Modern genomic techniques in the identification of genetic causes of cardiomyopathy

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ABSTRACT
Over the past three decades numerous disease-causing genes have been linked to the pathogenesis of heritable cardiomyopathies, but many causal genes are yet to be identified. Next-generation sequencing (NGS) platforms have revolutionised clinical testing capacity in familial cardiomyopathy. In this review, we summarise how NGS technologies have advanced our understanding of genetic non-syndromic cardiomyopathy over the last decade. First, 26 putative new disease-causing genes have been identified to date, mostly from whole-exome sequencing, and some of which (FLNC, MTO1, HCN4) have had a considerable clinical impact and are now included in routine diagnostic gene panels. Second, we consider challenges in variant interpretation and the importance of large-scale NGS population control cohorts for this purpose. Third, an emerging role of common variation in some forms of genetic cardiomyopathy is being elucidated through recent studies which have illustrated an additive effect of numerous polygenic loci on cardiac parameters; this may explain phenotypic variability and low rates of genetic diagnosis from sequencing studies. Finally, we discuss the clinical utility of genetic testing in cardiomyopathy in Western settings, where NGS includes targeted sequencing of candidate genes alongside genes not previously associated with cardiomyopathy. Advantages of targeted sequencing are that NGS panels are easily customisable and high accuracy can be achieved. Disadvantages are that targeted sequencing is unsuitable for novel gene discovery, and intronic and promoter regions are typically excluded. WES is a technique in which the protein-coding regions of ~25,000 genes are sequenced, allowing investigation of a ‘virtual panel’ of known disease genes alongside genes not previously associated with cardiomyopathy. An advantage of WGS over WES is substantially lower size of data generation as the exome is only around 1% of the genome’s size. Limitations include heterogeneity in capture efficiency, exclusion of intronic and promoter regions, and the difficulty of assessing variation in regulatory regions. This means that variation in regulatory regions can be assessed, although challenges in the interpretation of non-coding variants remain considerable. Coverage of exons tends to be more uniform in WGS compared with WES, and in other diseases WGS has proven more sensitive in the detection of rare exonic variants, although neither WES nor WGS entirely covers their genomic targets.

INTRODUCTION
Inherited cardiomyopathies are genetically heterogenous disorders of the heart muscle which may be classified morphologically and functionally into dilated (DCM) (table 1), hypertrophic (HCM), restrictive (RCM) and arrhythmogenic (ACM) cardiomyopathy. Left ventricular non-compaction cardiomyopathy (LVNC) and overlapping syndromes remain unclassified in this schema (figure 1). Beyond Morphofunctional phenotype, the MOGE(S) classification endorsed by the World Heart Federation in 2013 incorporates Organ involvement, Genetic inheritance, Etiology (specific gene variant) and State of cardiac function (New York Heart Association classification) where relevant. This framework helpfully integrates genetic information into the clinical management of cardiomyopathy.

Over the past three decades, numerous genes have been linked to aetiology/pathogenesis, chiefly using traditional Sanger-based sequencing of candidate genes. However, as many as 60% of patients with familial cardiomyopathy lack a conclusive genetic diagnosis. Next-generation sequencing (NGS) refers to massively parallel, non-Sanger-based, high-throughput DNA sequencing. Through NGS, novel variants and genes for cardiomyopathy have been discovered. NGS includes targeted sequencing of known or candidate disease genes, whole-exome sequencing (WES) and whole-genome sequencing (WGS) (figure 2).

Targeted sequencing refers to the application of NGS to known disease-associated genes. Advantages of targeted sequencing are that NGS panels are easily customisable and high accuracy can be achieved. Disadvantages are that targeted sequencing is unsuitable for novel gene discovery, and intronic and promoter regions are typically excluded. WES is a technique in which the protein-coding regions of ~25,000 genes are sequenced, allowing investigation of a ‘virtual panel’ of known disease genes alongside genes not previously associated with cardiomyopathy. An advantage of WGS over WES is substantially lower size of data generation as the exome is only around 1% of the genome’s size. Limitations include heterogeneity in capture efficiency, exclusion of intronic and promoter regions, and the difficulty of assessing genes with an unknown role in disease. WGS is a technique which enables unbiased sequencing of all genes and regulatory regions, including variation in intronic and intergenic regions. This means that variation in regulatory regions can be assessed, although challenges in the interpretation of non-coding variants remain considerable. Coverage of exons tends to be more uniform in WGS compared with WES, and in other diseases WGS has proven more sensitive in the detection of rare exonic variants, although neither WES nor WGS entirely covers their genomic targets.

In this review, we summarise how each of these NGS technologies has advanced our understanding of genetic non-syndromic cardiomyopathy in the last decade. We explore the emerging role of common variation in some forms of cardiomyopathy, the importance of large-scale NGS population control cohorts in variant interpretation and the clinical implications of genetic testing in cardiomyopathy.
for approximately 40% of patients with familial DCM. Several genes have been found in patients with biventricular dysfunction that cause left ventricular hypertrophy, are identified as this alters disease progression and clinical management.

LVNC is a form of cardiomyopathy in which the left ventricle has prominent trabeculations and a non-compacted layer of the myocardium, which can lead to heart failure, thromboembolisation, arrhythmias and SCD. The prevalence of LVNC is unknown, partly due to differing diagnostic criteria and phenotypic overlap with sarcomeric cardiomyopathy and congenital heart defects. Similar to DCM, LVNC has a heterogeneous genetic basis, with variants in cytoskeletal, ion channel, sarcomeric and nuclear membrane genes (table 2).

ACM is an arrhythmogenic heart muscle disorder not explained by ischaemic, hypertensive or valvular heart disease. It may present clinically as atrial fibrillation, conduction disease and/or arrhythmia arising from the left or right ventricles. ACM includes arrhythmogenic right ventricular cardiomyopathy (ARVC) and left-sided arrhythmogenic cardiomyopathy.

ARVC is the most genetically well-characterised phenotype, where causative variants occur in genes that encode components of the cardiac desmosome, with impaired electrical and mechanical stability of the myocardial tissue underlying pathogenesis (table 2). Genetic causes of ARVC have not been identified in up to 50% of cases. Several pathogenic variants in typical ACM genes have been found in patients with biventricular dysfunction and high risk of SCD.

RCM, thought to be the least common familial cardiomyopathy, is a rare disorder characterised by abnormal relaxation of the myocardium, while chamber size and systolic function usually remain normal, resulting in rapid increases in ventricular pressures. Impaired filling of the ventricles can lead to arrhythmias and symptoms of heart failure. It often overlaps with other phenotypes, particularly HCM, where many of the described causal genes are shared, although with poorer prognosis (table 2).

TARGETED SEQUENCING IN CARDIOMYOPATHY

Targeted sequencing has enabled diagnostic laboratories to configure gene panels that encompass multiple overlapping clinical entities, bringing genetic testing to routine use in clinical medicine. Exclusion of intronic and promoter regions may prove disadvantageous in the future, as variants in these regions may make important contributions to cardiomyopathy. For example, a recently discovered intronic variant in MYBPC3 may account for as much as 1% of HCM.
Panel size is a matter of some contention, and there is no clear consensus on whether larger cardiac panels are more useful than cardiomyopathy-specific panels or if they merely increase the number of unclear genetic findings. A recent UK study explored the added value of screening 51 non-sarcomeric candidate genes for HCM in 240 patients with HCM negative for NGS and identified only one additional variant. The incremental diagnostic yield of more extensive testing was thus determined to be negligible in HCM in this setting and would, in practice, be outweighed by increased cost and complexity, as well as the problems with reporting uninterpretable findings to patients. Similar findings have been described in a Dutch DCM study, where extended gene panels yielded more variation of uncertain significance (VUS) without a corresponding increase in clinically relevant variants.

Figure 2  Comparison of targeted sequencing, whole-exome sequencing and whole-genome sequencing. DCM, dilated cardiomyopathy.  

In a discrete choice experiment, UK practitioners demonstrated a strong preference for panel tests over WES or WGS. Key drivers of choice were the potential for higher yield, less VUS and lower cost, indicating a perception in the clinical context that panel sequencing offers better value for money than other types of NGS. However, when presented with an alternative of no testing at all, the preference was for NGS testing regardless of the type.

### WES in Cardiomyopathy

WES has been used to identify 24 putative new disease genes for genetic cardiomyopathies (table 3 and online supplemental table 1). Of these, 11 have been identified as causes of disease in more than one unrelated family (online supplemental tables 1–8). The muscle filamin gene FLNC in particular has emerged

<table>
<thead>
<tr>
<th>Cardiomyopathy subtype</th>
<th>Typically recommended gene panel</th>
<th>Selected clinical implications</th>
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<tbody>
<tr>
<td>ACM</td>
<td>Desmosomal genes (ARVC; DSC2, DSG2, DSP, JUP, PKP2). Other genes (biventricular/ALVC/ARVC; BAG3, DES, FLNC, LDB3, LMNA, NIK2-5, PLN, RBM20, SCNSA, TMEM43). New genes from NGS studies: consider including CDH2 and TJP1.</td>
<td>► Identification of desmosomal gene variant will confirm ARVC, which may be exacerbated by exercise.</td>
</tr>
<tr>
<td>DCM</td>
<td>19 high-confidence genes (much larger gene panels are available): ACTC1, ACTN2, BAG3, DES, DSP, FLNC, JPH2, LMNA, MYH7, NEXN, PLN, RBM20, SCNSA, TNNI3, TNNC1, TNNT2, TPM1, TTN, VCL. New genes from NGS studies: consider including ALPK3.</td>
<td>► FLNC, LMNA and DSP variant carriers are at higher risk of SCD and end-stage heart failure.</td>
</tr>
<tr>
<td>HCM</td>
<td>Sarcomeric genes: ACTC1, MYBPC3, MYH7, MYL2, MYL3, TNNI2, TNNI3, TPM1. Phenopecopies: GAA, GLA, LAMP2, PRKAG2, PTEN11, RAF1, TTR. New genes from NGS studies: consider including ALPK3 and FLNC.</td>
<td>► Identification of desmosomal gene variant will confirm ARVC, which may be exacerbated by exercise.</td>
</tr>
<tr>
<td>RCM</td>
<td>TTR + DCM/HCM genes. New genes from NGS studies: consider including FLNC.</td>
<td>► TTR variants may increase risk of severe heart failure in RCM.</td>
</tr>
<tr>
<td>LVNC</td>
<td>Varies, typically DCM/HCM panel, cardiac transcription factors NIK2-5 and TBNX. New genes from NGS studies: consider including FLNC and HCM4.</td>
<td>► Transthyretin amyloidosis caused by TTR variants may require different treatment.</td>
</tr>
<tr>
<td>Paediatric cardiomyopathy</td>
<td>Varies by subtype.</td>
<td>► Identification of underlying metabolic or syndromic causes of cardiomyopathy can inform diagnosis, prognosis and patient management.</td>
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HCN4 was linked to LVNC with sinus bradycardia\textsuperscript{27} and subse-
dand familial HCM,\textsuperscript{26} while the pace-
has been associated with both sporadic
chondrial gene

4 Spracklen TF, 2022; 0:1–8. doi:10.1136/heartjnl-2021-320424

WGS IN CARDIOMYOPATHY

Few WGS cardiomyopathy studies were found in the literature, presumably due to cost and the current bioinformatic burden relating to data storage and analysis. Two novel cardiomyopathy genes, MYBPHL and PP1R13L, have been identified through WGS in familial DCM,\textsuperscript{34} 35 but pathogenic variation in these genes has not been described in cardiomyopathy since their original report.

LARGE-SCALE POPULATION CONTROL COHORTS IN VARIANT INTERPRETATION

One of the early benefits of NGS in the genetic diagnosis of cardiomyopathy has, perhaps paradoxically, accrued from sequencing of large populations in studies exploring common complex diseases. Aggregation of these genetic sequences in a readily available format in the Exome Aggregation Consortium (ExAC) database, and later Genome Aggregation Database (gnomAD), has had a major impact on clinical variant interpretation in many areas of clinical genetics.\textsuperscript{36}

Because human population history has involved population bottlenecks followed by very rapid population expansion, the human genome contains numerous rare variants—the overwhelming majority of which are benign. Early genetic studies using hundreds of healthy controls set the bar much too low; tens of thousands of healthy controls are needed to have confidence that a rare variant present in a case family and absent in controls was likely pathogenic. The total number of sequences in the combined ExAC and gnomAD databases now exceeds 140,000. Although these cohorts may have been minimally phenotyped, these data have become a routine part of variant interpretation in clinical genetics with astonishing speed and clearly illustrate that the standard of proof for a variant in a new gene to be considered causal has, in some instances, been too low.\textsuperscript{2}

AN EMERGING FIELD: COMMON VARIATION IN GENETIC CARDIOMYOPATHY

The influence of rare, deleterious variants in cardiomyopathy has been emphasised by panel sequencing in affected individuals. However, common variation has long been suspected to play a role, particularly in the development of more frequent forms of cardiomyopathy (DCM and HCM) due to unresolved issues including phenotypic variability among carriers of the same disease-causing variant and the limited diagnostic yields of panel sequencing. NGS studies conducted to date indicate that even when new disease genes are identified in cardiomyopathy, they are unlikely to account for a substantial proportion of genotype-negative patients.

Genome-wide association study (GWAS) is a technique which involves genotyping of a fixed set of common variants (typical allele frequency >1%) known as single-nucleotide polymorphisms (SNPs). SNP arrays typically comprise hundreds of thousands of variants across the human genome. Imputation can be

| Table 3 Putative new cardiomyopathy genes identified through next-generation sequencing |
|---------------------------------|----------------|-----------------|-----------------|
| **Gene** | **Associated cardiomyopathy** | **Number of variants (number of probands)** | **Variant type(s)** | **Inheritance patterns** |
|----------------|----------------|----------------|----------------|
| ALPK3 | DCM (p), HCM (p) | 25 (17) | Truncating | AR, CH, AD |
| ASNA1 | DCM (p) | 2 (1) | Truncating, missense | CH |
| CDH2 | ACM | 8 (8) | Missense | AD |
| FKX102 | DCM (p) | 1 (1) | Missense | AR |
| FLNC | ACM, DCM, HCM, LVNC, RCM >70 (>70) | Truncating, missense | AD, CH, DN |
| GATA4 | DCM | 1 (1) | Missense | AR |
| HCN4 | LVNC | 8 (8) | Truncating, missense | AD |
| KCNJ12 | DCM | 1 (1) | Inframe deletion | |
| KLF20a | RCM (p) | 2 (1) | Truncating, missense | CH |
| mto1 | HCM (p) | 25 (16) | Truncating, missense | AR, CH |
| MYBPHL | DCM | 1 (1) | Truncating | AD |
| nnt | LVNC | 2 (2) | Truncating, missense | AD |
| NRAP | DCM | 17 (12) | Missense | AR |
| PLEXHM2 | DCM (p) | 1 (1) | Truncating | AR |
| PCS | DCM (p) | 3 (2) | Truncating, missense | AR |
| PP1R13L | DCM (p) | 7 (5) | Truncating, missense | AR, CH |
| PP3R1 | DCM | 1 (1) | Missense | AD |
| ptten | LVNC | 1 (1) | Missense | Digenic |
| sox2 | DCM (p) | 1 (1) | Missense | AR |
| SPEG | DCM (p) | 1 (1) | Missense | AR |
| SYNM | DCM | 1 (1) | Missense | AD |
| taf7IA | DCM (p) | 2 (1) | Missense | CH |
| TJP1 | ACM | 4 (4) | Missense | AD |
| TMEM87B | RCM (p) | 1 (1) | Missense | Hemizygous |
| SMYD1 | HCM, DCM (p), LVNC | 3 (3) | Truncating, missense | AR, DN |
| XIRP2 | DCM (p) | 2 (1) | Truncating, missense | Modifier |

*These variants were identified by whole-genome sequencing; all others were by whole-exome sequencing.

ACM, arrhythmogenic cardiomyopathy; AD, autosomal dominant; AR, autosomal recessive; CH, compound heterozygous; DCM, dilated cardiomyopathy; DN, de novo; HCM, hypertrophic cardiomyopathy; LVNC, left ventricular non-compaction; p, paediatric disease; RCM, restrictive cardiomyopathy.

as an important cause of genetic cardiomyopathy,\textsuperscript{24} with loss-of-
function variants identified in 1%–4.5% of patients with DCM and 3.2%–3.3% of patients with ACM, and missense variants occurring in 1.3%–9.7% of HCM.\textsuperscript{24} The autosomal mitoch-
donadal gene MTO1 has been associated with both sporadic
and familial HCM,\textsuperscript{26} while the pace-making ion channel gene
HCN4 was linked to LVNC with sinus bradycardia\textsuperscript{27} and subse-
quentl identified as the cause of disease in several other famil-
ies. Pathogenic CDH2 and TJP1 missense variants in various
ACM families indicate that the pathogenesis of adhesion-related cardiomyopathy may extend beyond the cardiac desmo-
some,\textsuperscript{28} 29 as both genes encode protein components of a type of
cell junction present only in the heart intercalated disc—the area

composita—comprising both desmosomal cadherins and N-cad-
herins. Screening of 500 patients recently showed that CDH2 variants account for up to 1.2% of ACM.\textsuperscript{30}

Despite familial DCM being inherited in a typically dominant pattern, all but 4 of the 16 putative new DCM genes were iden-
tified in families with autosomal recessive disease and most were
reported in paediatric cardiomyopathies. Most important among
these were ALPK3, NRAP and PCS, variants which have been
described in several Arabic and European families with early-
onset recessive cardiomyopathy.\textsuperscript{31–33}
used to call non-genotyped variants of lower allele frequency (currently as low as 0.1%) using population-level sequence data. At present, however, imputation cannot be used to infer pathogenic variants in cardiomyopathy genes, as these would typically have much lower allele frequencies than can be reliably imputed. Due to the large number of variants genotyped, creating multiple testing issues, several thousand unrelated cases and healthy controls are usually required for robust GWAS in order to achieve statistical significance levels that are both theoretically supported and have empirically been shown to deliver reproducible results (typically $p<5 \times 10^{-8}$). The bioinformatic and statistical genetic analysis pipelines for GWAS are well established. By comparing variant frequencies in cases and controls, GWAS may enable identification of SNPs that associate with common traits, rare disorders or protection from disease.

Two recent GWAS have demonstrated that common variants associate with cardiac parameters and cardiomyopathy. First, a GWAS by Pirruccello et al identified 57 genome-wide significant loci in association with cardiac MRI traits among 36,041 UK Biobank participants, of which 28 SNPs associated with indexed left ventricular end-systolic volume. A 28-SNP genetic risk score was found to associate with the risk of incident DCM among 362,922 participants who were disease-free at enrolment, 388 of whom subsequently developed DCM. Second, Harper et al conducted a GWAS of 2780 patients with HCM from the HCM Registry and Bioresource Rare Disease Study (BRRD) and 47,486 controls from the UK Biobank and BRRD, from which 12 genome-wide significant loci were identified. This information was similarly used to generate a genetic risk score comprising genotypes at the 12 risk SNPs, which associated with the odds of HCM both variant-positive and variant-negative patients in a meta-analysis of three replication cohorts. In both studies, the proportions of non-European participants were small and future research may involve determining the applicability of these polygenic susceptibility scores in additional populations; however, the 12-SNP HCM score was shown to have comparable results when the cohort was divided according to participant ethnicity.

Despite GWAS predating NGS by several years, previous GWAS in cardiomyopathy were limited by small sample sizes. These recent studies, however, demonstrate for the first time how multiple common genetic variants can influence susceptibility to cardiomyopathies previously thought to be Mendelian and the valuable insights that can be gleaned by genotyping both variant-positive and variant-negative patients in a meta-analysis of three replication cohorts. In both studies, the proportions of non-European participants were small and future research may involve determining the applicability of these polygenic susceptibility scores in additional populations; however, the 12-SNP HCM score was shown to have comparable results when the cohort was divided according to participant ethnicity.

CLINICAL IMPLICATIONS FOR GENETIC TESTING IN CARDIOMYOPATHY

The application of NGS to large cardiomyopathy cohorts has emphasised that a genetic cause of disease may be identified in at least a subset of patients with cardiomyopathy. Consequently, panel testing of core cardiomyopathy genes is now recommended in many settings on clinical suspicion or confirmation of a non-syndromic cardiomyopathy (figure 3)—this includes sporadic and familial forms of HCM and ACM, where clear clinical benefits of a genetic result have been described, while in DCM and other forms of cardiomyopathy genetic testing is usually limited to familial instances of disease or those with features of a genetic disorder. In all cases, genetic testing may be prioritised in patients with family members who could benefit from cascade genetic testing, patients with conduction defects or family history of SCD, or patients with an age of disease onset below 50 years. As WES and WGS become more accessible, these techniques may be preferable as NGS data can be stored and re-evaluated as new disease genes are reported.

Depending on the type of cardiomyopathy and the nature of the genetic test, between 30% and 60% of patients may be expected to carry a pathogenic variant that underlies their disease. Figure 4 illustrates the expected variant yields from panel testing and potential implications of a positive result for each genetic cardiomyopathy.

A key point from this review is that genetic causes of cardiomyopathy can be identified outside the core gene panels, occurring either as deleterious variants in potential new cardiomyopathy genes or as accumulated polygenic risk. When panel testing yields no result or a VUS, WES or WGS should be considered in cases of clearly familial disease (>2 similarly affected family members) or severe paediatric disease. WGS is currently recommended for paediatric cardiomyopathy in the UK. The clinical utility of polygenic risk assessment needs to be determined for unexplained DCM and HCM; these are important outstanding research questions.

Another consideration is the need for WES/WGS in patients of non-European ancestry due to the gaps in genetic studies in these populations. Patient and control genetic data from other populations will be fundamental in unravelling disease-causing sequence variants from benign variants, especially those that appear rare due to minimal sequencing of under-represented populations. Such information may also guide variant interpretation in European patients.

Genetic counsellors (GCs) play an important role in helping patients understand their genetic results and the potential consequences for themselves as well as their families. In addition to ensuring understanding of the genetic variant(s) identified, or lack thereof, GCs are uniquely placed to help individuals and their families make sense of the clinical and genetic information related to cardiomyopathies. By integrating the collection of accurate family history with personal experiences of diagnosis, they provide information about cardiomyopathy and the related complex genetic concepts in a way that assists individuals to cope with their diagnosis or the diagnosis of a family member.

In the era of WGS and WES, accurate family information is vital for variant interpretation and prioritisation. The roles of GCs in cardiomyopathy service provision include facilitating family communication, cascade screening and/or testing and predictive testing through the family. Through these processes they discuss important concepts such as variable expression, reduced penetrance, ways in which to manage risk and relevant lifestyle factors, potential insurance discrimination, and the options related to the risks for offspring, such as prenatal testing and preimplantation genetic diagnosis.

As the use of sequencing data in medical genetics is contingent on the ability to distinguish pathogenic from benign variants, NGS has also created new diagnostic challenges because of the...
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Figure 3  Recommendations for how genetic testing may be included in management of patients with cardiomyopathy. ACMG, American College of Medical Genetics and Genomics; gnomAD, Genome Aggregation Database; VUS, variant of uncertain significance; WES, whole-exome sequencing; WGS, whole-genome sequencing.
Figure 4  Expected yields of panel sequencing and potential clinical implications of a positive result. ACM, arrhythmogenic cardiomyopathy; BP, blood pressure; CMO, cardiomyopathy; DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; LVNC, left ventricular non-compaction; NGS, next-generation sequencing; RCM, restrictive cardiomyopathy; WES, whole-exome sequencing; WGS, whole-genome sequencing.

FUTURE DIRECTIONS
Genomic techniques have advanced our understanding of genetic cardiomyopathy through large-scale genetic testing of patient cohorts, identification of new disease genes and renewed appreciation of the role of common variants in pathogenesis. These techniques, however, also suggest many future avenues for research. The role of polygenic susceptibility scores in DCM and HCM needs further investigation—for example, the potential consequences on prognostication, patient and family management, and modified expression of known disease-causing variants. GWAS may now be warranted in other forms of cardiomyopathy such as ACM. In addition to common variants, the contributions of non-coding variation warrant further investigation. Future work may also seek to clarify the disease-causing potential of the 26 new genes described here, through refined functional assays or large-scale cohort sequencing studies.

CONCLUSION
NGS has proved of clear value through the elucidation of new disease genes, although this has been primarily in paediatric or recessive cardiomyopathy. At least 26 putative new cardiomyopathy genes were reported between 2011 and 2021, 24 of which were from WES experiments. HCN4 and FLNC now often appear on cardiomyopathy panels for diagnostic screening, demonstrating the potential power of WES. While NGS technologies have been used to successfully identify numerous novel cardiomyopathy genes, a large portion of the genetic predisposition to cardiomyopathies still needs to be accounted for. Furthermore, substantial phenotypic variability is often observed and unexplained. This may be due to genetic modifiers, a detailed discussion of which is beyond the scope of this review, although recent evidence strongly supports a role for common variation in disease susceptibility. As new genes are discovered, studies placing pathogenic variants in a broader context with transcriptomics, proteomics and epigenomics data are likely to be required to confirm the putative pathogenic nature of these variants.

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