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Diagnostic yield of genetic testing in heart transplant recipients with prior cardiomyopathy

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KEYWORDS:
cardiogenetics;
heart transplantation;
genetic screening;
cardiomyopathy;
dilated cardiomyopathy

BACKGROUND: The importance of genetic testing for cardiomyopathies has increased in the last decade. However, in heart transplant patients with former cardiomyopathy, genetic testing *in retrospect* is not routinely performed. We hypothesize that the yield of genetic testing in this population is considerable, and will have a major impact for both patients and relatives.

METHODS: Patients that underwent heart transplantation (HTx) between 1995 and 2020 and were still in follow-up, were offered genetic testing if the primary etiology was non-ischemic cardiomyopathy. Next generation sequencing (NGS) of known cardiomyopathy genes was performed and variants were classified as variant of unknown significance (class 3), likely pathogenic (class 4) or pathogenic (class 5) variant.

RESULTS: Of the 99 HTx patients in active follow-up, only 6 patients had a genetic diagnosis at the time of HTx. In this study, 31 selected patients with prior non-ischemic cardiomyopathy underwent genetic testing post HTx. 23/31 patients (74.2%) carried a variant that was classified as class 3 or higher. In 12/31 patients a class 4/5 variant (38.7%) was identified, and in 11/31 patients (35.5%) a class 3 variant. Class 5 Variants in *TTN* were the most prevalent (7/31), followed by class 5 variants in *MYBPC3* (2/31). A positive family history was present in 21/31 (67.7%) and a second precipitating factor (e.g., alcohol abuse, pregnancy) was present in 17/31 patients (54.8%). Diagnostic yield of genetic testing was similar between patients with or without familial history and/or second hit. Through cascade screening 48 family members were screened for presence of a class 4/5 variant, of whom 19 (39.6%) were genotype positive, of whom 10 (52.6%) showed a cardiac phenotype. Appropriate follow-up was offered.

Abbreviations: ACM, arrhythmogenic cardiomyopathy; AHA, American heart association; CM, cardiomyopathy; CM-panel, Haloplex gene panel including different cardiomyopathy genes; DCM, dilated cardiomyopathy; EACVI, European association of cardiovascular imaging; GLS, global longitudinal strain; HCM, hypertrophic cardiomyopathy; HTx, heart transplantation; ISHLT, the international society for heart and lung transplantation; LVEDD, left ventricle end diastolic diameter; LVESD, left ventricle end systolic diameter; LVEF, left ventricular ejection fraction; LVNC, left ventricular non-compaction cardiomyopathy; NGS, next-generation sequencing; PGT, pre-implantation genetic testing; RCM, restrictive cardiomyopathy; SCD, sudden cardiac death; *TTN*, titin; VUS, variant of unknown significance.

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CONCLUSIONS: Genetic testing for cardiomyopathy genes established a molecular diagnosis in 38.7% of patients post HTx. These results highlight the importance of genetic testing in this population as it is still often overlooked in patients that already underwent HTx in the past. Genetic testing is highly recommended, independent of family history or second precipitating factors, as it might identify relatives at risk.

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End-stage refractory heart failure is the main indication for heart transplantation (HTx).[1-4] Based on the ISHLT data from 2010 to 2018, the most frequent underlying etiology is non-ischemic cardiomyopathy (57.7%), followed by ischemic heart disease, valvular heart disease and a small group of other disease entities resulting in heart failure (e.g. congenital heart disease).[5] Within the cardiomyopathies, dilated cardiomyopathy (DCM) accounts for 50.8% of HTx, followed by hypertrophic cardiomyopathy (HCM; 3.4%) and restrictive cardiomyopathy (RCM; 3.5%).[5] Arrhythmogenic cardiomyopathy (ACM) occasionally results in HTx.[6] A fifth, unclassified cardiomyopathy, left ventricular non-compaction (LVNC), can lead to HTx in 10% to 30% of patients.[7,8]

During the last decade, genetic testing for cardiomyopathies has gained momentum and a genetic cause is identified in a growing number of cardiomyopathies. The inheritance pattern is most often autosomal dominant, although autosomal recessive, X-linked and polygenic mechanisms are seen as well.[9,10] In 2020, the American Heart Association (AHA) issued a statement recommending genetic testing for patients diagnosed with all forms of cardiomyopathy.[11] The diagnostic yield of genetic testing in HCM amounts to 40% and even up to 72% in patients with a positive family history of HCM or sudden cardiac death (SCD).[10,12,13] A rather small group of 8 genes encoding for sarcomeric proteins contain the most commonly identified pathogenic variants in HCM (*MYBPC3, MYH7, TNNI3, TNNT2, TPM1, MYL2, MYL3 and ACTC1*).[10,14] Also in patients with DCM, the importance of genetic testing has increased in the last years.[10,13,15] The European consensus document advises genetic testing in all non-ischemic DCM patients with important conduction disturbances and/or a positive family history of SCD.[16] However, more recent expert-consensus is to consider genetic testing in non-ischemic DCM irrespective of familial history.[17] Comparable, the American practice guidelines recommend genetic testing in all DCM patients irrespective of the presence of conduction disturbances or SCD.[15] In DCM, genetic yield is 15% to 25% in isolated cases and up to 25% to 40% in DCM patients with a positive family history.[10,15,16] DCM is also genetically very heterogeneous and over 30 genes have been linked to DCM.[9] *TTN* is the gene with the most identified causal variants, in up to 15% to 25% of patients.[10,18] Other genes that are often linked to DCM are *RBM20, LMNA, BAG3* and more recently *FLNC*.[10,19]

Genetic testing is a cost-effective tool to improve diagnosis, assist in precision treatment and counselling of patients and their families.[16,20] If a pathogenic variant is identified in a proband, strict clinical follow-up of family members carrying this variant is warranted, whereas family members without the variant can be reassured and omitted from routine controls.[15,17] Moreover, identification of asymptomatic variant-carriers allows for early diagnosis and treatment, resulting in a decrease in morbidity and mortality.[15,17]

In patients that underwent HTx for non-ischemic cardiomyopathies in the past, retrospective genetic testing often has been overlooked. This is even more true for isolated cases of DCM, for which genetic testing has not always been advised in the past.[21] Despite the probands being tsssransplanted, establishing a molecular genetic diagnosis can still be of importance for family members who may be susceptible to an inherited cardiomyopathy. Since HTx patients represent a severely affected group of patients with a cardiomyopathy refractory to treatment, it is plausible that an underlying genetic cause is present, either as the sole culprit or in combination with additional environmental factors. We therefore hypothesize that genetic testing in HTx patients will result in a high and clinically relevant diagnostic yield. In this study, we evaluated the diagnostic yield of genetic testing in a HTx cohort and assessed the subsequent clinical approach for the family members.

Methods

Patient cohort

In this single center cohort study, patients who underwent HTx for end-stage heart failure between 1995 and September 2020 at the Antwerp University Hospital and were still in active follow-up, were screened for eligibility. All patients with DCM, HCM, RCM, ACM or LVNC were deemed eligible for genetic testing, irrespective of the presence of an additional trigger (e.g., alcohol) or a positive family history. Heart failure due to ischemic, valvular of congenital heart disease, served as exclusion criteria.

Eligible patients in whom genetic testing was not performed before HTx were offered genetic testing, irrespective of the presence of a family history. This retrospective study was approved by the local Ethics Committee.

Clinical evaluation

Data on last clinical evaluation before HTx, electrocardiography, echocardiography and 24-hour Holter monitoring were collected from patient records. Family history and aggravating or eliciting

factors were retrieved systematically in all patients. Family history was considered positive if 1 or more additional family members had DCM/HCM/LVNC or experienced unexplained sudden cardiac death.

Classification of the cardiomyopathies was performed according to the definition of the European Society of Cardiology.[22]

Anatomopathological data

The anatomopathological report of the explanted heart was retrieved for evaluation of total weight and left- and right ventricular wall thickness. Fibrosis was classified as local or diffuse. The pathological diagnosis was mentioned.

Genetic analysis

A targeted gene panel for next-generation sequencing (NGS) of known cardiomyopathy genes (CM-panel) was performed and comprised 36, 51 or 59 genes, depending on the year of analysis (respectively 2017, 2018 and 2019). Table 1 shows the complete list of genes included in the CM- panel. Genomic DNA was extracted from EDTA blood using standard procedures (Chemagic DNA bloodkit special, Perkin Elmer, Waltham, MA). Genetic analysis was performed using an in-house HaloPlex target enrichment followed by NGS as previously described.[23]

All variants were annotated on the corresponding metatranscripts. Variants were classified as benign or likely benign (class 1 and 2), variant of unknown significance (class 3), likely pathogenic (class 4) or pathogenic (class 5) according to ACMG guidelines.[24] Only class 3, 4 and 5 variants were considered clinically actionable. For *TTN* only truncating variants were reported as the clinical significance of *TTN* missense variants is currently insufficiently clear. All reported variants were confirmed using Sanger sequencing if they did not comply to strictly defined NGS quality criteria.[25]

Cascade testing and segregation analysis

If a class 4 or 5 variant was identified, cascade screening of family members of the proband was conducted using Sanger sequencing of the specific identified variant. If a variant of unknown significance was identified, family members were invited for clinical and molecular segregation analysis. First degree family members of probands without a genetic diagnosis were referred for a clinical cardiac evaluation. Family members were considered phenotype positive if they fulfilled the criteria for cardiomyopathy (see above). Reference values for echocardiographic evaluation were based on the EACVI guidelines.[26]

Statistical analysis

Continuous variables are expressed as mean and range and frequencies are expressed as numbers and percentages. Normality was assessed based on Shapiro-Wilk test and Q-Q plots. All continuous variables mentioned in the paper showed normal distribution. Unpaired *t*-test was used for comparisons of continuous variables with normal distribution after equality of variances was assessed with Levene's test for Equality of variances. Chi-square test was used for comparisons of categorical variables. All analyses were performed using SPSS Statistics version 26 (IBM Corporation). A p-value of <0.05 was considered statistically significant.

Results

Between 1995 and September 2020, 166 patients underwent HTx of whom 99 patients were still in active follow-up at the Antwerp University Hospital at the time of this study. Of these, 45 patients (45.5%) had been diagnosed with a non-ischemic cardiomyopathy before HTx and were eligible (Figure 1). Of the 45 patients, only 6 patients (3 HCM, 3 DCM) already had a molecular diagnosis *pre HTx*. Of the 39 remaining eligible patients, 31 agreed to undergo genetic testing (*post HTx)*.

Characteristics of the patient cohort

Demographic, clinical and anatomopathological characteristics of the patients that underwent genetic testing (pre- and post HTx) are shown in Table 2. Mean age was 47.8 years (19-66) at time of HTx and patients were on average 10.6 years after HTx (<1-32 years).

Table 1 Overview of the Genes Included in the Targeted Next-Generation Sequence CM-Panel

	CM 36 gene panel	CM 51 gene panel- added genes	CM 59 gene panel- added genes
Genes	*ABCC9, ACTC1, ACTN2, ANKRD1, CSRP3, DES, DSC2, DSG2, DSP, GLA, JUP, LDB3, LMNA, MYBPC3, MYH6, MYH7, MYL2, MYL3, MYOZ2, NEXN, PKP2, PLN, RBM20, SCN5A, SGCD, TAZ, TCAP, TGFB3, TMEM43, TNNC1, TNNI3, TNNT2, TPM1, TTN, TTR, VCL*	*ALPK3, BAG3, CALR3, CAV3, CRYAB, CTNNA2, EMD (STA), FHL1, JPH2, LAMA4, LAMP2, MIB1, MYPN, PRDM16, PRKAG2*	*FHL2, FLNC, NEBL, PPA2, RAF1, RYR2, SDHA, SYNE1*
Number of patients tested	*2*	*23*	*6*
Number of identified variants	31	5	0

The Number of Patients Tested with this Panel and the Yield are Displayed.

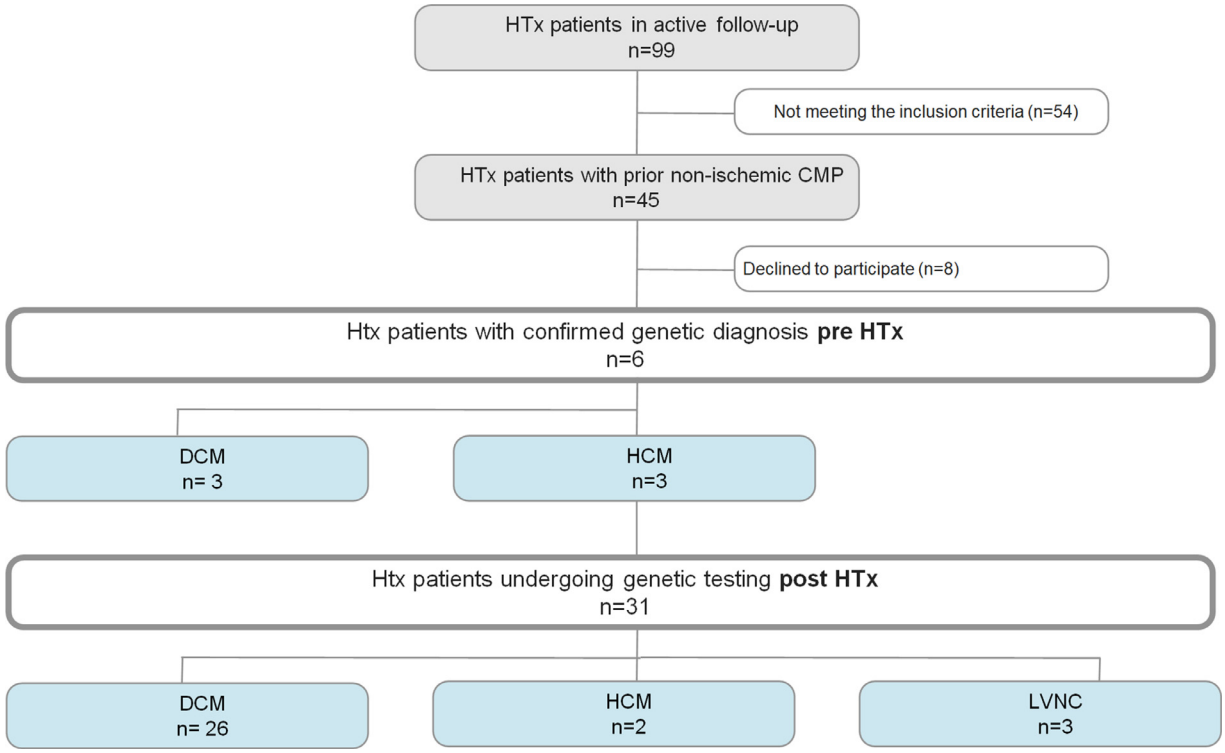

Figure 1 Flowchart of patient selection. Figure 1 From the total 99 HTx patients still in active follow-up we invited patients transplanted in light of a non-ischemic cardiomyopathy for genetic testing. From the 45 eligible patients, 8 patients declined and 6 patients already had received a genetic diagnosis before HTx. The remaining 31 patients underwent genetic testing. HTx = heart transplantation.

The majority of patients were diagnosed with DCM, followed by HCM and LVNC.

Diagnostic yield of genetic testing

Of the 31 patients that were tested with the CM-panel in the current study, 12 patients carried a class 5 variant (38.7%) and 11 patients carried a class 3 variant (35.5%) (Table 3).

When combining results of all genetic testing performed, both pre HTx and post HTx, a genetic diagnosis could be made in 45.9% of CM patients. In 32.4% of patients a class 3 variant was identified. Table 3 shows this overall diagnostic yield (both pre- and post HTx), and separately per type of cardiomyopathy.

Identified variants according to the phenotype

Table 4 shows the identified variants in the 37 patients. Truncating variants in *TTN* were most frequent in DCM patients (7/29 individuals). Five different *TTN* truncating variants were identified, 3 located in the A-band and 2 located in the I-band of Titin. Four patients with DCM had more than 1 variant, 2 had a class 5 variant accompanied by a class 3 in a different gene, 2 patients presented with 3 variants of unknown significance. One *TTN* variant was identified in 3 different patients, representing a Flemish founder variant (c.53918delG). One class 3 variant in *NEXN* was identified in both a DCM and unrelated HCM patient (c.1174C>T).

Characteristics of variant carriers

As shown in Table 5, clinical characteristics were similar between class 4/5 variant carriers and patients without a variant. On the explanted heart, fibrosis was more prevalent in patients carrying a class 4/5 variant compared to patients with no variant and fibrosis tended to be more widespread throughout the heart.

There was a tendency toward a higher diagnostic yield of genetic testing for a class 4/5 variant in patients with a positive family history (47.6% vs 20.0%; $p = 0.140$).

A substantial amount of DCM patients (15/26, 57.7%) presented with additional precipitating factors for the development of the cardiomyopathy phenotype (Table 2). Diagnostic yield of genetic testing (class 4 or 5) however, was similar in DCM patients with and without precipitating factors (respectively 5/15 and 5/11, $p = 0.530$).

Impact of genetic diagnosis on family members and further approach

Patients with a class 4/5 variant were offered genetic counselling and cascade screening for the 1st-degree relatives, which was performed in 82.4% of subjects (Figure 2). 39.6% of the family members was genotype positive, 60.4% genotype negative. There was no significant difference in demographic characteristics between genotype positive and genotype negative family members (Table 6). Of the 16 clinically evaluated genotype positive family

Table 2 Characteristics of the Total HTx Cohort that Underwent Genetic Testing

Characteristic	DCM (*N* = 29)	HCM (*N* = 5)	LVNC (*N* = 3)
Age at time of HTx (years)	49.4 [26-66]	46.8 [19-58]	33.0 [25-42]
Female	3 (10.3%)	3 (60%)	2 (66.7%)
Positive family history	21 (72.4%)	5 (100%)	1 (33.3%)
Precipitating factors (number of patients)			
Alcohol abuse	6 (20.7%)	0 (0%)	0 (0%)
Drug abuse	0 (0%)	0 (0%)	0 (0%)
Coronary atheromatosis	6 (20.7%)	1 (20.0%)	0 (0%)
Cardiotoxic chemotherapy	1 (3.4%)	0 (0%)	0 (0%)
Myocarditis	1 (3.4%)	0 (0%)	1 (33.3%)
Peripartum period	2 (6.9%)	0 (0%)	0 (0%)
Total	*16 (55.2%)*	*1 (20%)*	*1 (33.3%)*
Echocardiography			
LVEF (%)	17.4 [3-33]	43.8 [20-68]	23.0 [20-29]
LVEDD (mm)	72.0 [54.0-100.0]	52.2 [31.0-75.0]	68.5 [64.0-73.0]
LVESD (mm)	62.0 [41.5-79.0]	38.4 [23.1-66.0]	58.5 [54.0-63.0]
Pathological findings in explanted heart			
Number of patients	27 (93.1%)	5 (100%)	3 (100%)
Mass of explant (g)	511.4 [313.2-707.8]	477.6 [346.3-641.0]	394.0 [353.0-435.0]
LV wall thickness (mm)	16.8 [8.0-35.0]	24.3 [20.0-31.0]	12.5 [8.0-17.0]
RV wall thickness (mm)	6.6 [2.0-19.0]	10.0 [6.0-15.0]	8.0 [*n* = 1]
Fibrosis present	23 (85.2%)	5 (100%)	3 (100%)
Diffuse fibrosis (vs localised)	18 (66.7%)	1 (20.0%)	3 (100%)
Compensatory cardiomyocyte hypertrophy	25 (92.6%)	0 (0%)	3 (100%)

Demographic, clinical and anatomopathological characteristics of the patients that underwent genetic testing, both pre-HTX (*n* = 6) and post HTX (*n* = 31). Data are mean [range] or numbers (%). DCM was defined as left ventricular (LV) dilatation and the presence of systolic dysfunction defined as left ventricular ejection fraction (LVEF) <50% or global longitudinal strain (GLS) < -15.9%.[35] LV dilatation was defined as LV end diastolic diameter (LVEDD) >58 mm in male patients, >53 mm in female patients.[35] HCM was defined as LV myocardial hypertrophy (intraventricular septum thickness >12 mm) in the absence of hemodynamic stress sufficient to account for the degree of hypertrophy and systemic disease.[22] LVNC was defined as the presence of prominent LV trabeculae and deep trabecular recesses in the absence or presence of LV dilatation and/or LV dysfunction.[22]

Alcohol abuse was defined as >5E daily, drug abuse was defined as any prolonged use of stimulating drugs in the prior history. Coronary atheromatosis was defined as coronary stenosis and atheromatosis insufficient to explain the degree of systolic dysfunction. Peripartum period was defined as last month or pregnancy till the 5th mo postpartum[36].

LVESD, LV end-systolic diameter.

members, 10 (62.5%) had a cardiac phenotype (DCM, arrythmia). Although phenotype negative family members were on average younger than the phenotype positive family members, this was not significant, possibly due to the low sample size (Table 6). In addition, genetic counselling allowed for pre-implantation genetic testing (PGT), in which early-stage embryos are genetically tested for the familial variant and only those without the variant are implanted, ensuring offspring does not carry the pathogenic variant (3 patients).[11]

Familial combined molecular and clinical co-segregation analysis was offered in relatives of patients with an identified class 3 variant, when the variant was deemed more suspect. In 4 families segregation analysis was performed (36.4% of families in whom a VUS was identified). Absence of segregation in 1 family led to the reclassification of the ACTN2

Table 3 Overview of Diagnostic Yield of Genetic Testing

		n	*Class 4/5*	*Class 3*	*Total (Class 3+4+5)*
DCM	*Testing post HTx*	*26*	*10*	*8*	*18*
HCM	*Testing post HTx*	*2*	*0*	*2*	*2*
LVNC	*Testing post HTx*	*3*	*2*	*1*	*3*
Total	*Testing post HTx*	***31***	***12 (38.7%)***	***11 (35.5%)***	***23 (74.2%)***
	Testing pre HTx	*6*	*5*	*1*	*6*
	Total pre+post HTX	*37*	*17 (45.9%)*	*12 (32.4%)*	*29 (78.4%)*

Testing "post HTx" are the results of the genetic testing of the current study (*n* = 31). Testing "pre HTx" was the genetic result that was already available in the patients before HTx (*n* = 6).

n, number of patients. Numbers displayed in bold represent the total number of patients tested post HTx (DCM, HCM and LVNC combined).

Table 4 Overview of the Identified Variants in the HTx Cohort

	Patient	Gene	Variant (c.)	Variant (p.)	Class	ClinVar annotation	Comment
DCM	***1***	***DSP***	c.137G>A	p.Gly46Asp	3	VCV000451932.6	
		TTN	c.80514delA	p.Val26839Leufs*5	5	VCV001065192.1	
	2	***DSP***	c.344A>G	p.Asn115Ser	3	VCV000518982.11	
	3	***JUP***	c.56C>T	p.Thr19Ile	3	VCV000179756.9	
		PRDM16	c.872 C>T	p.Pro291Leu	3	VCV000060728.4	
		MYH7	c.1997A>G	p.His666Arg	3	VCV001065184.1	
	4	***LDB3***	c.91C>T	p.Arg31Trp	3	VCV000201857.5	
	5	***LDB3***	c.998G>A	p.Arg333His	3	VCV000201843.8	
		RBM20	c.2893G>A	p.Gly965Arg	3	VCV001065186.1	
		VCL	c.1961A>C	p.Asn654Thr	3	VCV001065185.1	
	6	***LMNA***	C.884C>T	p.Ser295Leu	3	VCV000518983.7	
	7	***LMNA***	c.1580G>C	p.Arg527Pro	5	VCV000014481.4	Identified pre-HTx
	8	***MYBPC3***	c.821+1G>A		5	VCV000042791.14	
	9	***MYH7***	c.1357C>T	p.Arg453Cys	5	VCV000014089.11	Identified pre-HTx
	10	***MYPN***	c.59A>G	p.Tyr20Cys	3	VCV000031811.11	
		TMEM43	c.1073C>T	p.Ser358Leu	5	VCV000000734.11	
	11	***NEXN***	c.1174C>T	p.Arg392*	3	VCV000229051.7	Identified in 1 HCM patient as well
	12	***PRDM16***	c.2362A>T	p.Met788Leu	3	VCV000953465.3	
	13	***PRKAG2***	c.1681G>C	p.Ala561Pro	3	VCV000520490.4	
	14	***RBM20***	c.2176C>T	p.Arg726Ter	5	VCV000538028.3	Identified pre-HTx
	15	***TTN***	c.83497G>T	p.Gly27833* (A-band)	5	VCV001065188.1	
	16	***TTN***	c.69522T>G	p.Tyr23174* (A-band)	5	VCV001065189.1	
	17	***TTN***	c.53918delG	p.Gly17973Glufs*18 (A-band)	5	VCV001065190.1	Identified in 3 different HTx patients
	18	***TTN***	c.53918delG	p.Gly17973Glufs*18 (A-band)	5	VCV001065190.1	Identified in 3 different HTx patients
	19	***TTN***	c.53918delG	p.Gly17973Glufs*18 (A-band)	5	VCV001065190.1	Identified in 3 different HTx patients
	20	***TTN***	c.41641C>T	p.Arg13881*(I-band)	5	VCV000655573.4	
	21	***TTN***	c.13592C>G	p.Ser4531* (I-band)	5	VCV001065191.1	
	22-29	No Class 3-5 variants identified					
LVNC	***30***	***MYBPC3***	c.1404delG	p.Gln469Serfs*19	5	VCV000254153.4	
	31	***LAMP2***	c.668dupA	p.Tyr223*	5	VCV001065183.1	
	32	***LDB3***	c.1366C>G	p.Pro456Ala	3	VCV000967764.3	
HCM	***33***	***ABCC9***	c.4640C>T	p.Thr1547Ile	3	VCV000030185.4	
	34	***MYBPC3***	c.772G>A	p.Glu258Lys	5	VCV000042792.16	Identified pre-HTx.
			c.1828G>A	p.Asp610Asn	3	VCV000042575.7	Identified pre-HTx.
	35	***MYBPC3***	c.2373dup	p.Gln791fs	5	VCV000042619.21	Identified pre-HTx.
	36	***NEXN***	c.1174C>T	p.Arg392*	3	VCV000229051.7	Identified in DCM patient as well
	37	**RBM20**	c.2746_2748delGAA	p.Glu918del	3	VCV000645954.3	Identified pre-HTx.
		TNNT2	c.411_412 delinsTA	p.Gln137_Gln138delinsHisLys	3	VCV001065187.1	Identified pre-HTx.

All variants were submitted to ClinVar: accessions for this submission are SCV001572558 - SCV001572591.
*indicates indicate a translation termination (stop) codon.

c.890T>A variant, identified in a DCM family, from class 3 to 2, according to ACMG guidelines.[27] For the other 3 families limited co-segregation was obtained, but this was insufficient to allow reclassification of the VUS.

Discussion

In this study, we assessed the diagnostic yield of genetic testing in a population of 31 HTx recipients with prior non-ischemic cardiomyopathy.

Overall, we could confirm a high diagnostic yield of genetic testing: 38.7% for class 4/5 variants. In 35.5% a VUS was identified. When this was extended to the entire HTx population, including those genetically tested before HTx, this yield was even higher with 45.9% for class 4/5 variants and 32.4% for VUS.

Although genetic counselling and screening are now advised for all cardiomyopathy patients, these findings show that this is still often overlooked in clinical practice.[11] Especially in patients in whom genetic analysis was not performed before transplantation, there is a chance that this will also be overlooked during follow-up.

For *DCM*, the diagnostic yield (38.5% for class 4/5 variants and an additional 30.8% for class 3 variants), is

Table 5 Differences in Risk Factors and Clinical & Anatomopathological Findings between Class 4/5 Variant Carriers and Patients without a Variant

	Class 4/5 variant	No variant	*p*-value pathogenic vs none
Number of patients	**17 (45.9%)**	**8 (21.6%)**	
Risk factors			
Positive family history	88.2%	75%	0.400
Female	23.5%	12.5%	0.520
2nd hit	41.2%	50.0%	0.678
Age at time of HTX	47.4 [19-66]	43.9 [35-58]	0.526
Anatomopathology			
Data available	15 (88.2%)	8 (100%)	
Fibrosis	100%	75%	**0.043**
Diffuse fibrosis	80%	37.5%	0.055
Compensatory cardiomyocyte hypertrophy	80%	100%	0.399
Massa (g)	508.9 [342.0-707.8]	511.0 [313.2-637.2]	0.484
LV wall (mm)	15.9 [8.0-31.0]	17.5 [15.0-20.0]	0.328
RV wall (mm)	6.0 [2.0-9.0]	6.0 [4.0-7.0]	0.500

Data are median [interquartile range] or numbers (%).
LVESD, LV end-systolic diameter.

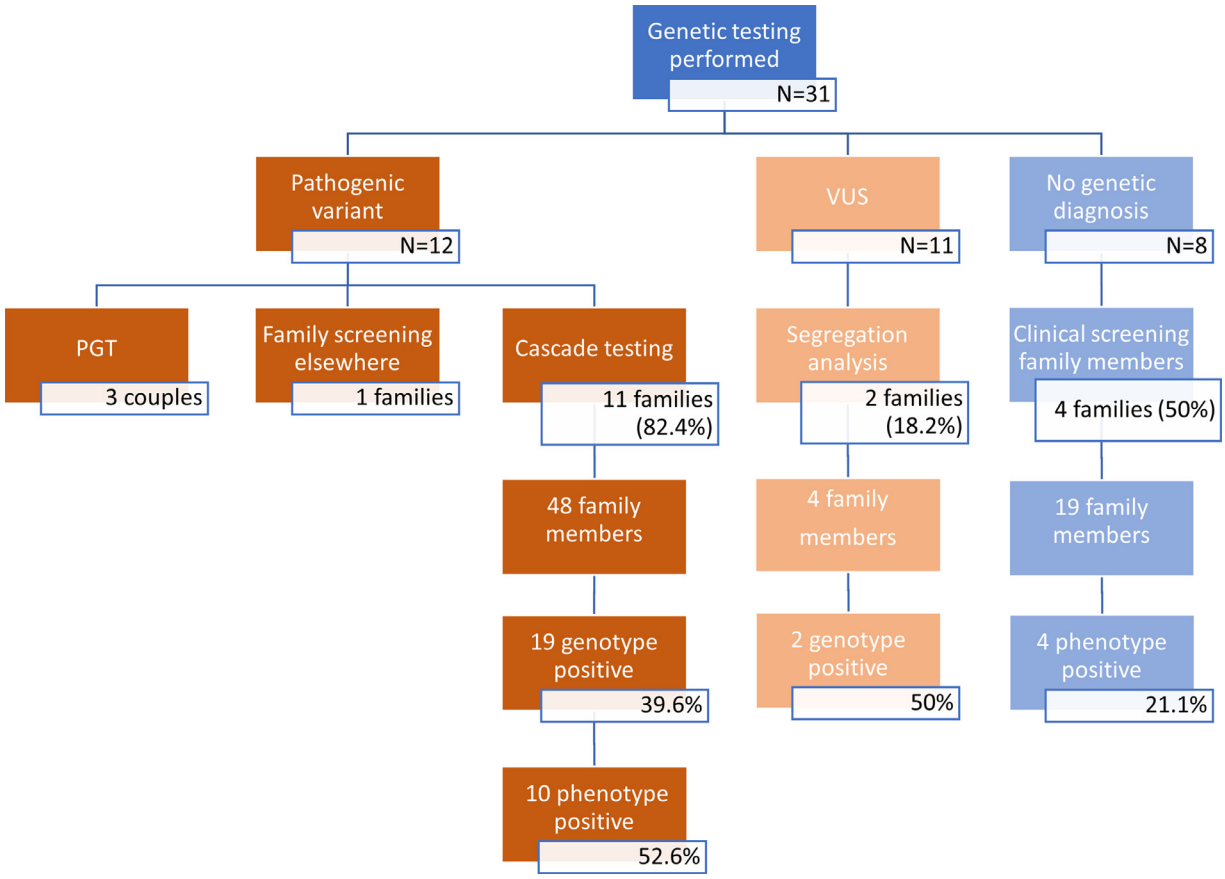

Figure 2 Overview of results of genetic testing of family members. Figure 2 Family members of probands were invited for genetic cascade screening (class 4,5 variants, (likely) pathogenic variants), segregation analysis (class 3 variants, VUS) or clinical screening (no variant identified) depending on the classification of the variant. In families carrying a class 5 variant, pre-implantation genetic testing (PGT) was offered as well.

Table 6 Characteristics of 1] Genotype Positive Family Members and Genotype Negative Family Members and 2] Phenotype Positive and Phenotype Negative Family Members

	All family members ($N = 48$)		
	Genotype negative $N = 29$ (60.4%)	Genotype positive $N = 19$ (39.6%)	p-value
Age at time of genetic diagnosis (y)	43.2 ± 16.5	43.2 ± 20.4	0.994
Current Age (y)	45.9 ± 16.2	46.0 ± 21.0	0.990
Gender: Male	11 (37.9%)	8 (42.1%)	0.772
Female	18 (62.1%)	11 (57.9%)	
Cardiac evaluation		3/19 patients no data available 7/19 DCM 3/19 arrhythmia	
	Genotype positive family members only ($N = 16$)		
	Phenotype negative $N = 6/19$ (31.6%)	Phenotype positive $N = 10/19$ (52.6%)	
Age at time of genetic diagnosis (y)	37.3 ± 20.0	47.0 ± 15.1	0.289
Current age (y)	39.7 ± 20.9	50.0 ± 15.1	0.270
Gender: Male	1 (16.7%)	6 (60%)	0.091
Female	5 (83.3%)	4(40%)	

Continuous variables are displayed as mean ± standard deviation with minimum and maximum values between brackets. Categorical variables are displayed as absolute number and percentages in between brackets. Pairwise comparison was performed using t-test for continuous variables with a normal distribution and using chi-square for categorical variables.

BMI, body mass index.

relatively high compared to the diagnostic yield in non-HTx DCM patients, where genetic yield is estimated at 15% to 25% in isolated cases and up to 40% in familial DCM.[10,15,16] This finding is in line with our hypothesis that HTx patients, who represent a severely affected group of patients with a cardiomyopathy refractory to treatment, more often harbour an underlying genetic cause.

To date, only a limited number of small studies have assessed the genetic background of DCM patients receiving HTx. In a small study of 13 DCM patients undergoing HTx, Martins et al. reported a class 3 variant in 5 patients and a class 4/5 variant in only 1 patient (7.7%).[28] The remarkably lower yield might be explained by the use of a smaller targeted gene panel (15 genes) and the exclusion of *TTN,* which was the most frequently affected gene in our population.[28] Especially when taking into consideration that *TTN* is the largest gene in the human genome and variants in *TTN* are therefore more frequent.[18,29]

Seidelmann et al. conducted genetic testing in 10 DCM patients, preselected for a positive family history. In this small group of patients, a diagnostic yield of 50% (5/10) for class 4/5 variants and 60% (6/10) for class 3, 4 or 5 variants could be achieved, similar to our findings.[21] Cuenca S et al. investigated the genetic background of 52 HTx patients with DCM, again preselected by the presence of a positive family history.[30] By NGS of 126 genes related to cardiac function they showed a genetic yield of 73% for class 4/5 variants and an additional 10% for class 3 variants.[30] Their higher diagnostic yield might be due to the fact that they only included patients with familial DCM. Indeed, also in our study, the presence of a familial history was higher in patients with a class 4/5 variants compared to those without variants (although not statistically significant).

For *HCM*, the diagnostic yield within our cohort was 40% for class 4/5 variants. In the other 60% of patients a class 3 variant was identified. These yields are comparable with other HCM populations (40% and even up to 72% in patients with a positive family history).[10,12,13] To our knowledge, this is the first study that performed cardiogenetic testing in HTx recipients with a previous HCM.

Overall, these data show the importance of offering genetic testing in cardiomyopathy patients. Currently, genetic counselling and genetic testing is recommended for all confirmed or suspected inherited HCM, DCM and ARVC patients.[11] However, as our data show there are still patients with severe cardiomyopathy to whom genetic counselling had not yet been offered.

When offering genetic testing, it is also important to regularly update the set of genes of interest, according to the latest advances in the field.[11]

Variants in TTN in a HTx cohort

In DCM HTx patients, variants in *TTN* were the most frequent, similar to previous findings in non-HTx DCM cohorts. Indeed, *TTN* truncating variants can be identified in about 25% of familial DCM and 18% of sporadic DCM patients.[18] Although *TTN* has been linked to DCM almost 1 decade ago,[18] this gene remained incompletely studied in large prior populations, due to technical difficulties. Indeed, *TTN* is a giant gene, over 108kb spread over 363 exons, which for long impeded fast and high-throughput

sequencing. However, due to technical improvements and large data cohorts showing the importance of *TTN* in DCM, it now needs to be an essential part of genetic testing in DCM patients.[17,18]

More recently increasing evidence is available that *TTN* truncating variants also play an important role in "secondary" DCM, where a precipitating, environmental factor is present. In fact, about 10% of patients with peripartum cardiomyopathy or toxic cardiomyopathy (caused by excess alcohol intake or cardiotoxic chemotherapy) carry a *TTN* truncating variant.[31-34]

In this cohort of DCM patients, with advanced heart failure requiring HTx, a *TTN* variant was identified in 7/29 patients (24.1%). This is comparable to the findings of Cuenca S. et. al who identified a variant in *TTN* in 10 individuals (19.2%) in a highly selected cohort of HTx patients with familial DCM.[30]

The role of *TTN* in DCM might even still be underestimated at this moment since *TTN* missense variants have not been included so far.

Genetic testing even useful in the presence of a precipitating factor

Our study is the first to investigate a widely diverse HTx cohort, including patients with "secondary" DCM as well as "non-familial" cases. Even in this unselected population, diagnostic yield was high.

More than half of the DCM patients (55%) in our cohort had a precipitating factor ("second hit theory") for the development of DCM. Interestingly, these patients showed a similar diagnostic yield compared to patients without a precipitating factor. Our findings illustrate that, even in HTx patients, a precipitating factor does not exclude a genetic cause for DCM. Of the 12 patients with a class 3 variant, 8 (66.7%) had a "second hit", either an environmental precipitating factor or an additional class 3 variant. Class 3 variants might need a second hit to reveal a cardiac phenotype, a hypothesis that deserves further investigation.

Impact of a genetic diagnosis on family members

Even more than the index patient, who already underwent HTx for the underlying condition, the result of genetic analysis greatly impacts family members. Familial cascade screening and genetic counselling for family members of HTx patients with class 4/5 variants, identified additional variant carriers. Recognition of a cardiac phenotype early in the course of the disease, allows for prompt treatment that is substantial to decrease morbidity and mortality.[16,35] In this particular study, 39.6% of the family members was genotype positive, of whom 52.6% were found to have an abnormal cardiac evaluation although all were asymptomatic at the time of evaluation. Due to genetic screening, these patients could be identified early and were all offered adequate clinical follow-up, allowing early detection and treatment to prevent disease progression as is recommended by the recent guidelines.[11] In addition, 29 family members (60.4%) were found to be genotype negative, providing reassurance and refrain from clinical follow-up,[11] thereby resulting in personal and health-economic benefit. In line with the current consensus, PGT can be offered in genotype positive family members when appropriate.

As a VUS is not considered directly actionable for predictive testing, it is recommended that family members undergo regular clinical follow-up irrespective of their genotype.[11] In addition, segregation testing, when performed, can be very informative and could assist in the reclassification of variants. Importantly long-term follow-up with regular clinical checkup should be performed, because with development of a cardiac phenotype, the classification of VUS can change. Patients should be updated about changes in classification and management should be adapted accordingly.[11]

The use of 3 different gene panels is recognized as a limitation of this study. In fact, in this cohort, 5 variants would not have been identified when we would have limited the analysis to the initial panel containing 36 CM genes. This confirms that updating the set of genes of interest according to the most recent genetic insights is important and will help molecularly diagnosing more patients

Conclusion

Diagnostic yield of genetic testing in HTx recipients is very high, with the majority of variants identified in *TTN*. Therefore, genetic testing (including *TTN)* must not be overlooked in HTx recipients due to DCM, HCM or LVNC, and should be irrespective of prior family history or the presence of another precipitating factor. By doing so, relatives at risk can be identified in an early stage, which, will have a major impact on their clinical outcome.

Author contributions

Dr Hanne M Boen: Data analysis and writing of manuscript. Prof Dr Bart L Loeys: Study design, data analysis, counselling and follow-up of patients. Prof Dr Maaike Alaerts: Data analysis. Prof Dr Johan B Saenen: data collection, counselling and follow-up of patients. Inge Goovaerts: data collection, counselling and follow-up of patients. Prof Dr Lut Van Laer: Data analysis. Dr Anne Vorlat: Data collection. Dr Tom Vermeulen: Data collection. Dr Constantijn Franssen: Data collection. Prof Dr Patrick Pauwels: anatomopathological data collection. Prof Dr Inez Rodrigus: Data collection. Prof Dr Hein Heidbuchel: Data analysis, acquisition of funding. Prof Dr Emeline M. Van Craenenbroeck: Study design, data analysis, writing of manuscript, data collection, counselling and follow-up of patients, acquisition of funding.

Disclosure statement

The authors have no conflict of interest to declare.

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References

1. Mehra MR, Canter CE, Hannan MM, et al. The 2016 international society for heart lung transplantation listing criteria for heart transplantation: a 10-year update. J Heart Lung Transplant 2016;35:1-23.
2. Mehra MR, Kobashigawa J, Starling R, et al. Listing criteria for heart transplantation: international society for heart and lung transplantation guidelines for the care of cardiac transplant candidates—2006. J Heart Lung Transplant 2006;25:1024-42.
3. Yancy CW, Jessup M, Bozkurt B, et al. 2013 ACCF/AHA guideline for the management of heart failure: a report of the American college of cardiology foundation/american heart association task force on practice guidelines. Circulation 2013;128:e240-327.
4. Ponikowski P, Voors AA, Anker SD, et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC)Developed with the special contribution of the heart failure association (HFA) of the ESC. Eur Heart J 2016;37:2129-200.
5. Khush KK, Cherikh WS, Chambers DC, et al. The international thoracic organ transplant registry of the international society for heart and lung transplantation: thirty-sixth adult heart transplantation report - 2019; focus theme: donor and recipient size match. J Heart Lung Transplant 2019;38:1056-66.
6. Gilljam T, Haugaa KH, Jensen HK, et al. Heart transplantation in arrhythmogenic right ventricular cardiomyopathy - experience from the Nordic ARVC registry. Int J Cardiol 2018;250:201-6.
7. Liu S, Xie Y, Zhang H, et al. Multiple genetic variants in adolescent patients with left ventricular noncompaction cardiomyopathy. Int J Cardiol 2020;302:117-23.
8. Sedaghat-Hamedani F, Haas J, Zhu F, et al. Clinical genetics and outcome of left ventricular non-compaction cardiomyopathy. Eur Heart J 2017;38:3449-60.
9. Hershberger RE, Hedges DJ, Morales A. Dilated cardiomyopathy: the complexity of a diverse genetic architecture. Nat Rev Cardiol 2013;10:531-47.
10. Wilcox JE, Hershberger RE. Genetic cardiomyopathies. Curr Opin Cardiol 2018;33:354-62.
11. Musunuru K, Hershberger RE, Day SM, et al. Genetic testing for inherited cardiovascular diseases: a scientific statement from the American heart association. Circ Genom Precis Med 2020;13:e000067.
12. Ingles J, Sarina T, Yeates L, et al. Clinical predictors of genetic testing outcomes in hypertrophic cardiomyopathy. Genet Med 2013;15:972-7.
13. Louis C, Calamaro E, Vinocur JM. Hereditary arrhythmias and cardiomyopathies: decision-making about genetic testing. Curr Opin Cardiol 2018;33:78-86.
14. Van Driest SL, Ommen SR, Tajik AJ, Gersh BJ, Ackerman MJ. Yield of genetic testing in hypertrophic cardiomyopathy. Mayo Clin Proc 2005;80:739-44.
15. Hershberger RE, Givertz MM, Ho CY, et al. Genetic evaluation of cardiomyopathy: a clinical practice resource of the American college of medical genetics and genomics (ACMG). Genet Med 2018;20:899-909.
16. Ackerman MJ, Priori SG, Willems S, et al. HRS/EHRA expert consensus statement on the state of genetic testing for the channelopathies and cardiomyopathies this document was developed as a partnership between the heart rhythm society (HRS) and the European heart rhythm association (EHRA). Heart Rhythm 2011;8:1308-39.
17. Tayal U, Ware JS, Lakdawala NK, Heymans S, Prasad SK. Understanding the genetics of adult-onset dilated cardiomyopathy: what a clinician needs to know. Eur Heart J 2021;42:2384-96.
18. Herman DS, Lam L, Taylor MRG, et al. Truncations of titin causing dilated cardiomyopathy. N Engl J Med 2012;366:619-28.
19. Verdonschot JAJ, Vanhoutte EK, Claes GRF, et al. A mutation update for the FLNC gene in myopathies and cardiomyopathies. Hum Mutat 2020;41:1091-111.
20. Wordsworth S, Leal J, Blair E, et al. DNA testing for hypertrophic cardiomyopathy: a cost-effectiveness model. Eur Heart J 2010;31:926-35.
21. Seidelmann SB, Laur O, Hwa J, et al. Familial dilated cardiomyopathy diagnosis is commonly overlooked at the time of transplant listing. J Heart Lung Transplant 2016;35:474-80.
22. Elliott P, Andersson B, Arbustini E, et al. Classification of the cardiomyopathies: a position statement from the european society of cardiology working group on myocardial and pericardial diseases. Eur Heart J 2007;29:270-6.
23. Proost D, Vandeweyer G, Meester JA, et al. Performant mutation identification using targeted next-generation sequencing of 14 thoracic aortic aneurysm genes. Hum Mutat 2015;36:808-14.
24. Li MM, Datto M, Duncavage EJ, et al. Standards and guidelines for the interpretation and reporting of sequence variants in cancer: a joint consensus recommendation of the association for molecular pathology, American society of clinical oncology, and college of American pathologists. J Mol Diagn 2017;19:4-23.
25. Proost D, Saenen J, Vandeweyer G, et al. Targeted next-generation sequencing of 51 genes involved in primary electrical disease. J Mol Diagn 2017;19:445-59.
26. Lancellotti P, Cosyns B, European Society of C. The EACVI Echo Handbook. 2016.
27. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American college of medical genetics and genomics and the association for molecular pathology. Genet Med 2015;17:405-24.
28. Martins E, Sousa A, Canedo P, et al. Genetic variants identified by target next-generation sequencing in heart transplant patients with dilated cardiomyopathy. Rev Port Cardiol 2019;38:441-7.
29. Ware JS, Cook SA. Role of titin in cardiomyopathy: from DNA variants to patient stratification. Nat Rev Cardiol 2018;15:241-52.
30. Cuenca S, Ruiz-Cano MJ, Gimeno-Blanes JR, et al. Genetic basis of familial dilated cardiomyopathy patients undergoing heart transplantation. J Heart Lung Transplant 2016;35:625-35.
31. Ware JS, Li J, Mazaika E, et al. Shared genetic predisposition in peripartum and dilated cardiomyopathies. N Engl J Med 2016;374:233-41.
32. van Spaendonck-Zwarts KY, Posafalvi A, van den Berg MP, et al. Titin gene mutations are common in families with both peripartum cardiomyopathy and dilated cardiomyopathy. Eur Heart J 2014;35:2165-73.
33. Ware JS, Amor-Salamanca A, Tayal U, et al. Genetic etiology for alcohol-induced cardiac toxicity. J Am Coll Cardiol 2018;71:2293-302.
34. Garcia-Pavia P, Kim Y, Restrepo-Cordoba MA, et al. Genetic variants associated with cancer therapy–induced cardiomyopathy. Circulation 2019;140:31-41.
35. Hershberger RE, Morales A. Dilated cardiomyopathy overview. In: Adam MP, Ardinger HH, Pagon RA, et al., eds. GeneReviews(®). Seattle (WA): University of Washington, Seattle Copyright © 1993-2020, University of Washington, Seattle. GeneReviews is a registered trademark of the University of Washington, Seattle. All rights reserved.; 1993.
36. Sliwa Karen, Frett James, Elkayam Uri. Peripartum cardiomyopathy. Lancet 2006. https://doi.org/10.1016/S0140-6736(06)69253-2. In press.