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ABSTRACT

Background: Next-generation sequencing (NGS) is increasingly used for clinical evaluation of cardiomyopathy patients as it allows for simultaneous screening of multiple cardiomyopathy-associated genes. Adding copy number variant (CNV) analysis of NGS data is not routine yet and may contribute to the diagnostic yield.
Objectives: Determine the diagnostic yield of our targeted NGS gene panel in routine clinical diagnostics of Dutch cardiomyopathy patients and explore the impact of exon CNVs on diagnostic yield.
Methods: Patients ($N = 2002$) referred for clinical genetic analysis underwent diagnostic testing of 55–61 genes associated with cardiomyopathies. Samples were analyzed and evaluated for single nucleotide variants (SNVs), indels and CNVs. CNVs identified in the NGS data and suspected of being pathogenic based on type, size and location were confirmed by additional molecular tests.
Results: A (likely) pathogenic (L)P variant was detected in 22.7% of patients, including 3 with CNVs and 25 where a variant was identified in a gene currently not associated with the patient's cardiomyopathy subtype. Only 15 out of 2002 patients (0.8%) were found to carry two (L)P variants.
Conclusion: The yield of routine clinical diagnostics of cardiomyopathies was relatively low when compared to literature. This is likely due to the fact that our study reports the outcome of patients in daily routine diagnostics, therefore also including patients not fully fulfilling (subtype specific) cardiomyopathy criteria. This may also explain why (L)P variants were identified in genes not associated with the reported subtype. The added value of CNV analysis was shown to be limited but not negligible.

1. Introduction

Cardiomyopathies are disorders that affect the functioning of the cardiac muscle. Primary cardiomyopathies often share phenotypic characteristics and are clinically differentiated based on morphology and pathophysiology [1]. Cardiomyopathy subtypes include hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), arrhythmogenic cardiomyopathy (ACM), left ventricular non-compaction cardiomyopathy (LVNC) and restrictive cardiomyopathy (RCM) [2]. Cardiomyopathies are genetically heterogeneous [3], and over 100 genes have been associated with inherited forms of these disorders [4]. These cardiomyopathy genes encode for proteins present at various cellular locations, but mainly impact the structural components of the myocyte [5].

Current clinical practices for genetic testing of cardiomyopathy patients are largely based on simultaneous screening of coding regions of multiple genes using next-generation sequencing (NGS) gene panels. Various reports have documented the outcome of screening multiple genes (19 to 84 genes) in a single test, often focusing on reporting variants identified within a specific cardiomyopathy subtype [6–9]. Screening for single nucleotide variants (SNVs) and small indels using these multi-gene cardiomyopathy panels has led to an increase in genetic diagnoses of patients with cardiomyopathies, as compared to Sanger sequencing [10,11]. Nonetheless, only 30–60% of underlying genetic causes are identified in HCM, ACM or DCM and <20% are identified in LVNC or RCM [12–14].

We have previously demonstrated that targeted NGS gene panel sequencing matches the quality of Sanger sequencing and can be used as a stand-alone tool in clinical genetic diagnostics of cardiomyopathies [15]. We have subsequently upgraded and expanded this targeted gene panel

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to screen for variants in 55–61 genes (numbers increasing with panel upgrades over time) associated with cardiomyopathies and applied it in routine clinical genetic diagnostics. Although deletions or duplications of one or multiple exons occur in several cardiomyopathy genes, explaining <1% of cases [6,16], these structural variations are currently not routinely analyzed in clinical genetic diagnostics in many laboratories. In order to assess the contribution of analyzing exonic deletions/duplications to diagnostic yield, we developed and validated CoNVaDING, a tool to detect such deletions and duplications in targeted NGS data [17].

We evaluated the outcome of cardiomyopathy diagnostics in a large Dutch cohort of 2002 patients. The aim of this study was: (1) to determine and evaluate the diagnostic yield of our custom targeted NGS gene panel in routine clinical diagnostics and (2) to explore the impact of the analyses of exonic CNVs on diagnostic yield.

2. Materials

2.1. Patients and materials

This study was performed in accordance with UMCG and Dutch national ethical guidelines and complies with the regulations stated in the *Declaration of Helsinki*. Informed consent was obtained for all patients referred to our clinical genetics laboratory. Peripheral blood was collected from patients as a routine diagnostic procedure. DNA was isolated using the Maxwell 16 DNA purification kit and a Maxwell rapid sample concentrator instrument (Promega, Madison WI, USA) following protocols provided by the manufacturer (AS1010, AS4500).

2.2. Disease criteria

Patients included in this study were referred to our clinical genetics laboratory for genetic testing between 2012 and 2017, with referral diagnoses for various types of cardiomyopathies and therefore do not necessarily meet all the criteria for the specific subtypes (DCM, ACM, HCM, LVNC, and RCM). Patients with cardiomyopathy symptoms, but insufficient to categorize to a specific subtype were labeled as unclassified cardiomyopathy (CM). Additional patients from the same family were excluded from our research unless: (1) they presented with a different phenotype from the relative already included, or (2) they had been shown *not* to carry the (likely) pathogenic variant previously detected in the proband from their family.

Details of the gene panel design is provided in Supplemental Table 1, criteria of genetic variant classification in Supplemental Table 2 and genes grouped per functional role in Supplemental Table 3. Variant filtering and classification flowchart are detailed in Supplemental Fig. 1, CNV decision tree in Supplemental Fig. 2 and genes in the targeted NGS panel implicated per cardiomyopathy subtype in Supplemental Fig. 3.

A comprehensive genetic analysis and methodology is available in the Supplemental file under the methods section.

3. Results

3.1. Cohort description

In total, 2002 patients from 1967 families (60.4% male and 39.6% female) were included in our study. Mean age for males and females combined was 56 (SD 16.5) years. Of the 2002 patients, 962 were referred for DCM, 590 for HCM, 115 for ACM, 98 for LVNC, 233 for CM and 4 for RCM.

3.2. Yield in routine clinical diagnostics of cardiomyopathy patients

The overall diagnostic yield using targeted gene panels for patients with cardiomyopathy referred to our laboratory was 21.5% (430/2002), following RDC (Table 1). When taking all patients into consideration (including patients carrying (L)P variants not explaining their phenotype), 13.1% (263/2002) carried one P variant, 9.0% (181/2002) carried one LP variant, 0.8% (15/2002) carried multiple (L)P variants, 39.1% (783/2002) carried one or more VUS and 38.0% (760/2002) carried no VUS/(L)P variant. Following RDC, a total of 74.5% (1419/1904) of total class 3–5 variants were VUS and a total of 25.5% (485/1904) were LP/P. The number of class 3–5 variants detected per gene is shown in Supplemental Table 4. Diagnostic yield per subtype following RDC ranged from a maximum of 28.0% for HCM to a minimum of 17.2% for unclassified CM, with DCM, ACM, LVNC and RCM at 19.3%, 17.4%, 18.4% and 25.0%, respectively (Fig. 1). Notably, founder mutations provided a genetic diagnosis in 35.5% (172/485; notably the founder mutation *PKP2* c.2386 T > C; p.(Cys796Arg) was found once in an HCM patient and not considered to explain that phenotype) of clinically solved cases.

A secondary finding, i.e., unrelated to the primary purpose of the tested cardiomyopathy panel, was detected in 0.2% (4/2002), all resulting from CNV analysis. Following quality control, 148 of the 2002 samples were discarded from CNV analyses. In the remaining 1854 samples, putative CNVs were initially reported in 34 cases. Of these, 50.0% (17/34) were confirmed and 26.5% (9/34) were not confirmed. Validation was inconclusive for 23.5% (8/34) samples, involving five putative *PLN* duplications and three putative *TTN* CNVs (two deletions and one duplication) (Supplemental Fig. 2 and Supplemental Table 5). Of the confirmed CNV calls, six were classified as LB, two as VUS, one as LP and four as P. Secondary findings ($N = 4$; 1 monosomy X, 2 males with an additional X chromosome and 1 mosaic trisomy 12) were not taken into consideration for determining diagnostic yield. CNV analyses resulted in three (*DSP* del exons 1–24, *RYR3* exon 3 and *PLN* del exon 1) additional diagnoses in patients previously not reported to carry any (L)P variant, following RDC (Fig. 1, Table 1, Supplemental Table 5). An (L)P SNV was also identified in the remaining two patients with an (L)P CNV, therefore the diagnostic yield did not increase by these two cases. CNV analyses increased the diagnostic yield by 0.1% (3/2002).

(L)P variants that did not explain the patient's phenotype were not included in our initial diagnostic yield calculation. However, if we would take these into account because they do provide a genetic diagnosis, in an additional 25 patients (10 DCM, 5 HCM, 6 LVNC and 4 ACM), 24 variants in 17 different genes that were classified as (L)P were identified (Table 1, Fig. 1). This would have increased the diagnostic yield by 1.1% (22/2002) (Table 1, Fig. 1). In the remaining three patients (one HCM and two LVNC), an (L)P variant in a gene associated to the subtype was already accounted for in the diagnostic yield (Supplemental Table 6).

Evaluation of diagnostic yield differences per cardiomyopathy subtype showed HCM patients having a statistically significant higher proportion of (L)P variants while DCM and CM patients had a statistically significant lower proportion of (L)P variants. No difference was detected for LVNC and ACM patients (Supplemental Fig. 4). Analyses of diagnostic yield with respect to sex and age categories showed no significant difference for sex or age (Supplemental Fig. 5 and 6). Interestingly, the average age of patients without an (L)P was significantly higher than patients with an (L)P (Supplemental Fig. 7).

3.3. Prevalence of genes affected in cardiomyopathy subtypes

Half of the diagnostic yield following RDC was contributed by (L)P variants in *MYBPC3* and *TTN*, representing 26% (126/485) and 22.9% (111/485) of total (L)P variants respectively. The prevalence of (L)P variants in genes per subtype is shown in Table 2 and the contribution of (L)P variants per gene in our total cohort is shown in Supplemental Fig. 8. Variants in *TTN* were most prevalent in the DCM subtype, with 40.8% of total (L)P variants detected in this gene, while variants in *MYBPC3* and *MYH7* were most prevalent in the HCM subtype, with

Table 1
Results of clinical diagnostics in cardiomyopathy patients.

Subtype	No. of patients	(A) RDC-classified SNV and indel variants			(B) CNV Analyses			(C) RDC-classified LP/P variants in genes not implicated in subtype	
		Patients with only VUS	LP/P explaining phenotype	Patients with no Variants	VUS	LP/P	Sec. Findings	Patient yield change	Patient no yield change
DCM	962	404	186	372	–	3	2	10	0
HCM	590	209	165	216	1	–	–	4	1
ACM	115	46	20	49	–	–	1	4	0
CM	233	101	40	92	–	–	–	–	–
RCM	4	2	1	1	–	–	–	–	–
LVNC	98	50	18	30	1	2	1	4	2
TOTAL	**2002**	**812**	**430**	**760**	**2**	**5**	**4**	**22**	**3**
Diagnostic Yield		430 (21.5%)			3 (0.1%)			22 (1.1%)	

DCM – dilated cardiomyopathy, HCM – hypertrophic cardiomyopathy, ACM - arrhythmogenic cardiomyopathy, LVNC – left ventricular non-compaction cardiomyopathy, RCM - restrictive cardiomyopathy.
Overview of class 3–5 variants (VUS – variant of uncertain significance, LP – likely pathogenic and P – pathogenic) identified in all patients per subtype and diagnostic yield calculations: A) following routine diagnostic criteria (RDC) for single nucleotide variants (SNVs) and indels in genes implicated per subtype, B) Copy number variation (CNV) analyses and C) class 4–5 SNVs and indels following RDC in genes not implicated in their cardiomyopathy (CM) subtype. Variants in patients classified as VUS or LP/P explaining phenotype may be one or more. An (L)P SNV was identified in 2 of the 5 patients in which a CNV was detected and therefore did not increase the diagnostic yield for these two cases. Of the 30 patients in which RDC-classified LP/P variants were detected in genes not implicated in subtype, 4 patients had an (L)P variant in a gene associated to the subtype and therefore were not accounted for diagnostic yield change.

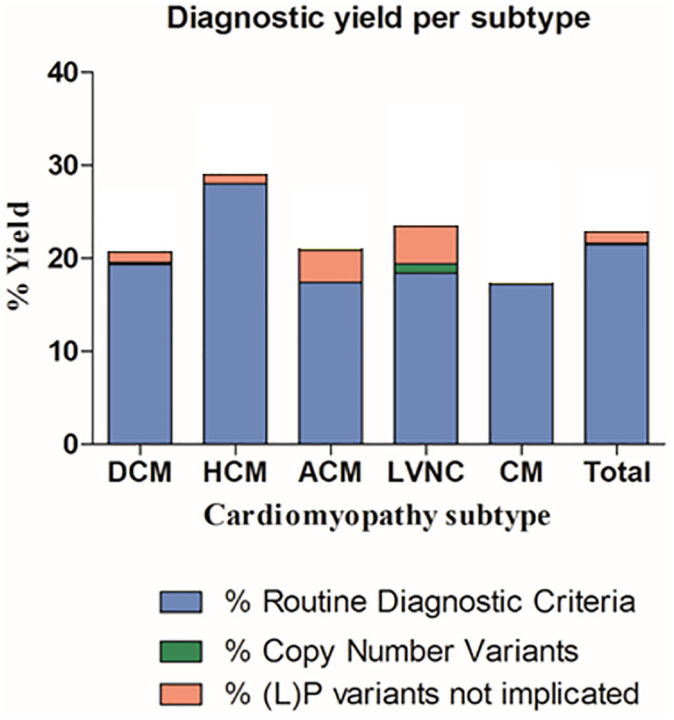

Fig. 1. Diagnostic yield for different cardiomyopathy subtypes. Blue: yield contributed by variants classified as (L)P according to RDC in genes implicated in the patient's subtype. Green: additional diagnostic yield contributed by CNVs (1 LVNC and 2 DCM patients). Pink: provisional increase in yield when including variants classified as (L)P according to RDC in genes not implicated in the patient's subtype.

60.5% and 14.1% of total (L)P variants detected in these genes, respectively. Variants in *PKP2* were most prevalent in the ACM subtype. *MYH7* and *TTN* contributed most (L)P variants for the LVNC subtype. In addition, (L)P variants in *PLN*, the p.(Arg14del) founder mutation in particular, were prevalent in CM, ACM and DCM, accounting for 26.8%, 14.8% and 10.8%, respectively, of total (L)P variants detected in these subtypes.

Table 2
Prevalence of variants classified as (L)P in genes per cardiomyopathy subtype.

DCM	Variants		HCM	Variants		CM	Variants	
	%	(L)P		%	(L)P		%	(L)P
TTN	40.8	87	*MYBPC3*	60.5	107	*PLN*	26.8	11
PLN	10.8	23	*MYH7*	14.1	25	*TTN*	26.8	11
LMNA	8.0	17	*TNNI3*	4.5	8	*DSP*	9.8	4
MYBPC3	6.6	14	*TTN*	4.5	8	*DES*	7.3	3
MYH7	5.6	12	*CSRP3*	3.4	6	*MYBPC3*	7.3	3
DSP	5.2	11	*TNNT2*	2.8	5	*LMNA*	4.9	2
MYPN	2.3	5	*MIB1*	1.7	3	*SCN5A*	4.9	2
SCN5A	2.3	5	*DSP*	1.1	2	*HCN4*	2.4	1
RBM20	1.9	4	*GLA*	1.1	2	*MIB1*	2.4	1
TNNI3	1.9	4	*LAMP2*	1.1	2	*RBM20*	2.4	1
MIB1	1.4	3	*DES*	0.6	1	*TMEM43*	2.4	1
MYL2	1.4	3	*FHL1*	0.6	1	*TNNT2*	2.4	1
BAG3	0.9	2	*MYL2*	0.6	1		100.0	41
DSC2	0.9	2	*MYL3*	0.6	1			
PKP2	0.9	2	*PKP2*	0.6	1	**LVNC**	**%**	**(L)P**
TNNC1	0.9	2	*PRKAG2*	0.6	1	*MYH7*	19.2	5
TNNT2	0.9	2	*SCN5A*	0.6	1	*TTN*	19.2	5
TPM1	0.9	2	*TCAP*	0.6	1	*DSP*	11.5	3
*47, XXY**	0.9	2	*VCL*	0.6	1	*DMD*	7.7	2
ACTC1	0.5	1		100.0	177	*MYBPC3*	7.7	2
CSRP3	0.5	1				*DES*	3.8	1
DMD	0.5	1	**ACM**	**%**	**(L)P**	*HCN4*	3.8	1
GLA	0.5	1	*PKP2*	48.1	13	*MYPN*	3.8	1
LAMP2	0.5	1	*PLN*	14.8	4	*PLN*	3.8	1
MYL3	0.5	1	*DSC2*	7.4	2	*RBM20*	3.8	1
PRKAG2	0.5	1	*CSRP3*	3.7	1	*RYR2*	3.8	1
TBX20	0.5	1	*DSP*	3.7	1	*SCN5A*	3.8	1
TTR	0.5	1	*LMNA*	3.7	1	*TAZ*	3.8	1
VCL	0.5	1	*MYH7*	3.7	1	*ACTN2*	3.8	1
PSEN1	0.5	1	*MYL2*	3.7	1		100.0	26
	100.0	213	*RBM20*	3.7	1			
			SCN5A	3.7	1	**RCM**	**%**	**(L)P**
			*45, X**	3.7	1	*DES*	100.0	1
				100.0	27			

(%) percentage that gene adds to (L)P. *represents chromosomal duplication or deletion of the X chromosome.

3.4. Genetic variant spectrum

From a total of 485 (L)P variants detected, we identified 234 unique variants in 37 genes. Of these, 79 (33.8%) were classified as P and the

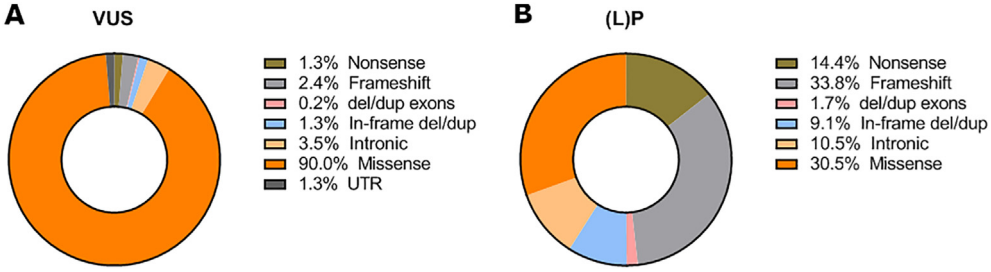

Fig. 2. Genetic variant spectrum. Contribution of different variant types to the VUS and (L)P variant categories. A) prevalence of variant types identified within the group of variants of uncertain significance and B) within the group of (likely) pathogenic variants.

remaining 155 (66.2%) as LP. Truncating/LoF variants had a higher impact on diagnostic yield, contributing 60.4% of total LP/P variants. The distribution of variants classified as VUS or (L)P within different mutational classes is shown in Fig. 2. Notably, missense variants are the largest contributor to the VUS group, contributing 90% of variants (Fig. 2), and this class constituted 75% of total variants detected. Variants in the UTR regions were minor contributors to the total VUSs detected (1%), as only a minor part (±20 bases) of 3′ and 5' UTR sequences were targeted.

3.5. Prevalence of functionally categorized genes contributing to diagnostic yield

We analyzed, per cardiomyopathy subtype, how (L)P variants in functionally assigned groups contributed to the diagnostic yield (as shown in Supplemental Fig. 9). Variants in the sarcomeric gene group were the largest contributors to yield in general, with 64% (L)P variants detected in that group. Most of these were in DCM, HCM and LVNC subtypes, accounting for 60%, 88% and 46% of the diagnoses, respectively. Genes in the desmosome group particularly impacted ACM, with 60% of (L)P variants identified in that functional group. In addition, variants in the ion channel/calcium handling gene group, in particular in *PLN*, significantly contributed to CM (34.1% of (L)P variants) and LVNC (15.4% of (L)P variants) subtypes, while variants in the nuclear gene group, in particular in *LMNA*, significantly contributed to DCM (9.9% of (L)P variants) and CM (9.8% of (L)P variants) subtypes.

4. Discussion

Diagnostic NGS panel testing in our 2002 cardiomyopathy patients demonstrated a yield of 21.5%, when using RDC, which is relatively low compared to the mostly subtype-specific and disease-criteria-fulfilling cohort studies published thus far. In contrast to these studies the patients in our center presented a broad spectrum of CM. Adding NGS-data-based CNV analyses to the SNV and (small) indel testing produced a minor increase in yield of 0.1%.

Results comparing diagnostic yield within cardiomyopathy subtypes in our cohort with those of studies using comparable NGS panels to analyze similar cardiomyopathy subtypes is shown in Supplemental Table 7. The lower diagnostic yield compared to studies in literature is most likely due to the fact that, within the daily practice of a routine diagnostic laboratory, all referred cases will be included using CM type reported by the cardiologist/clinical geneticist in the referral letter without knowing whether these patients indeed completely fulfill the diagnostic criteria for these cardiomyopathy subtypes. Moreover, standards and guidelines for variant interpretation and classification [18,19] may have been used differently in previous studies, as insights into variant classification have evolved over time and these differences could have resulted in an overestimation of overall diagnostic yield in past publications. Furthermore, our relatively low yield can also be partly explained by the fact that cardiomyopathy screening in the Netherlands has been available since 1996, and the pathogenic variants in many of the more severe cases were already identified by diagnostic screening in the pre-NGS era. Over time, more patients with unclear CM subtype diagnoses, i.e., unclear to the referring cardiologists, have been referred, often from relatively smaller families, which makes the interpretation of identified variants more difficult and thus leading to lower diagnostic yields [20].

Patients with HCM had a diagnostic yield of 28.0% (165/590). This is slightly lower than in previous report of 32% on an HCM cohort [8]. In patients with HCM, (likely) pathogenic variants in *MYBPC3* (60.5%) and *MYH7* (14.1%) contributed to 74.6% of positive cases. Our findings are in agreement with previous reports [21]. The high prevalence of mutations in *MYBPC3* in our cohort is also explained by the presence of Dutch *MYBPC3* founder mutations [22] as shown in Supplemental Table 8.

The diagnostic yield in patients with DCM was 19.3% (186/962). This is lower than in previous NGS studies in DCM cohorts, which ranged between 30 and 40% diagnostic yield [23,24]. Of the patients with DCM, the highest proportion of (likely) pathogenic variants listed were in the *TTN* gene: 9% (87/962). This is in agreement with previous reports [23,25,26]. In our cohort, we detected a total of 706 variants (575 unique) in *TTN* from a total of 1904 variants (37.1%). Of these, 111 variants (15.7%) contributed to a positive genetic diagnosis, with 95 classified as likely pathogenic and 16 as pathogenic. Another interesting finding within the DCM cases is the high prevalence of variants in the *PLN* and *MYBPC3* genes, with 10.8% and 6.6% of (likely) pathogenic variants identified respectively. Together these accounted for 3.6% of positive DCM cases, also largely explained by the prevalence of founder mutations: p.(Pro955Argfs*95), p.(Arg943*), p.(Trp792Val*fs**41), p.(Gln1259Arg*fs**72) and p.(Gly148Arg) in *MYBPC3* [24] and p.(Arg14del) in *PLN* [27].

We found an overall diagnostic yield of 17.4% (20/115) in ACM cases. This is low compared to previous studies, where yields between 26 and 63% were reported [14,28,29]. Our low yield could be explained by the high mean age of ACM patients in our cohort (mean = 50 years) since desmosomal/ACM associated gene mutations are associated with a younger onset of ACM [30]. As mentioned in general also above, most likely causal mutations in younger, more severe cases were already identified in the pre-NGS era. Of the (likely) pathogenic variants, 48.1% were detected in *PKP2* and 14.8% in *PLN*. Our findings are in agreement with reports describing a high prevalence of variants in *PKP2* accounting for 42–46% of ACM cases [14,31].

The diagnostic yield achieved for patients with LVNC, 18.4% (18/98), was lower than in previous reports, which found 27–32% yield [32,33]. Of the (likely) pathogenic variants detected, the *MYH7*/*TTN* (19.2% each), *DSP* (11.5%) and *MYBPC3*/*DMD* (7.7% each) genes showed the highest contribution. Our findings are comparable to reports by Takasaki et al., 2018 [32] and van Waning et al., 2018 [33] in that 63–71% of pathogenic variants were identified in the *MYH7*, *TTN* and *MYBPC3* genes for LVNC.

Only 15 out of 2002 patients (0.8%) were found to carry two (likely) pathogenic variants, suggesting a minor role of multiple mutations in genetic cardiomyopathies in this cohort. The phenotype in multiple variant carriers is generally believed to be more severe and/or to manifest at younger age. However, our group of multiple variant carriers is too small and therefore makes it difficult to draw any conclusions from their phenotypes.

Our findings reinforce previous suggestions to employ low thresholds for offering genetic testing in CM, given that different population cohorts have substantial variation in gene/(L)P variants prevalence [34,35]. In addition, variants in genes not implicated for the cardiomyopathy subtype were found in 25 patients (1.25%). Given the diverse and overlapping genotype heterogeneity, variable morphological phenotypes and the growing number of genes linked to different cardiomyopathy subtypes, only additional evidence will determine whether these variants actually have a previously undocumented role in the disease phenotypes of cardiomyopathy subtypes.

The prevalence of CNVs in our cardiomyopathy patients was low, 0.92% (17/1854), of which nine were classified as (L)P (including secondary findings). This is consistent with a previous report [16] identifying 0.63% (9/1425) of patients having a CNV. Moreover, a low rate of CNV's in cardiomyopathies was recently also confirmed in another study [36]. Nonetheless, as these kinds of variants can be deduced from available NGS panel data, this could be a valuable addition to regular variant detection and should be considered to be performed as part of the routine genetic testing. Although founder CNV mutations such as *RYR2* exon 3 deletion and *PLN* duplication have been previously reported [37,38], there are limited reports available on identifying CNVs. Our understanding of the role of these variants in causing cardiomyopathies is limited compared to that of the roles of SNVs and small indels. Follow-up studies on CNVs are therefore recommended to further evaluate their role and mechanism.

Categorizing (L)P variants with respect to the functional role of genes demonstrated that sarcomeric gene variants affect a majority of DCM (60%), HCM (88%) and LVNC (46%) cases, with an overall effect in 64% of total positive cases. These results are similar to previous findings [21,25,32,33,39] and reinforce the value of a greater emphasis on targeting new therapies for sarcomeric cardiomyopathies, that may delay onset and limit disease progression [40]. In ACM patients, 60% of (L)P variants were found in desmosomal genes, also endorsing earlier findings [14].

Finally, no (L)P CM-associated variant was found in more than 3 out of 4 of our patients. There are several reasons to explain this: (1) the cause of disease is not genetic (2) variants identified as VUS may be reclassified as pathogenic or likely pathogenic in the future; (3) variants lie in regulatory regions of currently screened genes or in genes currently not included in our gene panel; and (4) the underlying cause is oligogenic and difficult to deduce from gene-panel based NGS analyses. Other strategies have to be used to identify the cause of disease in those patients, including whole exome, whole genome or RNA sequencing, linkage(−like) methodologies, methods to identify regulatory variants, etc.

4.1. Limitations

We have only screened for variants in coding regions and surrounding regions of interest, ±20 bases, in ~60 selected cardiomyopathy genes. Deep intronic or regulatory (5'and 3'UTR) variants and variants in novel genes with potential functional roles in cardiomyopathies were not included. For example, only relatively recently reports emerged showing a role of truncating *FLNC* mutations in DCM in particular [41]. We therefore recently included this gene in our cardiomyopathy panel. In addition, patient phenotypic information was obtained from referral forms and may have been limited. This could have affected disease classification and may have made variant interpretation in some cases less optimal.

5. Conclusion

In cardiomyopathy patients referred for clinical genetic testing, a pathogenic or likely pathogenic gene variant was detected in 22.7% of cases, diagnostic yield, i.e. (L)P variant in genes with known association with the patients CM subtypes, was 21.5%. A limited number of patients (0.1%) are diagnosed through CNV analysis. However, as this information can be deduced from existing NGS data, CNV analysis should be considered to be part of routine clinical genetic testing in cardiomyopathy patients. In 25 patients a (likely) pathogenic variant was identified in a gene currently not associated with the patient's cardiomyopathy subtype. The yield of routine clinical diagnostics of cardiomyopathies was relatively low when compared to literature. This is most likely due to the fact that our study reports the outcome of patients in daily routine diagnostics, therefore also including patients not fully fulfilling (subtype specific) cardiomyopathy criteria. This may also explain why (likely) pathogenic variants were identified in genes not associated with the reported but not always perfectly matching subtype.

Author contribution

M.Z.A, H.W., R.J.S, R.H.S., B. S-R, J.D.H.J. and Pvd.Z. contributed to conceptualization and project administration and supervision; M.Z.A., L.F.J., A.P., L.G.B., K.K. v D., L.W., Y.J.V., Y.M.H., J.D.H.J. and P.vd.Z. contributed to data curation, formal analysis investigation and methodology; M.Z.A, L.F.J, B. S-R, J.D.H.J. and Pvd.Z. contributed to data validation and visualization M.Z.A, L.F.J, H.W, B.S-R, J.D.H.J. and P.vd.Z. contributed to writing – original draft, all authors contributed to review and editing critically for important intellectual content; and all authors gave final approval of the version to be published.

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Disclosures

The authors report no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijcard.2021.02.069.

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