dit proefschrift begint met een overzicht over de genetische achtergrond van NCCM, en hoe genetische onderzoek en familieonderzoek van NCCM verloopt. Dit hoofdstuk kan worden beschouwd als een leidraad voor klinisch genetici en cardiologen. In ten minste de helft van de patiënten met NCCM speelt erfelijkheid een belangrijke rol speelt. Bij erfelijke NCCM is net als bij andere erfelijke cardiomyopathieën tijdige herkenning en behandeling van risicogroepen belangrijk. De genetische oorzaken voor NCCM worden besproken, zoals de cardiomyopathie-genen, andere – meer zeldzame- genetische oorzaken, en structurele chromosoom defecten, evenals de nieuwe DNA-analyse technieken en de interpretatie van de resultaten van DNA-diagnostiek. Ook is er aandacht voor de overervingspatronen en het informeren van familieleden. Dit hoofdstuk geeft het belang van het genetisch counselen van NCCM-patiënten en hun familieleden weer.

**Hoofdstuk 2.** Dit hoofdstuk beschrijft de grootste NCCM studie wereldwijd tot op heden. Dit onderzoek had ten doel te onderzoeken wat de relatie is tussen de genetische achtergrond van NCCM en de ernst van de symptomen in de NCCM patiënten. Hiermee wilden we bekijken of genetische achtergrond kon worden gebruikt voor het bepalen van de prognose van verschillende patiënten. Een retrospectief multi-centeronderzoek werd uitgevoerd in samenwerking met de vier belangrijkste cardiogenetica centra in Nederland (Erasmus Medisch Centrum, Universitair Medisch Centrum Groningen, Universitair Medisch Centrum Utrecht en Amsterdam universitair Medische Centra, locatie AMC) om correlaties tussen erfelijke oorzaken, klinische kenmerken en complicaties vast te kunnen stellen. Deze studie populatie bestond uit 327 kinderen en volwassenen met NCCM, die geen familie van elkaar waren. We observeerden dat er verschillen in genetische oorzaken waren tussen kinderen en volwassenen. Kinderen hadden vaker complexe genetische oorzaken met aangeboren hartafwijkingen, terwijl volwassenen NCCM patiënten vooral aminozuur veranderingen (missense-mutaties) in sarcomeer genen hadden. NCCM was erfelijk in de helft van de gevallen; bij 32% werd een mutatie gevonden en bij 16% was er sprake was van meerder patiënten in de familie (familiare cardiomyopathie). In de laatste groep konden we ondanks het feit dat meerdere mensen NCCM hadden geen mutatie identificeren. Het meest voorkomend waren mutaties in *MYH7*, *MYBPC3* en *TTN* (71%). Mutaties kwamen vaker voor bij kinderen en werden geassocieerd met ernstige cardiale complicaties. Volwassen patiënten hadden vaker sporadische NCCM, dat wil zeggen dat zij geen mutatie hadden of familiaire cardiomyopathie. Bij sporadische

NCCM kwam vaker hypertensie (hoge bloeddruk) en linker bundeltakblok (ritmestoornis) voor. In patiënten met een erfelijke vorm van NCCM was er een verhoogd risico op een verminderde hartfunctie door een linker ventriculaire systolische dysfunctie (minder bloed wordt weg gepompt door de linkerhartkamer), met het hoogste risico voor patiënten met meerdere mutaties of *TTN*-mutaties. Kinderen waarbij een genetische oorzaak werd gevonden hadden een hoger risico op ernstige cardiale complicaties. Bij volwassenen was het risico op ernstige cardiale complicaties gerelateerd aan linkerventrikel systolische dysfunctie bij mutatiedragers, maar niet bij sporadische patiënten. Patiënten met *MYH7*-mutaties hadden een laag risico op complicaties. Deze resultaten benadrukken dat voor het voorspellen van het risico op complicaties het nuttig is een onderscheid te kunnen maken tussen erfelijke en niet-erfelijke NCCM, en dat zo een genetische stratificatie een belangrijke rol kan gaan spelen in de klinische zorg van de patiënt. Deze verschillen tussen sporadische en genetische patiënten lieten ook zien dat het NCCM-fenotype waarschijnlijk het verschil in symptomen (de morfologische expressie) is van verschillende factoren (pathofysiologische mechanismen). Deze studie benadrukte het klinische belang van genetische onderzoek bij NCCM patiënten.

**Hoofdstuk 3.** Het derde hoofdstuk van dit proefschrift richtte zich op het bepalen van het risico voor de familieleden van NCCM patiënten. Het doel was om te kijken of de prognose en ernst van cardiomyopathie bij familieleden van NCCM-patiënten kon worden voorspeld. Ook waren we geïnteresseerd of specifieke NCCM-fenotypes: NCCM met DCM, NCCM met HCM en geïsoleerde NCCM (zonder HCM of DCM, zie figuur 1), altijd samengaat in families. Ook bekeken we of deze specifieke NCCM-fenotypes gekoppeld waren aan bepaalde genetische defecten en cardiologische complicaties voorspelde. Dit deden we met behulp van de gegevens van cardiale- en genetische- familiescreening van 114 NCCM-indexpatiënten uit het Erasmus Medisch Centrum. Uit cardiologische familiescreening bleek dat 23% van de 472 gescreende familieleden een cardiomyopathie hadden. In ongeveer de helft van de families waren er aangedane familieleden (familieleden met een cardiomyopathie). De aangedane familieleden werden voornamelijk geïdentificeerd in families waarin de erfelijke oorzaak bekend was. Opvallend was dat een derde van de familieleden, die drager waren van de familiaire mutatie, (nog) geen cardiomyopathie (non-penetrantie) had. Dit is belangrijke informatie voor het counselen van familieleden. De familieleden bij wie de diagnose NCCM werd gesteld, hadden minder kans op ventriculaire systolische dysfunctie of ernstige cardiale complicaties dan de index-patiënten. Van

alle NCCM-patiënten had 56% NCCM met DCM. Dit fenotype was geassocieerd met linkerventrikel systolische dysfunctie, mutaties in de staart van *MYH7* en slechts 38% was asymptomatisch. Geïsoleerde NCCM (43%) werd geassocieerd met een milder beloop van de ziekte, met mutaties in de kop van *MYH7* en 42% was asymptomatisch. NCCM met HCM was geassocieerd met *MYBPC3*-mutaties. Familiare segregatie van verschillende NCCM-fenotypes werd waargenomen bij 47% van de aangedane familieleden; 55% had geïsoleerde NCCM, 34% NCCM met DCM en 33% NCCM en HCM. Zesendertig familieleden uit 24 families hadden HCM of DCM zonder hypertrabecularisatie. De conclusie was dat het NCCM-fenotype en het daar bijhorende risico voor familieleden kon worden voorspeld door het fenotype en het genotype van de index-patiënt. Deze inzichten kunnen belangrijk zijn voor het inschatten van risico's voor patiënten en familieleden.

**Hoofdstuk 4.** In dit hoofdstuk hebben we onderzocht of cardiale magnetic resonance imaging (CMR) kan helpen bij het voorspellen of een NCCM-patiënt een mutatie heeft, d.w.z. dat er bij DNA-onderzoek een (waarschijnlijk) pathogene variant wordt ontdekt, zoals bij ongeveer een derde van de NCCM-patiënten. Aangezien NCCM vaak wordt gediagnosticeerd via CMR, waren we geïnteresseerd of aan de hand van de CMR-beelden kon worden voorspeld of een NCCM patiënt een genetisch defect had. Hiervoor hebben we 62 NCCM-patiënten onderzocht die tussen 2005 en 2017 werden verwezen voor CMR en ook een genetisch onderzoek hebben gehad in het Erasmus Medisch Centrum. Van deze NCCM patiënten had 53% een mutatie. Alle patiënten werden beoordeeld volgens een aantal bekende diagnostische CMR-criteria beoordeeld; de Petersen (korte en lange as), Stacey, Jacquier, Captur en Choi criteria. De correlatie tussen verschillende diagnostische CMR-criteria varieerde van matig tot zeer sterk. Gemiddeld voldeden de NCCM-patiënten aan vier van de verschillende diagnostische criteria. Genetische patiënten voldeden gemiddeld aan meer diagnostische criteria dan patiënten zonder een mutatie. Positieve voorspellers voor een genetisch defect waren: familiare cardiomyopathie, trabekel massa en het voldoen aan Petersen-criteria in ≥2 van de 3 lange-as beelden. Een linker bundeltakblok en hypertensie waren negatieve voorspellers. De conclusie was dat CMR-criteria samen met andere cardiale kenmerk kunnen helpen met het voorspellen of een patiënt een genetisch defect heeft. Op deze manier kunnen cardiologen op grond van de CMR-resultaten een indruk krijgen welke patiënten en familieleden met voorrang geïnformeerd moeten worden over de erfelijkheid en voor welke patiënten genetische diagnostiek van NCCM het belangrijkste is.

**Hoofdstuk 5.** Aangezien NCCM een relatief zeldzame genetisch cardiomyopathie is, zijn er weinig onderzoeken groot genoeg om met voldoende statistische power een verband tussen genetische defecten en de klinische kenmerken vast te kunnen stellen. Door het uitvoeren van een systematische review van de literatuur, waarbij NCCM-patiënten met zowel genotype als fenotypes werden geselecteerd, konden we de resultaten van kleinere studies combineren en analyseren. Met deze data konden we het genetische spectrum van NCCM bepalen. Ook bekeken we of de genetische oorzaak bijdraagt aan de risico voorspelling op ernstige cardiale complicaties in NCCM. Het literatuuronderzoek naar patiënten met de diagnose NCCM leverde 1978 wetenschappelijke publicaties op. Na het verwijderen van duplicaten uit de verschillende zoekopdrachten bleven 990 artikelen over, waarvan 172 voldeden aan onze inclusiecriteria. De klinische en genetische gegevens van 561 patiënten konden op deze manier worden geïncludeerd. In totaal waren er 244 (43%) kinderen, die vaak een aangeboren hartaandoeningen hadden en ook een verhoogd risico op cardiale complicaties bleken te hebben in vergelijking met volwassen patiënten. Kinderen hadden vaker een mutatie in van een gen dat op het X-chromosoom ligt, een mitochondrieel defect of een chromosoomafwijking. Bij volwassen NCCM-patiënten waren de belangrijkste oorzaken aminozuur veranderingen (missense-mutaties) in sarcomeer genen, waarvan bij 48% het *MYH7*gen betrokken was. Patiënten met *MYH7*- en *ACTC1*-mutaties hadden een lager risico op ernstige cardiale complicaties dan die met *MYBPC3*- en *TTN*-mutaties. In deze studie kwam het NCCM/DCM-fenotype het meest voor en dit fenotype werd geassocieerd met een verhoogd risico op cardiale complicaties en een hoog risico op systolische disfunctie van de linkerventrikel. De literatuurstudie bevestigde derhalve dat genetische oorzaken gerelateerd waren aan leeftijd bij presentatie, prevalentie van aangeboren hartafwijkingen en cardiale complicaties zoals wij hebben beschreven in hoofdstuk 2. Deze observaties benadrukte dan ook de conclusies uit hoofdstuk 2, dat DNA-diagnostiek voor NCCM patiënten van belang is voor risico predictie en counseling.

**Hoofdstuk 6**. In hoofdstuk zes presenteren we een nieuw NCCM-gen in twee families. In bijna de helft van de families met familiare NCCM wordt geen oorzaak gevonden bij reguliere DNA-diagnostiek. In twee van dit soort families werd 'whole exome sequencing' verricht om alsnog de oorzaak te vinden. Eén van deze families was bekend in het Erasmus Medisch Centrum en de ander in het Universitair Medisch Centrum Groningen. In deze twee families segregeerden varianten in het filamine C-gen (*FLNC*) met het NCCM-fenotype. In beide families kwamen patiënten voor met coarctatio van de aorta (aangeboren vernauwing in de **aorta**) of een afwijking aan de mitralisklep (de hartklep tussen linkerboezem en

linkerkamer). Ziekteverwekkende varianten in *FLNC* werden eerder ontdekt bij patiënten met HCM of DCM en ook in patiënten met skeletspierziekte. Op basis van deze resultaten presenteren we *FLNC* als een nieuw NCCM-gen. Hiermee werd het spectrum van mogelijke genetische oorzaken voor NCCM nog verder uitgebreid, waardoor mogelijk in de toekomst in meer patiënten de genetische oorzaak kan worden geïdentificeerd.

**Conclusie**

Het is van belang voor patiënten met NCCM en hun familieleden om een onderscheid te kunnen maken tussen patiënten met een hoog genetisch risico op NCCM en die met een laag genetisch risico. Het genetische risico kan worden ingeschat met behulp van DNA-onderzoek, familiegeschiedenis en klinische kenmerken en cardiale magnetic resonance imaging. De uitslag van het DNA-onderzoek kan ook gebruikt worden om NCCM-fenotypes te voorspellen binnen families en geeft een beeld van de kans op ernstige complicaties. We hopen dat vooral de patiënten en de families van de patiënten van de resultaten van dit onderzoek profijt zullen hebben. We hopen dat deze informatie toegepast zal worden in de dagelijkse praktijk en mede aanleiding zijn tot en bijdragen aan het tot stand komen van nieuwe diagnostische criteria en leidraden voor diagnostiek en behandeling.

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5. **van Waning JI**, Hoedemaekers YM, te Rijdt WP, IJpma A, Heijsman D, Caliskan K, Hoendermis ES, Willems TP, van den Wijngaard A, Suurmeijer A, van Slegtenhorst MA, Jongbloed JDH, Majoor-Krakauer D, van der Zwaag PA. Missense FLNC Mutations associated with familial noncompaction Cardiomyopathy and Congenital Heart Defects, Cardiogenetics, 2019;9(1).
6. **van Waning JI**, Moesker J, Heijsman D, Boersma E, Majoor-Krakauer D. Meta-analysis of the genotype- phenotype correlation in noncompaction cardiomyopathy. J Am Heart Assoc. 2019 Dec 3;8(23):e012993.

## Submitted manuscripts

7. **van Waning JI**, Caliskan K, Chelu RG, van der Velde N, Pezzato A, Michels M, van Slegtenhorst MA, Boersma E, Nieman K, Majoor-Krakauer D, Hirsch A**.** Cardiac magnetic resonance imaging analyses of genetic and sporadic noncompaction cardiomyopathy, submitted

## Book chapter

8. **van Waning JI,** Majoor-Krakauer D. Cardio- genetics and family screening of Noncompaction Cardiomyopathy. In monography : Noncompaction cardiomyopathy, ed. K. Caliskan. Springer Nature. 2019

**PhD portfolio**

| | |
|---|---|
| Name | Jaap I. van Waning |
| Department | Clinical genetics |
| Research school | COEUR, Erasmus MC |
| PhD period | 2014-2018 |
| Title thesis | Noncompaction Cardiomyopathy |
| Promotor | Prof. Dr. R.M.W. Hofstra |
| Copromotor | Dr. D.F. Majoor-Krakauer |
| Date of thesis defense | April 27, 1987 |

| | Year | Workload |
|---|---|---|
| **General academic and research skills:** | | |
| NIHES - Biostatistics for clinicians (17 days) | 2014 | 5.7 |
| Rules and organization of clinical research (BROK) course (5 days) | 2015 | 1.5 |
| Good-clincial practice (GCP) course | 2016 | 0.3 |
| Scientific integrity (1 day) | 2016 | 0.3 |
| English writing course (10 days) | 2016 | 3.0 |
| **In-depth courses:** | | |
| Windows Acces (2 days) | 2014 | 0.6 |
| Windows Excel (2 days) | 2014 | 0.6 |
| Echocardiography course (5 days) | 2015 | 1.5 |
| PhD-management (2 days) | 2015 | 0.6 |
| Medical business Masterclass 2016 (3 days) | 2016 | 1.8 |
| Photoshop (1 day) | 2016 | 0.3 |
| **COEUR courses:** | | |
| Vascular clinical epidemiology | 2014 | 1.5 |
| Congenital heart disease and the left side: an introduction to congenital heart disease | 2015 | 1.5 |
| Cardiovascular pharmacology | 2015 | 1.5 |
| Cardiomyopathies | 2016 | 1.5 |

| | | |
|---|---|---|
| **International congresses:** | | |
| ESHG conference, Glasgow, United Kingdom | 2015 | 2.0 |
| ESC conference, London, United Kingdom | 2015 | 2.0 |
| Cardiomyopathies, conference Florence, Italy | 2015 | 2.0 |
| ESHG conference, Copenhagen, Denmark | 2017 | 2.0 |
| ESC conference, Barcelona, Spain | 2017 | 2.0 |
| | | |
| **National congresses:** | | |
| NVVC najaars congress | 2014 | 0.3 |
| NVVC voorjaars congres | 2015 | 0.6 |
| NVVC najaars congress | 2015 | 0.3 |
| NVVC voorjaars congres | 2016 | 0.3 |
| NVVC najaars congress | 2016 | 0.3 |
| 50 years of cardiogenetics | 2016 | 0.3 |
| NVHG | 2016 | 0.6 |
| | | |
| **Organized congresses:** | | |
| Sophia Research Day 2015 | 2015 | 1.6 |
| COEUR Day 2016 | 2016 | 1.6 |
| COEUR Day 2017 | 2017 | 1.6 |
| COEUR Day 2018 | 2018 | 1.6 |
| | | |
| **Committees:** | | |
| COEUR PhD committee 2016 | 2016 | 0.3 |
| COEUR PhD committee 2017 | 2017 | 0.3 |
| COEUR PhD committee 2018 | 2018 | 0.3 |
| | | |
| **Student guidance:** | | |
| Supervising writing a systematic review of 2nd year medical student | 2015 | 0.6 |
| Supervising writing a systematic review of 2nd year medical student | 2016 | 0.6 |

| | | |
|---|---|---|
| Supervising bachelor thesis psychology: genetics, depression and heredity | 2016 | 0.6 |
| Supervising master thesis of 6th year medical student: meta-analysis genetics in noncompaction cardiomyopathy | 2018 | 2.4 |
| **Total** | | 46.4 |

| | | **No. of presentations** |
|---|---|---|
| **Oral presentations** | | |
| European society of cardiology (ESC) conference, London, United Kingdom | 2015 | 1 |
| Cardiomyopathies conference, Florence, Italy | 2015 | 1 |
| Dutch society of human genetics (NVHG) conference, oral presentation, Utrecht, The Netherlands | 2016 | 1 |
| COEUR symposium cardiomyopathies, Rotterdam, The Netherlands | 2016 | 1 |
| European society of cardiology (ESC) conference, Barcelona, Spain | 2017 | 1 |
| **Poster presentations** | | |
| Dutch society of cardiology (NVVC) conference, Noordwijkerhout, The Netherlands | 2015 | 1 |
| European society of human genetics (ESHG) conference, Glasgow, United Kingdom | 2015 | 2 |
| European society of human genetics (ESHG) conference, Copenhagen, Denmark | 2017 | 2 |
| European society of human genetics (ESHG) conference, Milan, Italy | 2018 | 1 |

## About the author

Jacob Isaäc van Waning was born on April 27th 1987, in Rotterdam, the Netherlands. After graduating high school (Rotterdams Montessori Lyceum), he started medical school at Leiden Univerity. After obtaining his Medical Doctor's degree, he started working as a resident at the department of cardiology in Haaglanden Medisch Centrum, The Hague. In 2014, he started with the research project described in this thesis 'Genotype-phenotype correlations of noncompaction cardiomyopathy', supervised by dr. Danielle Majoor-Krakauer and Prof. Robert Hofstra at the Erasmus Medical Center, Rotterdam. During this PhD project, he had the opportunity to present his work on several national and international conferences and to publish manuscripts in peer reviewed international journals. As of October 2019, Jaap is working as a resident at the department of cardiology at the Radboud University Medical Centre, Nijmegen.

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