Determining the Likelihood of Disease Pathogenicity Among Incidentally Identified Genetic Variants in Rare Dilated Cardiomyopathy-Associated Genes

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BACKGROUND: As utilization of clinical exome sequencing (ES) has expanded, criteria for evaluating the diagnostic weight of incidentally identified variants are critical to guide clinicians and researchers. This is particularly important in genes associated with dilated cardiomyopathy (DCM), which can cause heart failure and sudden death. We sought to compare the frequency and distribution of incidentally identified variants in DCM-associated genes between a clinical referral cohort with those in control and known case cohorts to determine the likelihood of pathogenicity among those undergoing genetic testing for non-DCM indications.

METHODS AND RESULTS: A total of 39 rare, non-TTN DCM-associated genes were identified and evaluated from a clinical ES testing referral cohort (n=14,005, Baylor Genetic Laboratories) and compared with a DCM case cohort (n=9,442) as well as a control cohort of population variants (n=141,456) derived from the gnomAD database. Variant frequencies in each cohort were compared. Signal-to-noise ratios were calculated comparing the DCM and ES cohort with the gnomAD cohort. The likely pathogenic/pathogenic variant yield in the DCM cohort (8.2%) was significantly higher than in the ES cohort (1.9%). Based on signal-to-noise and correlation analysis, incidental variants found in FLNC, RBM20, MYH6, DSP, ABCC9, JPH2, and NEXN had the greatest chance of being DCM-associated.

CONCLUSIONS: The distribution of pathogenic variants between the ES cohort and the DCM case cohort was gene specific, and variants found in the ES cohort were similar to variants found in the control cohort. Incidentally identified variants in specific genes are more associated with DCM than others.

Key Words: dilated cardiomyopathy | exome sequencing | genetics | incidental finding | secondary finding

Nonischemic dilated cardiomyopathy (DCM) is a primary disease of the myocardium characterized by left ventricular dilation and contractile dysfunction in the absence of coronary artery disease or abnormal loading conditions. DCM is estimated to occur in 1 in 2500 individuals with a higher incidence in men for the European and American populations. The primary clinical manifestations of DCM include heart failure, arrhythmia, cardiac conduction abnormalities, and sudden cardiac death. The development of non-ischemic DCM is believed to be the result of an interplay between genetics and environmental insults. Indeed, pathogenic variants are identified in ~35% of nonischemic DCM cases, with pathogenic variants identified in ~40% of familial DCM cases and in ~60% of pediatric DCM cases. Pathogenic DCM variants occur in genes...
that encode a variety of proteins, including cytoskeletal, sarcomere, nuclear envelope, mitochondrial, and RNA-binding proteins. For example, variants of the sarcomere genes MYH7, TNNT2, and ACTC1, which encode components of the thick and thin filament contractile sarcomere proteins, are associated with DCM.9

In the rapidly developing field of genomic medicine, exome sequencing (ES) demonstrates multiple advantages for facilitating the diagnosis of rare Mendelian disorders, with a higher molecular diagnostic yield and a lower commercial cost compared with other high-throughput sequencing techniques such as genome sequencing (GS). ES provides a highly effective way to sequence all protein coding exons of the human genome and can identify genetic causes of disease, enabling both the identification of novel genetic loci for single gene diseases, which may be clinically difficult to diagnose, and of diseases in which multiple genes may be causative.10 However, the large number of genes analyzed, combined with a growing number of individuals sequenced for a variety of indications, has led to a dramatic increase in the number of incidentally identified variants, which can pose a diagnostic dilemma when these are found in known disease-associated genes among individuals with no pretest suspicion for the disease in question.11 In fact, we have previously shown that the burden incidental variants found in cardiac channelopathy and cardiomyopathy disease associated genes far outweighs the population frequencies of these diseases.12 To address this, in 2015, the American College of Medical Genetics and Genomics (ACMG) recommended reporting only likely pathogenic/pathogenic (LP/P) variants found in 59 genes believed to be clinically actionable—so-called secondary findings.13,14 While some DCM-associated genes are included in this ACMG-59 list, many of the rare DCM-associated genes are not included in the list. Moreover, the list is evolving, and the 2021 revision of the ACMG-59 added 2 additional DCM-associated genes.15,16 This highlights the need to understand the prevalence of incidentally identified variants in rare DCM-associated genes and to identify which genes, if any, have a higher diagnostic yield.

We have previously found that variants in TTN-encoded titin, the most common genetic cause of DCM, had a relatively high prevalence of being found incidentally.17 These variants were similar in class and overall frequency to rare population variation, and our findings suggest that variant location along the primary sequence may be informative in determining the probability of true disease association. While TTN variants have been well characterized, non-TTN DCM-associated genes remain largely unexplored and, when found, present a major diagnostic dilemma.

To this end, we systematically assessed non-TTN DCM-associated genes in a large ES referral cohort and compared them against a cohort of clinically pathologic cases and a cohort of healthy controls. We identified the prevalence of genetic variation of DCM-related genes among the 3 cohorts and determined the likelihood of variant pathogenicity utilizing both a gene-level signal-to-noise (S:N) analysis and a correlation analysis comparing variant frequency and protein length among the cohorts. We demonstrate that for DCM, variants in FLNC, RBM20, and MYH6 are associated with the highest probability of pathogenicity.

**CLINICAL PERSPECTIVE**

**What Is New?**
- Incidentally identified variants in genes associated with cardiomyopathy are an increasing problem in the era of broad genetic testing.
- Incidently variants found in specific dilated cardiomyopathy genes have a higher chance of being associated with disease.

**What Are the Clinical Implications?**
- Signal-to-noise analysis can be informative when determining the probability an incidentally identified variant will cause dilated cardiomyopathy.
- Incidently identified variants in genes such as FLNC, RBM20, MYH6, DSP, ABCC9, JPH2, and NEXN have a higher likelihood of being associated with development of dilated cardiomyopathy.

**Nonstandard Abbreviations and Acronyms**

| Abbreviation | Description |
|--------------|-------------|
| ACMG | American College of Medical Genetics and Genomics |
| DCM | dilated cardiomyopathy |
| ES | exome sequencing |
| gnomAD | Genome Aggregation Database |
| GS | genome sequencing |
| LP/P | likely pathogenic/pathogenic |
| S:N | signal-to-noise |
| VUS | variant of unknown significance |
Study Cohorts
The current study was approved by an institutional review board. As no identifiable clinical data were included, consent was waived.

ES Cohort
This research study was approved by the Baylor College of Medicine institutional review board and the Duke University Hospital institutional review board. From October 2011 through September 2019, individuals referred for clinical ES at Baylor Genetics Laboratories (Houston, Texas) were studied. Inclusion criteria were: (1) referred for diagnostic ES for any indication (including DCM), and (2) unrelated probands. Exclusion criteria were: (1) nonproband family members, (2) those whose samples were derived for platform validation studies or from oncological samples, and (3) participants with variant calls with poor read depth (variant-positive reads/total reads ≤10% or total reads <20). This cohort was composed of genetic testing probands. Approximately 90% were referred from institutions within the United States and ~47% were referred from within the state of Texas. Most of the cohort underwent ES testing during childhood. Sequencing and variant analyses were performed as previously described. Briefly, extracted DNA was subjected to VCRome version 2.1, an in-house exome capture platform (targeting ~20 000 genes, including the coding and untranslated region exons) and was sequenced using a HiSeq. The platform used has a minimum depth coverage of 20x and a practical detection rate of single nucleotide variants and insertion/deletions of ~95%. As previously detailed, neurological concerns, primarily developmental delay, were the most common indications for genetic testing in this cohort. Abnormal muscle tone, dysmorphic facial features, and seizures were other common ES referral indications. Only 13% of the cases referred for ES testing included a cardiac diagnosis as part of a multifaceted phenotype, and an even smaller proportion (5%) were referred exclusively with a cardiac diagnosis. Thus, the population of the ES cohort with cardiomyopathy, arrhythmia, and/or sudden death is likely only a very small part of the overall cohort.

The variants included were: (1) identified in the coding nucleotide sequence or intronic variants no more than 2 nucleotides from the splice boundary of a DCM-associated gene locus; (2) interpreted as “pathogenic,” “likely pathogenic,” or “variant of unknown significance (VUS)” at the time of genetic testing according to 2015 ACMG interpretation guidelines, and (3) included on the clinical report sent to the referring provider as an actionable, incidental finding or as a VUS without implications for pathogenicity on an “expanded report” provided to the clinician on request. The variants excluded from this study were: (1) deemed “benign,” “likely benign,” or “not reported” at the time of genetic testing; (2) variants >2 nucleotides from the splice boundary; (3) 5′ or 3′ untranslated region variants; or (4) synonymous variants. Variants in the ES cohort were labeled according to pathogenicity data available through the Baylor Miraca database at the time of analysis and maintained the initial interpretation of pathogenicity at the time of genetic testing.

DCM Pathologic Case Cohort
A DCM pathologic case cohort was aggregated with variants from the literature, as well as genetic testing data from Invitae’s database (San Francisco, California), which were denoted as “likely pathogenic” or “pathogenic.” For literature studies (Table S1), the included studies were: (1) cohort-based individuals who underwent genotyping, (2) reported cases meeting diagnostic criteria for clinical nonischemic DCM, and (3) reported cases that conducted comprehensive sequencing analysis for at least 1 non-TTN DCM-associated gene. Given the time span encompassed by cases in the DCM case cohort, to account for the evolution of variant interpretation over time, each variant was referenced against ClinVar, if present, and pathogenicity was reassigned based on ClinVar classification if necessary. Likely benign/benign and VUS variants were excluded to ensure that all variants included in the DCM pathologic case cohort had sufficient evidence of pathogenicity and association with DCM. In cases where a variant was found in the pathologic case cohort but not in ClinVar, we included those interpreted as LP/P and excluded those interpreted as VUS by modified ACMG criteria.

To validate and expand pathogenic variants, we queried all patients with LP/P variants from cohort data from Invitae’s clinical genetic testing database. Inclusion criteria were: (1) referral to Invitae for clinical genetic testing, (2) referral diagnosis of nonischemic DCM based on International Classification of Diseases, Ninth Revision (ICD-9), code 425.4 and International Classification of Diseases, Tenth Revision (ICD-10), code I42.0, and (3) a “likely pathogenic” or “pathogenic” variant identified in a non-TTN DCM-associated gene according to ACMG interpretation guidelines. The exclusion criteria were: (1) variants determined to be VUS, likely benign, and benign at the time of genetic testing; and (2) variants found to be singly heterozygous in genes with a solely autosomal recessive mode of inheritance. Furthermore, studies with identical authors and those in which the institution where the cohort was derived was the same as subsequent DCM cohort studies were excluded. The DCM-associated minor allele frequency between the DCM literature cohort and Invitae cases was similar.
GnomAD/Control Variant Cohort

To compare the ES cohort and the DCM pathologic cohort with a control cohort of population variants, we utilized the Genome Aggregation Database (gnomAD) with a total of 141,456 individuals with 125,748 ES and 15,708 GS. While gnomAD does contain data from case-control studies, these studies are of common adult-onset diseases and thus gnomAD represents a population control cohort and is routinely used as such. Individuals affected by severe pediatric-onset disease are excluded as are their first-degree relatives.

Non-TTN variants present in gnomAD with an allele frequency of <0.0003 were included. This threshold was determined based on the highest frequency of a pathologic cohort variant that was also identified in gnomAD. This approach, which has been previously validated, also potentially includes disease-susceptibility alleles with incomplete penetrance in the population.

To make direct comparisons between variant yield in the gnomAD, DCM, and ES cohorts, we assigned pathogenicity to gnomAD variants based on ClinVar assessments (as of September 2021). While gnomAD is considered here as a healthy control cohort, LP/P variants in genes associated with cardiomyopathy would still be expected in this cohort as a result of incomplete disease penetrance. Detailed information regarding assignment of variant pathogenicity of gnomAD variants is described in Data S1.

Genetic Analysis

A total of 39 DCM-associated genes and encoded proteins were analyzed. These included genes previously designated as definitive, strong, or moderate evidence of pathogenicity for DCM as put forth by The Dilated Cardiomyopathy Precision Medicine Study, as well as by independent multi-institutional teams, were included in the current analysis. In order to set the model, a set of limited/disputed genes from these previous studies were also included as a limited set of genes associated with syndromes that can have a primary presentation of nonischemic DCM. Genes that met these criteria were: ABCC9, ACTC1, ACTN2, ANKRD1, BAG3, CAV3, CRYPB, CSRP3, DES, DMD, DSG2, DSP, FKTN, FLNC, FYN, ILK, JPH2, LAMA4, LAMP2, LDB3, LMNA, MYBP3, MYH6, MYH7, NEXN, PDLIM3, PKP2, PLN, RBM20, SCN5A, SGCD, SYNE1, TAZ, TCAP, TNNC1, TNNT3, TNNT2, TPM1, and VCL. Further details are included in Data S1.

Nomenclature

A “variant” is a change in DNA that has been identified through genetic testing and differs from the standard reference genome. Variants can be classified into VUS, pathogenic, likely pathogenic, benign, or likely benign. LP/P is frequently used to describe variants that fall into either pathogenic or likely pathogenic categories and are thus of high clinical interest. A “rare DCM-associated variant” is defined as any variant in a gene that has been associated with DCM and found in <5% of probands with DCM; this excludes TTN variants as TTN is the only gene with a genetic yield of >5% among patients with DCM. Variants were included in the gnomAD cohort based on a minor allele frequency <0.0003, calculated using a previously described methodology: the LP/P variant in the DCM cohort with the highest minor allele frequency also present in gnomAD. “Radical” was used to describe variants that cause changes in the protein product, other than a single amino acid change, including nonsense (early termination), insertion/deletion (both in-frame and out-of-frame) and predicted canonical splice site mutations. “Incidental variant” was used to describe a variant identified in a rare DCM-associated, non-TTN gene found in the ES cohort. As the majority of these genes are not part of the ACMG list of reportable gene variants, this term was used as opposed to “secondary variant.” Protein size refers to protein length in amino acids. This information was obtained from Ensembl (https://www.ensembl.org/index.html).

Statistical Analysis

The variant yield by gene was calculated for each cohort. Yield for a given gene in each cohort was calculated as the number of individuals found with at least one variant in that gene, divided by the number of people who received genetic sequencing that included that gene. Individuals hosting multiple variants in one gene of interest is a rare occurrence. Data were presented as yield by gene with 95% CIs calculated using the formula for SE of a proportion, multiplied by 1.96. Yield for LP/P and VUS variants were directly compared between cohorts using chi-square analysis and t test of proportions for post hoc testing with a Bonferroni adjustment. Chi-square analysis was used as an omnibus statistical test, which was followed up with a pairwise, post hoc t test of proportions to compare proportions between cohorts. Yield of VUS was only compared between ES and gnomAD cohorts as the DCM cohort included LP/P variants exclusively.

Correlational sensitivity analyses using Pearson r coefficient of the relationship between variant yield by gene and multiple indicators, including yield in the gnomAD cohort and protein size, were performed for the DCM case cohort and the ES cohort. For gene-level S:N calculations, the yield of each gene for the ES and DCM cohorts, by pathogenicity, was normalized to the corresponding gene and pathogenicity yield found incidentally in the gnomAD cohort as previously
RESULTS

Prevalence of Variants in DCM-Associated Genes in the ES Cohort

To evaluate the prevalence of incidentally identified variants in non-TTN DCM-associated genes, we established an ES referral cohort that consisted of 14,005 individuals who met inclusion/exclusion criteria, with 7518 male (53.7%), 6211 female (44.3%), 263 fetal (1.9%), and 13 sex unknown/undocumented individuals (0.1%, Table). The median age at genetic testing was 9.6 years (age range of newborn to 90.1 years; fetal participants were excluded for this calculation). Within the ES cohort, a total of 9154 (65.4% [64.6, 66.2]) unrelated probands had at least 1 LP/P or VUS variant in 1 of the 39 non-TTN DCM-associated genes with a total of 19,196 variants identified (Figures 1A and 1B). Among these, 269 (1.9% [95% CI, 1.7–2.2]) probands carried at least 1 LP/P variant (Details in Table S2), and 8885 (63.4% [95% CI, 62.6–64.2]) individuals carried a VUS (Figure 1B). VUS made up the majority of all identified variants (98.3% [95% CI, 98.1–98.5]).

Among variant-positive probands including those with LP/P or VUS variants, 4632 (50.6% [95% CI, 49.6–51.6]) carried a single variant, while 2847 (31.1% [95% CI, 30.2–32.1]) carried 2 variants, 1165 (12.7% [95% CI, 12.1–13.4]) carried 3 variants, and 510 (5.6% [95% CI, 5.1–6.1]) carried >4 variants (Figure 1C). When analyzed by individual gene, SYNE1 had the highest yield in the ES cohort, with 23.5% of probands carrying a VUS in this gene, followed by FLNC (9.7%) and DSP (8.8%) (Figure 1D). Taken together, this suggests that there is a high variant yield (65.4%) in the ES cohort, with the majority of variants being VUS and ~2% of individuals carrying incidentally identified LP/P variants.

DCM and gnomAD Cohort Variant Characteristics Compared With ES Cohort Variant Characteristics

To determine the prevalence of non-TTN, DCM-associated gene variants in the DCM and gnomAD cohorts, we described qualities of each cohort, including sample size, variant type (ie, missense versus radical), and yield, defined as the proportion of individuals with a variant of a given pathogenicity. The DCM case cohort included 6359 DCM cases from 25 independent studies (additional data and references in Table S1), as well as 3083 individuals from the Invitae database who received genetic testing for an indication of nonschemic DCM. The combination of these 2 data sources created a total DCM cohort of 9442 cases, among whom 771 (8.2% [95% CI, 7.9%–8.5%]) were genotype-positive for an LP/P variant. To confirm that cases obtained from the literature approximated the deidentified genetic testing data obtained from Invitae, we compared gene-specific allele frequencies and found them to be similar between the 2 subgroups (Figure S1).

In the control cohort of 141,456 individuals from gnomAD, we identified 72,568 individuals with a rare variant in a cardiomyopathy-associated gene. As individual-level data are not available in gnomAD, our estimate does not account for individuals with multiple variants. We found a gnomAD rare variant yield of 51.3% (95% CI, 51.0%–51.6%).

The variants in the ES and control cohorts share several features that were divergent from the DCM case cohort. Missense variants made up the majority of all identified variants within the ES cohort as well as the control cohort, while both had a relatively low frequency of radical variants: 6.9% (95% CI, 6.5%–7.3%) in the ES cohort and 5.5% (95% CI, 5.3%–5.6%) in the control cohort. By comparison, the DCM cases from the literature displayed a higher frequency of radical variants, accounting for 38.4% (95% CI, 34.3%–42.6%) of total variants, which is over 5-fold higher than the ES or control cohorts.

Finally, we compared variant yield, by pathogenicity status, between cohorts. We split the gnomAD cohort variants by pathogenicity, based on ClinVar pathogenicity assignments. We found that there was a significantly higher LP/P variant yield in the DCM cohort (8.2% [95% CI, 7.9%–8.5%]) compared with the ES cohort (1.9% [95% CI, 1.7%–2.2%]), both of which were

| Table 1. Clinical ES Cohort Characteristics and Demographics |
|-----------------------------------------------|
| **ES cohort** | **No. (%)** |
| --- | --- |
| Total probands | 14,005 |
| Male | 7518 (53.7%) |
| Female | 6211 (44.3%) |
| Fetal | 263 (1.9%) |
| Unknown | 13 (0.1%) |
| Median age at genetic test (SD), y | 9.6 (12.0) |
| Variant-positive probands | 9154 (65.4% [64.1%–66.7%]) |
| Likely pathogenic/pathogenic probands | 269 (1.9% [1.7%–2.2%]) |
| VUS probands | 8885 (63.5% [62.2%–64.8%]) |

Values in square brackets represent 95% CIs. ES indicates exome sequencing; and VUS, variant of uncertain significance.
significant higher than the gnomAD cohort LP/P yield (0.31% [95% CI, 0.28%–0.33%], P<0.017) (Figure 2A). We also found that there was a significantly higher VUS variant yield in the ES cohort (63.4% [95% CI, 62.6%–64.2%]) compared with the gnomAD cohort (16.8% [95% CI, 16.6%–17.0%], P<0.017) (Figure 2B). A subanalysis was performed including only the 10 genes determined to have a definitive association with cardiomyopathy according to the most recent ClinGen analysis (BAG3, DES, FLNC, LMNA, MYH7, PLN, RBM20, SCN5A, SGCD, SYNE1, TAZ, TCAP, TNNT1, TNNI3, TNNT2, TPM1, VCL).30 Similar to the analysis of the overall cohort, there was a significantly higher LP/P yield in the DCM cohort compared with the ES cohort, both of which had significantly higher LP/P yields than the gnomAD cohort (Figure S2). In summary, we found a significantly lower LP/P variant yield in the ES cohort compared with the DCM cohort, but a significantly higher yield in the ES cohort compared with the gnomAD cohort.

**Gene-Specific Variant Prevalence Suggests Similarities Between the ES and gnomAD Cohorts**

To determine genes that may have higher diagnostic yield for DCM, we next assessed the gene-specific variant yields among the cohorts. In the DCM case cohort, the genes with the highest variant yields were FLNC (2.0% [95% CI, 1.6%–2.5%]), LMNA (1.8% [95% CI, 1.5%–2.1%]), TNNT2 (1.1% [95% CI, 0.9%–1.4%]), MYH7 (1.0% [95% CI, 0.8%–1.3%]), and DSP (0.9% [95% CI, 0.6%–1.1%]) (Figure 3A). This gene distribution was markedly different in the ES cohort, which demonstrated highest LP/P variant yield in ABCC9.
(0.19% [95% CI, 0.12%–0.27%]), SCN5A (0.19% [95% CI, 0.11%–0.25%]), DSP (0.17% [95% CI, 0.08%–0.28%]), and SYNE1 (0.14% [95% CI, 0.13%–0.15%]) (Figure 3B).

In alignment with our previous observation that the ES-VUS and gnomAD cohorts appeared to be similar overall, gene-specific VUS variation in the ES cohort was similar to that of gnomAD. The genes demonstrating the highest VUS yield in the ES cohort were SYNE1 (23.5% [95% CI, 22.8%–24.2%]), FLNC (9.7% [95% CI, 9.2%–10.1%]), DSP (8.8% [95% CI, 8.3%–9.3%]), MYBPC3 (7.1% [95% CI, 6.7%–7.5%]), and MYH6 (6.5% [95% CI, 6.1%–6.9%]) (Figure 3C). Similarly, the genes with the highest overall yield in the gnomAD cohort were SYNE1 (11.9% [95% CI, 11.7%–12.0%]), FLNC (3.7% [95% CI, 3.6%–3.8%]), DSP (3.5% [95% CI, 3.4%–3.6%]), MYBPC3 (3.2% [95% CI, 3.1%–3.3%]), and SCN5A (2.8% [95% CI, 2.7%–2.9%]) (Figure 3D).

Overall, the distribution of genes with the highest variant yield was similar between ES-VUS variants and gnomAD variants, although the numeric percent yield was consistently higher in the ES cohort for VUS variants.

Recent studies have compared pathogenic variant yield, normalized against population yield, as a methodology for determining genes that are more likely to be associated with disease. Using this approach, we next applied an S:N analysis, by normalizing the pathogenicity-specific variant yield for each gene from the DCM and ES cohorts against the corresponding pathogenicity-specific yield in the control (gnomAD) cohort, to identify which non-CCN DCM-associated genes may hold more diagnostic weight. In the DCM case cohort, 25 genes had an S:N ratio significantly greater than 1 when normalized to gnomAD LP/P yield, suggesting a higher likelihood that a variant localizing to the gene may be DCM-associated (P<0.0013). The genes with the greatest S:N ratios were FLNC (626 [95% CI, 651–602]), RBM20 (533 [95% CI, 526–540]), and MYH6 (180 [95% CI, 178–181]) (Figure S3A). Among identified variants deemed to be LP/P within the ES cohort, the genes with the greatest S:N ratios were ABC9 (242.3 [95% CI, 241.4–243.2]), LAMA4 (38.1 [95% CI, 38.0–38.2]), and MYH6 (32.9 [95% CI, 32.9–33.0]), which partially overlap with the DCM case cohort (Figure S3B). Using a population prevalence of 1:2500 for familial DCM, the genes with the greatest positive predictive value for LP/P variants were FLNC (20.0% [95% CI, 19.3%–20.8%]), RBM20 (17.6% [95% CI, 17.3%–17.8%]), and MYH7 (6.7% [95% CI, 6.6%–6.8%]) (Figure S3C). Taken together, this analysis demonstrates that rare variants localizing to FLNC, RBM20, and MYH6 were most likely to be disease-associated based on S:N analysis.
Prevalence of Individuals With Incidentally Identified VUS is Strongly Correlated With Healthy Cohort and Size of Protein

We next sought to identify other features of non-\textit{TTN} DCM genes that were associated with disease. We compared variant yield from the DCM and ES cohorts, respectively, with the healthy gnomAD control cohort using correlational analysis. Likely because of a subset of genes, primarily \textit{SYNE1}, which demonstrated a higher variant frequency in the DCM and ES LP/P cohorts, respectively, compared with controls (Figures 4A), we did not identify a tight correlation between LP/P variant yield by gene from the DCM cohort versus the gnomAD control cohort ($R^2=0.018$) and ES LP/P variant yield versus the gnomAD control cohort ($R^2=0.271$) (Figures 4B and 4C). In addition to \textit{SYNE1}, \textit{FLNC}, \textit{DSP}, \textit{LMNA}, \textit{MYBPC3}, \textit{MYH6}, \textit{MYH7} and \textit{TNNT2} were also found to have variant frequencies above the anticipated linear model from DCM and ES LP/P cohorts. Moreover, in the DCM case cohort, variants in \textit{FLNC} had the highest likelihood of associating with DCM compared with the gnomAD control cohort, while an LP/P variant in \textit{ABCC9} was most likely to be present in the ES cohort compared with the gnomAD control cohort. A similar calculation of correlation with \textit{SYNE1}, an outlier in this analysis, excluded can be found in Figure S4. In contrast, and in alignment with previous findings, we identified a tight correlation between the yield of ES-VUS and the gnomAD control cohort ($R^2=0.931$), suggesting that ES VUS are found more frequently in genes with higher variant yield in gnomAD among all DCM genes analyzed (Figure 4D).

We next evaluated the correlation between gene-specific variant yield and encoded protein size by cohort. We hypothesized that in cohorts with rare disease-associated variants, gene-specific yield would be more strongly correlated with protein size, while in
cohorts with a higher proportion of disease-associated variants, variant yield would have less of a correlation with protein size. We found a weak correlation between the observed frequencies of DCM case cohort and ES LP/P variants compared with protein size ($R^2=0.023$ and $R^2=0.304$, respectively) (Figure 5A and 5B). However, DCM-associated genes in the ES-VUS cohort and the gnomAD control cohort were tightly correlated with the corresponding encoded protein size ($R^2=0.872$ and $R^2=0.930$, respectively; Figure 5C and 5D). A similar calculation of correlation with $SYNE1$, an outlier in this analysis, excluded can be found in Figure S5. Taken together, this correlation analysis identified $FLNC$, $RBM20$, and $MYH6$ as having a higher variant yield in the DCM cohort than anticipated based on population variant frequencies or coding protein size.

**Integration of Analyses to Identify the Highest Probability of DCM-Associated Genes Among ES Incidentally Identified Variants**

Finally, we sought to integrate the previous analyses to determine which non-$TTN$ genes had the highest
probability of hosting a DCM-causal incidentally identified variant. To do this, we first identified genes from the DCM cohort with the highest frequency of variation relative to the gnomAD cohort (S:N ratio) and protein size. This yielded FLNC, RBM20, MYH6, TPM1, LMNA, DSP, ABCC9, JPH2, NEXN, and PLN. We then compared this gene distribution with that of genespecific LP/P variant yields in the ES cohort (ABCC9, LAMA4, MYH6, ACTC1, RBM20, FLNC, DSP, NEXN, JPH2, and DMD). We identified partial overlap between the identified genes with the highest S:N from the DCM and ES LP/P cohorts: FLNC, RBM20, MYH6, DSP, ABCC9, JPH2, and NEXN (Figure 6). Overall, these genes are predicted to have the highest likelihood of being associated with DCM when incidentally identified variants in these genes are found.

DISCUSSION
Genetic testing is integral in helping to facilitate the diagnosis and risk evaluation of heritable cardiomyopathies. Genetic testing is useful not only in identifying the
affected proband but also in familial cascade screening where it may facilitate discovery of the disease-associated variant in asymptomatic family members who have not yet manifest disease. Early identification of such at-risk individuals is critical for planning preventive and disease management strategies such as lifestyle recommendations and medical therapy. Genetic testing is recommended for both familial DCM and sporadic DCM cases according to the 2016 American Heart Association's scientific statement on DCM.31 DCM subtypes with arrhythmias are highly correlated to specific genetic variants including those found in LMNA or SCN5A.32-34 However, there is no specific relationship between genetic variants and distinguishing phenotypic characteristics in most DCM cases. Currently, there still remains a vast, unexplored field of potential unknown DCM pathogenic genes attributable to the possibility of low penetrance alleles, de novo variants, copy number variation, and intronic variants.35 A broad cardiomyopathy gene panel consisting of 20 to 50 genes is recommended following clinical diagnosis of DCM.36 Next-generation sequencing such as ES or GS may be considered when the gene panel is negative to identify rare mutations.31,37,38 As expanded genetic sequencing techniques become more routinely employed across all specialties for a variety of indications, the number of variants identified overall, and VUS in particular, will continue to increase dramatically. This will significantly increase the difficulty in interpretation of results for clinicians and researchers.36 For example, in our study, we found that ~65% of individuals who underwent clinical ES were found to have at least 1 variant of either pathogenic or uncertain significance or had an actionable incidental finding. Thus, as enhanced methods of expanded, next-generation sequencing develop, the ability to interpret the results will become increasingly urgent.

Results from ES should not be viewed as binary “positive versus negative” results, but instead, the “positive” result of finding a genetic variant should be interpreted based on whether the variant is likely to be disease causing. According to ACMG guidelines, genetic variants can be classified from benign to pathogenic based on evidence including population data identifying variant association with disease, computational data, functional/experimental data, and family segregation data.19 After excluding benign/likely benign variants from the ES and control cohorts, we found that both cohorts had the highest frequency of VUSs in the SYNE1, FLNC, DSP, and MYBPC3 genes. However, the distribution of LP/P variants in DCM-associated genes within the DCM cohort did not follow this pattern. The DCM case cohort showed the highest frequency of pathogenic variants occurring in FLNC, LMNA, TNNT2, MYH7, and DSP. These results suggest that incidentally identified variants from clinical ES, specifically VUSs, are more likely to represent normal population variants instead of disease-associated variants.

After normalizing against control variants, most DCM-associated genes including CAV3, RBM20, BAG3, TCAP, PKP2, DSG2, and MYBPC3 showed high S:N (>1) for VUS discovered from ES results. The high S:N values of VUS from the ES cohort are expected since the utilization of ES increases the reporting yield of VUS. The highest gene-level S:N was observed in FLNC, RBM20, and MYH6 in the DCM case cohort, while ABCC9, LAMA4, and MYH6 had the highest S:N values in LP/P variants of the ES cohort. The discrepant distribution patterns between the 2 cohorts indicates incidentally identified variants by ES do not reliably predict a pathogenic phenotype for DCM. Moreover, the high correlation between the frequency of VUS in the ES and control cohorts, and the correlation of VUS frequency in the ES cohort and the protein size, suggest that incidentally identified VUSs more likely reflect rare population variation. These results further demonstrate that VUSs identified in most non-TTN DCM genes from ES testing should not be reported clinically. This is in agreement with current ACMG variant reporting guidelines.13,19

On further analysis of the relationship between the prevalence of variants in common DCM-associated genes and protein size, we identified 7 genes that have a higher likelihood of DCM pathogenicity when found incidentally: FLNC, RBM20, DSP, ABCC9, JPH2, MYH6, and NEXN. This suggests that LP/P variants localizing to these genes should be followed clinically. Moreover, we previously demonstrated that incidental variants of TTN in the A band had a high S:N value and were more likely to be associated with cardiomyopathy.17 Among the 7 genes, we found that FLNC variants represented in the DCM cohort had the highest yield.
while in the ES LP/P cohort, ABCC9 variants had the highest possibility of pathogenicity. FLNC-encoded filamin C is a crucial protein in sarcomere structure and signaling. Variants in this gene have been associated with both arrhythmias and cardiomyopathies and cases of FLNC-associated DCM are associated with severe clinical course and high risk of sudden cardiac death.39 Thus, incidental variants found in this gene should warrant clinical evaluation. Additionally, the ACMG recently added incidentally found variants in FLNC to its list of reportable secondary findings.15

One important possibility is that individuals in the DCM and ES cohorts may have had more than one rare variant in a cardiomyopathy-associated gene. These variants may represent a single disease causative variant with others that are not causing disease, or multiple variants that add to confer greater DCM risk. There is existing evidence that multiple disease-associated variants may worsen cardiomyopathy prognosis.40,41 The S:N approach detailed here can be applied in this setting and used to evaluate each variant in question. Even if variants represent additive risk of DCM, burden of rare variation in DCM versus control cohorts would still likely be closely associated with the relevance of a given gene to disease.

Importantly, our findings highlight a probabilistic approach to variant interpretation in incidental variants localizing to DCM-associated genes. Careful clinical phenotyping of all individuals with incidentally identified LP/P variants, and particularly those listed as actionable by the ACMG, should be performed and evaluation and follow-up individualized to the patient based on existing clinical guidelines.31,42–44 Among individuals with a normal evaluation, the identification of an LP/P variant necessitates continued clinical follow-up to look for future manifestation of disease. The diagnostic strength of ES-identified LP/P variants will be maximized when combined with clinical phenotype and personal or family history, which support a DCM diagnosis, and for young patients with incidentally identified LP/P variants these findings should be used to guide surveillance. Similarly, when VUS are identified, a careful reevaluation of the variant should ensue. This includes a careful reevaluation of the variant and gene locus in the context of an individualized evaluation. VUS should only be acted upon clinically should this reevaluation elevate the likelihood of pathogenicity to >90%, which is the threshold for redesignation of LP. Additionally, as pathogenicity interpretation can evolve over time, it is important for clinicians following patients with a VUS in a DCM-associated gene to periodically revisit its latest pathogenicity determination. The strength of disease association in many genes classically associated with DCM is rapidly changing as new evidence is found.30 Future work should continue to strengthen this probabilistic model. Further studies, conducted in large ES or GS-based longitudinal cohorts independent from ours, are needed to fully validate our findings, and broad consensus-based guidance on how to systematically evaluate incidentally identified variants in DCM-associated genes is needed.

**Limitations**

An important limitation of the current study is that both the DCM case cohort and the ES cohort were predominantly made up of individuals of European descent. This limits the generalizability of our conclusions. Variability in calling/filtering of genetic variants between the cohorts exists and represents a limitation. For example, while the early variant calls in the ES cohort were not used for analysis, there is undoubtedly evolution in variant calls with time. Moreover, while ES in this study was clinical grade (at least 100–150× depth of coverage), it may still not have the same resolution as gene panels (usually 350× depth of coverage) at the single-exon level with respect to copy number variants and complex rearrangement (ie, MYBPC3 and LMNA). While this difference in coverage may not yield a statistically significant difference in detection, these types of variants were excluded from our study and remain unexplored. Additionally, individuals in the gnomAD cohort may have hosted more than one variant in DCM-associated genes; however, this information was not available. For consistency, in the DCM and ES cohorts, if an individual hosted more than one variant in DCM-associated genes, an LP/P variant would take precedence and these individuals were only included in the LP/P yield. These numbers were exceedingly small with <0.025% of the ES cohort hosting more than one variant DCM-associated genes. The young age of individuals in the ES cohort is an additional limitation. DCM may present later in life and thus while these individuals did not have DCM as a clinical diagnosis at the time of testing, they may develop this diagnosis later in life. Moreover, the differences in race, ethnicity, and ancestry, as well as the differences in proportion of familial DCM between cohorts is challenging to directly compare and may account for some differences in variant frequency.

**ARTICLE INFORMATION**

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SUPPLEMENTAL MATERIAL
SUPPLEMENTAL METHODS

Genetic Analysis

39 DCM-associated genes, with respectively encoded proteins, were analyzed in this study. Gene abbreviations were consistent with HUGO Gene Nomenclature Committee. Consensus primary sequences from Ensembl browser included \textit{ABCC9}-encoded ATP binding cassette subfamily C member 9 (NM\_005691, MIM 608569)\textsuperscript{45,46}, \textit{ACTC1}-encoded actin alpha cardiac muscle 1 (NM\_005159, MIM 613424)\textsuperscript{47,48}, \textit{ACTN2}-encoded alpha actinin 2 (NM\_001103, MIM 612158)\textsuperscript{49,50}, \textit{ANKRD1}-encoded ankyrin repeat domain-containing protein 1 (NM\_014391, MIM 615248)\textsuperscript{51,52}, \textit{BAG3}-encoded BAG family molecular chaperone regulator 3 (NM\_004281, MIM 613881)\textsuperscript{53,54}, \textit{CAV3}-encoded caveolin 3 (NM\_033337, MIM 601253)\textsuperscript{55,56}, \textit{CRYAB}-encoded crystallin alpha B (NM\_001289807, MIM 615184)\textsuperscript{57,58}, \textit{CSRP3}-encoded cysteine and glycine rich protein 3 (NM\_003476, MIM 607482)\textsuperscript{59,60}, \textit{DES}-encoded desmin (NM\_001927, MIM 604765)\textsuperscript{60,61}, \textit{DMD}-encoded dystrophin (NM\_004006, MIM 302045)\textsuperscript{62,63}, \textit{DSG2}-encoded desmoglein 2 (NM\_001943, MIM 612877)\textsuperscript{64,65}, \textit{DSP}-encoded desmoplakin (NM\_004415, MIM 125647)\textsuperscript{66,67}, \textit{FKTN}-encoded fukutin (NM\_006731, MIM 611615)\textsuperscript{68}, \textit{FLNC}-encoded filamin C (NM\_001458.5, MIM 617047)\textsuperscript{69}, \textit{FXN}-encoded frataxin (NM\_000144, MIM 229300)\textsuperscript{70,71}, \textit{ILK}-encoded integrin linked kinase (NM\_004517, MIM 115200)\textsuperscript{72,73}, \textit{JPH2}-encoded junctophilin 2 (NM\_020433.5, MIM 619492)\textsuperscript{74}, \textit{LAMA4}-encoded laminin subunit alpha 4 (NM\_001105206, MIM 615235)\textsuperscript{75,76}, \textit{LAMP2}-encoded lysosomal associated membrane protein 2 (NM\_001122606, MIM 300257)\textsuperscript{77}, \textit{LDB3}-encoded LIM domain binding protein 3 (NM\_001080114, MIM 601493)\textsuperscript{78,79}, \textit{LMNA}-encoded prelamin-A/C (NM\_170707, MIM115200)\textsuperscript{80,81}, \textit{MYBPC3}-encoded
myosin-binding protein C, cardiac-type (NM_000256, MIM 600958)\textsuperscript{82,83}, \textit{MYH6}-encoded myosin heavy chain 6 (NM_002471.4, MIM 613252)\textsuperscript{84}, \textit{MYH7}-encoded myosin heavy chain 7 (NM_000257, MIM 613426)\textsuperscript{85,86}, \textit{NEXN}-encoded nexilin F-actin binding protein (NM_144573, MIM 613122)\textsuperscript{87,88}, \textit{PDLIM3}-encoded PDZ and LIM domain 3 (NM_014476, MIM 605889)\textsuperscript{89,90}, \textit{PLN}-encoded cardiac phospholamban (NM_002667, MIM 609909)\textsuperscript{91,93}, \textit{PKP2}-encoded plakophilin 2 (NM_001005242.3, MIM 609040)\textsuperscript{93}, \textit{RBM20}-encoded RNA binding protein 20 (NM_001134363, MIM 613172)\textsuperscript{94}, \textit{SCN5A}-encoded sodium channel protein type 5 subunit alpha (NM_198056, MIM 601154)\textsuperscript{95,96}, \textit{SGCD}-encoded delta sarcoglycan (NM_000337, MIM 606685)\textsuperscript{97,98}, \textit{SYNE1}-encoded spectrin repeat containing nuclear envelope protein 1 (NM_182961, MIM 612998)\textsuperscript{99,100}, \textit{TAZ}-encoded tafazzin (NM_000116, MIM 302060)\textsuperscript{101,102}, \textit{TCAP}-encoded telethonin (NM_003673, MIM 607487)\textsuperscript{103,104}, \textit{TNNC1}-encoded troponin C, slow skeletal and cardiac muscles (NM_003280, MIM 611879)\textsuperscript{105}, \textit{TNNI3}-encoded troponin I, cardiac muscle (NM_000363, MIM 613286)\textsuperscript{106,107}, \textit{TNNT2}-encoded troponin T2, cardiac type (NM_000364)\textsuperscript{108}, \textit{TPM1}-encoded tropomyosin alpha-1 chain (NM_001018004, MIM 601494)\textsuperscript{109,110}, and \textit{VCL}-encoded vinculin (NM_003373, MIM 611407)\textsuperscript{111,112}. 
**Table S1. Literature Studies.**

| Study | J. Ramchand et al. | T. Shin et al. | A. Long et al. | M. R. Hazebroek et al. | F. Mazzarotto et al. | R. Walsh et al. | T. J. Pugh et al. | A. Sousa et al. | J. Herkert et al. | A. Minoche et al. | S. Cuenda et al. | M. Dal Ferro et al. | O. Akkarine et al. | J. Verdonschot et al. | N. Lakdawala et al. | X.-L. Zhang et al. | C. Veselovskaya et al. | Y. Zhao et al. | R. Hershberg et al. | C. Horvat et al. |
|-------|--------------------|----------------|---------------|------------------------|---------------------|----------------|-------------------|---------------|-----------------|-----------------|----------------|-------------------|-----------------|-------------------|-------------------|----------------|-------------------|----------------|----------------|
| DCM cohort (N) | 83 | 26 | 18 | 262 | 1040 | 559 | 766 | 21 | 31 | 42 | 52 | 148 | 145 | 303 | 264 | 118 | 37 | 21 | 312 | 532 |
| DCM variant positive patients | 10 | 3 | 9 | 43 | 279 | 157 | 283 | 6 | 15 | 21 | 21 | 87 | 51 | 49 | 64 | 41 | 9 | 12 | 34 | 407 |
| Total number of individuals with LPM variants | 4 | 1 | 5 | 23 | 25 | 42 | 86 | 1 | 5 | 6 | 11 | 19 | 22 | 16 | 31 | 13 | 3 | 4 | 25 | 76 |

| Number of variant positive individuals by gene |
|-----------------------------------------------|
| ABC9 | 0 | 0 | 0 | N/A* | 0 | N/A | 0 | N/A | 0 | 1 | 1 | 0 | N/A | N/A | N/A | N/A | 0 | 0 | 0 | N/A | 0 |
| ACTN2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | N/A | 0 | 0 | 0 | 1 | 0 | N/A | N/A | 0 |
| ANKRD1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | N/A | 0 | 0 | 0 | 0 | N/A | 0 | N/A | 0 | N/A | 1 | 0 | N/A | N/A | 0 |
| BAG3 | 1 | 0 | 0 | 1 | 2 | N/A | N/A | N/A | 0 | 2 | 4 | 0 | 0 | 1 | N/A | 0 | 0 | 0 | N/A | 2 |
| CAV3 | 0 | 0 | 0 | 0 | N/A | N/A | N/A | 0 | N/A | 0 | 0 | 0 | 0 | N/A | 0 | N/A | 0 | N/A | 0 | N/A | 0 |
| CRYAB | 0 | 0 | 0 | 0 | 0 | 0 | 0 | N/A | 0 | N/A | 0 | 0 | N/A | 0 | N/A | 0 | N/A | 0 | N/A | 0 |
| CSRPR3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | N/A | 0 | N/A | 0 | N/A | 0 | N/A | 0 |
| DE9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | N/A | 0 | 0 | 0 | 0 | 0 | 1 | N/A | 0 | N/A | 1 | 0 | 1 |
| DMD | 0 | 0 | 0 | 1 | 0 | N/A | N/A | N/A | 0 | 0 | 1 | 0 | N/A | 1 | N/A | 0 | N/A | 0 | N/A | 0 |
| DSG2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | N/A | 0 | N/A | 0 | 0 | 0 | 0 | 1 | 0 | N/A | 0 | N/A | 0 |
| DSP | 0 | 0 | 0 | 0 | 2 | 2 | 9 | 3 | N/A | 0 | 1 | 0 | 4 | 8 | 1 | N/A | 0 | N/A | 0 | N/A | 0 |
| FIGN | 0 | 0 | 0 | 0 | N/A | 3 | N/A | N/A | N/A | 0 | 0 | 0 | 0 | 0 | 0 | N/A | N/A | N/A | 0 | N/A | 0 |
| FXN | 0 | 0 | 0 | 0 | 0 | N/A | N/A | N/A | 0 | 0 | 0 | 0 | N/A | N/A | N/A | N/A | N/A | N/A | 0 | N/A | 0 |
| ILK | 0 | 0 | 0 | N/A | 0 | N/A | N/A | N/A | 0 | 0 | 0 | 0 | N/A | N/A | N/A | 0 | N/A | 0 | N/A | 0 |
| LAM4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | N/A | 0 | N/A | 0 | 0 | 0 | 0 | 1 | 0 | N/A | 0 | 0 | 0 | N/A |
| LAMP2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | N/A | 0 | 0 | 0 | 0 | N/A | 0 | N/A | 0 | 0 | 0 | N/A | 0 |
| LDB3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | N/A | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | N/A | 0 | 0 | 0 | N/A |
| LMNA | 1 | 0 | 0 | 4 | 6 | 8 | 27 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | N/A | 0 |
| MYH7 | 0 | 0 | 0 | 3 | 4 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 1 | 0 | 0 |
| NEK5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | N/A | 0 | N/A | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | N/A | 0 |
| PDLIM3 | 0 | 0 | 0 | N/A | 0 | N/A | N/A | N/A | 0 | 0 | 0 | 0 | 0 | 0 | 0 | N/A | N/A | N/A | 0 | N/A | 0 |
| PLN | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | N/A | 1 | 0 | 0 | 0 | N/A |
| PRDM16 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | N/A | 0 | N/A | 0 | 0 | 0 | N/A | 0 | N/A | 0 | N/A | 0 | N/A | 0 |
| RBM29 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | N/A | 0 | N/A | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 2 |
| SCN5A | 0 | 0 | 0 | 0 | 0 | 0 | 0 | N/A | 1 | 0 | 0 | 0 | 2 | 0 | 0 | N/A | 1 | 0 | 0 | 0 | N/A |
| SDCD | 0 | 0 | 0 | 0 | 0 | 0 | 0 | N/A | 0 | 0 | 0 | 0 | 0 | 0 | 0 | N/A | 0 | 0 | 0 | 0 | N/A |
| SYNE1 | 0 | 0 | 0 | N/A | 0 | N/A | N/A | N/A | 0 | 0 | 0 | 0 | N/A | N/A | N/A | N/A | N/A | N/A | 0 | N/A | 0 |
| TAZ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | N/A | 0 | 0 | 0 | 0 | 0 | N/A | 0 | 0 | 0 | 0 | 1 | N/A | 0 |
| TCP1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | N/A | 0 | 0 | 0 | 0 | 0 | 0 | 0 | N/A | 0 | 0 | 0 | 0 | N/A |
| TNN1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | N/A | 0 | 0 | 0 | 0 | 0 | 0 | N/A | 0 | 0 | 0 | 0 | N/A |
| TNN2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | N/A | 0 | 0 | 0 | 0 | 0 | 0 | N/A | 0 | 0 | 0 | 0 | N/A |
| TPM1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | N/A | 0 | 0 | 0 | 0 | 0 | 0 | 0 | N/A | 0 | 0 | 0 | 0 | N/A |
| VCL | 0 | 0 | 0 | 0 | 0 | 0 | 0 | N/A | 1 | N/A | 0 | 0 | 0 | 0 | 0 | N/A | 1 | 0 | 0 | 0 | N/A |

*N/A means that this specific gene was not included or sequenced in the gene panel included in the respective study*
| ID     | Sex | Age | Variant type | Gene  | Location     | Nucleotide     | AminoAcid     | Zygosity |
|--------|-----|-----|--------------|-------|--------------|----------------|---------------|----------|
| 9316726| F   | 2   | P            | ABCC9 | exon2        | c.158delG      | p.S53fs       | Het      |
| 9314741| F   | 16  | P            | ABCC9 | intron18     | c.2238-1G>A    | N/A           | Het      |
| 9231516| F   | 2   | P            | ABCC9 | intron7      | c.1012-2A>G    | N/A           | Het      |
| 9217184| F   | 4   | P            | ABCC9 | intron18     | c.2238-1G>A    | N/A           | Het      |
| 9334925| F   | 24  | P            | ABCC9 | intron18     | c.2238-1G>A    | N/A           | Het      |
| 9333397| M   | 1   | P            | ABCC9 | exon38       | c.4573_4574insT| p.V1525fs     | Het      |
| 9256771| F   | 0   | P            | ABCC9 | exon38       | c.4570_4572delinsAAAT | p.L1524fs | Het      |
| 9250257| F   | 17  | P            | ABCC9 | exon24       | c.2928_2929dup | p.M977fs      | Het      |
| 9167818| M   | 6   | P            | ABCC9 | exon38       | c.4570_4572delTTAinsAAAT | p.L1524fs | Het      |
| 9049917| F   | 1   | P            | ABCC9 | intronic     | c.2238-1G>A    | N/A           | Het      |
| 9037848| F   | 0   | LP           | ABCC9 | exon12       | c.1664T>C      | p.F555S       | Het      |
| 9327245| F   | 65  | P            | ABCC9 | exon38       | c.4570_4572delinsAAAT | p.L1524fs | Het      |
| 9288771| M   | 6   | P            | ABCC9 | exon38       | c.4570_4572delinsAAAT | p.L1524fs | Het      |
| 9340902| M   | 18  | P            | ABCC9 | exon21       | c.2554C>T      | p.Q852X       | Het      |
| 9308534| F   | 17  | P            | ABCC9 | exon38       | c.4570_4572delTTAinsAAAT | p.L1524fs | Het      |
| 9250671| F   | 13  | P            | ABCC9 | exon38       | c.4570_4572delinsAAAT | p.L1524fs | Het      |
| 9238280| F   | 1   | P            | ABCC9 | exon38       | c.4570_4572delinsAAAT | p.L1524fs | Het      |
| 9210263| F   | 1   | P            | ABCC9 | exon28       | c.3524delT     | p.F1175fs     | Het      |
| 9348212| M   | 9   | P            | ABCC9 | exon38       | c.4570_4572delTTAinsAAAT | p.V1525fs | Het      |
| 9267625| M   | 1   | P            | ABCC9 | exon13       | c.1828_1829del | p.610_610del  | Het      |
| 9248450| M   | 7   | P            | ABCC9 | exon38       | c.4517_4526del | p.R1506fs     | Het      |
| 9252555| M   | 16  | P            | ABCC9 | intron18     | c.2238-1G>A    | N/A           | Het      |
| 9341118| F   | 0   | P            | ABCC9 | Intronic     | c.2238-1G>A    | N/A           | Het      |
| 9313207| M   | 7   | P            | ABCC9 | exon27       | c.3347G>A      | p.R1116H      | Het      |
| 9267877| M   | 12  | P            | ABCC9 | intron18     | c.2238-1G>A    | N/A           | Het      |
| 9190256| F   | 6   | P            | ABCC9 | exon13       | c.1828_1829del | p.610_610del  | Hom      |
| 9181181| F   | 0   | P            | ABCC9 | intron37     | c.4450-1G>A    | N/A           | Het      |
| 9327644| M   | 4   | P            | ACTC1 | exon2        | c.57_58insCA   | p.V19fs       | Het      |
| 9010016| M   | 0   | LP           | ACTC1 | exon3        | c.382A>G       | p.T128A       | Het      |
| 9341906| M   | 1   | P            | ACTC1 | exon2        | c.57_58insCA   | p.V19fs       | Het      |
| 9213684| M   | 0   | LP           | ACTC1 | exon5        | c.635G>A       | p.R212H       | Het      |
| SampleID | Sex | Exon | Gene | Description | Mutation | Status |
|----------|-----|------|------|-------------|----------|--------|
| 9299657  | M   | 70   | ACTN2 | exon21     | c.2554_2566del | p.852_856del | Het  |
| 9233371  | M   | 5    | ANKRD1| exon3      | c.222dupA   | p.L75fs  | Het  |
| 9338761  | M   | 28   | ANKRD1| exon8      | c.827C>T    | p.A276V  | Het  |
| 9337167  | M   | 4    | BAG3  | exon3      | c.626C>T    | p.P209L  | Het  |
| 9218587  | M   | 8    | BAG3  | exon2      | c.367C>T    | p.R123X  | Het  |
| 9204409  | F   | 13   | LP BAG3| exon4     | c.1363G>A   | p.E455K  | Het  |
| 9310491  | M   | 1    | CAV3  | exon1      | c.7_14del   | p.E4fs   | Het  |
| 9026205  | M   | 18   | CAV3  | exon2      | c.315delA   | p.C106Afs*6 | Het  |
| 9224711  | F   | 9    | CAV3  | exon1      | c.7_14del   | p.3_5del | Het  |
| 9297610  | M   | 1    | CRYAB | exon3      | c.499_505del| p.P166_167del | Het  |
| 9208949  | M   | 3    | CRYAB | exon4      | c.498_499del| p.166_167del | Het  |
| 9278460  | M   | 2    | CSRP3 | exon5      | c.286_287del| p.96_96del | Het  |
| 9272504  | M   | 9    | CSRP3 | exon3      | c.54C>A     | p.Y18X   | Het  |
| 9313275  | F   | 3    | CSRP3 | exon6      | c.457_458del| p.S153fs | Het  |
| 9235255  | M   | 17   | DES   | intron3    | c.897+2T>~  | N/A      | Het  |
| 9268947  | M   | 14   | DES   | exon2      | c.634C>T    | p.R212X  | Hom  |
| 9307121  | M   | 47   | DES   | exon1      | c.38C>T     | p.S13F   | Het  |
| 9292929  | F   | 3    | DES   | exon3      | c.733_734insAGGT | p.E245fs | Het  |
| 9168228  | M   | 19   | DES   | exon7      | c.1255_1271del17 | p.P167fs | Het  |
| 9329422  | F   | 24   | DMD   | exon16     | c.1806_1818del| p.D602_606del | Het  |
| 9307631  | F   | 5    | DMD   | exon16     | c.1809_1818del| p.D602fs  | Het  |
| 9267632  | F   | 3    | DMD   | exon1      | c.3G>T      | p.M1?    | Het  |
| 9324509  | F   | 0    | DMD   | intron31   | c.4233+2C>T | N/A      | Het  |
| 9270340  | M   | 14   | DMD   | exon40     | c.5652delG  | p.R1884fs | Hem  |
| 9219223  | M   | 23   | DMD   | exon7      | c.583C>T    | p.R195X  | Mosaic |
| 9322023  | F   | 5    | DMD   | intron31   | c.4233+2C>T | N/A      | Het  |
| 9308936  | M   | 0    | DMD   | deletion   | exon13-25   | N/A      | Hem  |
| 9268175  | M   | 16   | DMD   | intron69   | c.9975-2A>G | N/A      | Hem  |
| 9110527  | M   | 2    | DMD   | exon75     | c.10554-4_10571del | N/A | Hemi |
| 8980490  | M   | 5    | DMD   | exon45-52del | N/A    | N/A      | Hem  |
| 9278301  | M   | 7    | DMD   | exon49     | N/A      | N/A      | Hem  |
| 9283117  | M   | 1    | DMD   | exon51     | N/A      | N/A      | Hem  |
| 9214896  | M   | 2    | DMD   | exon66     | c.9568C>T  | p.R13190X | Hem  |
| 9341558  | M   | 10   | DSG2  | exon15     | c.3059_3062del | p.E1020fs | Het  |
| ID     | Sex | Chromosome Location | Gene          | Position | Mutation       | Type  |
|--------|-----|---------------------|---------------|----------|----------------|-------|
| 9292888 F | 3   | P                    | DSG2          | intron2  | c.82-1G>T      | N/A   |
| 9303877 M | 47  | P                    | DSG2          | intron12 | c.1880-2A>G    | N/A   |
| 9317499 M | 2   | P                    | DSG2          | exon15   | c.3039C>A      | p.Y1013X |
| 9292599 M | 0   | P                    | DSG2          | exon15   | c.3144-3147delAGA | p.R1049fs |
| 9184634 M | 2   | P                    | DSG2          | exon15   | c.2761_2764dupGCTA | p.T922fs |
| 9326883 F | 1   | P                    | DSG2          | exon15   | c.3144-3147del | p.R1049fs |
| 9251836 M | 5   | P                    | DSG2          | exon7    | c.797A>G       | p.N266S |
| 9328444 M | 13  | P                    | DSP           | exon8    | c.967G>T       | p.E323X |
| 9273021 M | 2   | P                    | DSP           | exon1    | c.88G>A        | p.V30M |
| 9291478 M | 4   | P                    | DSP           | exon1    | c.88G>A        | p.V30M |
| 9263671 F | 2   | P                    | DSP           | exon2    | c.269A>G       | p.Q90R |
| 9305153 M | 3   | P                    | DSP           | exon1    | c.88G>A        | p.V30M |
| 9284283 M | 1   | P                    | DSP           | exon1    | c.88G>A        | p.V30M |
| 9192058 M | 36  | P                    | DSP           | exon24   | c.7312G>T      | p.E2438X |
| 9220897 M | 8   | P                    | DSP           | exon23   | c.4008delG     | p.E1336fs |
| 9343179 F | 2   | P                    | DSP           | exon1    | c.G88A         | p.V30M |
| 9346862 F | 2   | P                    | DSP           | exon1    | c.G88A         | p.V30M |
| 9357604 F | 11  | P                    | DSP           | exon2    | c.A269G        | p.Q90R |
| 9297413 F | 2   | P                    | DSP           | exon1    | c.88G>A        | p.V30M |
| 9298153 F | 0   | P                    | DSP           | exon4    | c.478C>T       | p.R160X |
| 9284192 F | 4   | P                    | DSP           | exon24   | c.6954_6955del | p.G2319fs |
| 9236434 F | 5   | P                    | DSP           | exon23   | c.3931delC     | p.Q1311fs |
| 9006005 F | 33  | P                    | DSP           | exon11   | c.1273C>T      | p.R425X |
| 8983521 M | 4   | P                    | DSP           | exon24(Lastexon) | c.7623delG | p.K2542fs*19 |
| 9355087 F | 46  | P                    | DSP           | exon1    | c.G88A         | p.V30M |
| 8989081 F | 3   | P                    | DSP           | exon24(Lastexon) | c.5664_5667delTGAG | p.S1888Rfs*40 |
| 9243739 M | 18  | P                    | FKTN          | exon6    | c.642dupT      | p.D215fs |
| 9099262 M | 0   | P                    | FKTN          | exon5    | c.346C>T       | p.Q116X |
| 9207584 M | 20  | P                    | FLNC          | exon41   | c.6889G>A      | p.V2297M |
| 9087692 F | 0   | P                    | FLNC          | exon21   | c.3695_3698delGGCA | p.G1232fs |
| 9303246 F | 3   | P                    | FLNC          | intron44 | c.7384+1G>C    | N/A   |
| 9170761 F | 21  | P                    | FLNC          | exon28   | c.4926_4927insACGTCACA | p.V1643fs |
| 8980876 M | 18  | P                    | FLNC          | intronic | c.3791-1G>C    | N/A   |
| 9239511 F | 30  | P                    | FXN           | exon4    | c.389G>T       | p.G130V |
| Patient ID | Sex | Age | Gene | Exon | Mutation Type | Nucleotide Change | Protein Change | Allele

| 9327552 | F | 25 | JPH2 | exon1 | c.55delG | p.E19fs | Het |
| 9241033 | F | 6 | LAMA4 | exon2 | c.326C>G | p.S109X | Het |
| 9274105 | M | 6 | LAMA4 | exon2 | c.326C>G | p.S109X | Het |
| 9269909 | F | 5 | LAMA4 | exon2 | c.303_304insCCTT | p.E102_S103delinsPX | Het |
| 9223001 | M | 16 | LAMA4 | intron38 | c.5185+1G>A | N/A | Het |
| 9295716 | F | 11 | LAMA4 | exon2 | c.326C>G | p.S109X | Het |
| 9294085 | M | 0 | LAMA4 | exon11 | c.1240delG | p.V414fs | Het |
| 9175270 | F | 15 | LAMA4 | exon39 | c.5423dupT | p.V1808fs | Het |
| 9159593 | M | 7 | LAMA4 | intron4 | c.297+1_+5delinsTTAAC | N/A | Het |
| 9253979 | M | 15 | LAMA4 | exon19 | c.2395C>T | p.R799X | Het |
| 922936 | F | 4 | LAMA4 | exon15 | c.1831C>T | p.Q611X | Het |
| 9298955 | M | 1 | LAMA4 | intron38 | c.5185+1G>A | N/A | Het |
| 9297266 | M | 18 | LAMA4 | exon39 | c.5424_5425insT | p.V1808fs | Het |
| 9288782 | F | 1 | LAMA4 | exon2 | c.326C>G | p.S109X | Het |
| 9185605 | M | 14 | LAMA4 | exon39 | c.5423dupT | p.V1808fs | Het |
| 9195259 | F | 5 | LAMA4 | intron4 | c.297+1G>T | N/A | Het |
| 9272110 | F | 9 | LAMA4 | exon2 | c.326C>G | p.S109X | Het |
| 9003033 | M | 6 | LAMA4 | exon4 | c.406delC | p.L136Cfs*62 | Het |
| 9288515 | M | 15 | LAMP2 | intron8 | c.929-1G>C | N/A | Het |
| 8980119 | M | 1 | LAMP2 | exon5 | c.614_615delTG | p.V205Afs*21 | Hem |
| 9294290 | F | 8 | LAMP2 | exon5 | c.584_591delinsA | p.S195X | Het |
| 9338390 | F | 4 | LAMP2 | exon2 | c.138G>A | p.W46X | Het |
| 9189982 | F | 0 | LDB3 | intron4 | c.344+2T>C | N/A | Het |
| 9004410 | M | 7 | LDB3 | exon8 (Last exon) | c.850T>C | p.*284Qext*35 | Het |
| 9201784 | F | 21 | LMNA | exon2 | c.364A>T | p.K122X | Het |
| 9128770 | F | 6 | LP | LMNA | exon1 | c.158A>G | p.E53G | Het |
| 9271256 | F | 12 | LMNA | intron4 | c.810+1G>A | N/A | Het |
| 9197389 | F | 47 | LMNA | exon5 | c.892C>T | p.R298C | Het |
| 9290863 | M | 13 | LMNA | exon6 | c.1072G>A | p.E358K | Het |
| 9276918 | M | 16 | LMNA | exon7 | c.1357C>T | p.R453W | Het |
| 9152052 | M | 30 | LMNA | exon6 | c.1003delC | p.R335Gfs*2 | Het |
| 9348028 | F | 3 | MYBPC3 | exon17 | c.61505A | p.R502Q | Het |
| 9274105 | M | 6 | MYBPC3 | intron30 | c.3330+5G>C | N/A | Het |
| 9141487 | F | 4 | MYBPC3 | exon32 | c.3811C>T | p.R1271X | Het |
|   |   |   |   |   |   |
|---|---|---|---|---|---|
| 9084556 | M | 0 | P | MYBPC3 | intronic | c.1624+2T>C | N/A | Het |
| 9041363 | M | 11 | P | MYBPC3 | exon17 | c.1624G>C | p.E542Q | Het |
| 9276259 | M | 5 | P | MYBPC3 | intron22 | c.2148+1G>A | N/A | Het |
| 9151345 | M | 14 | P | MYBPC3 | intron11 | c.927-9G>A | N/A | Het |
| 9113089 | M | 3 | P | MYBPC3 | exon9 | c.901A>T | p.K301X | Het |
| 9089555 | M | 9 | P | MYBPC3 | exon32 | c.3697C>T | p.Q1233X | Het |
| 9075069 | M | 6 | P | MYBPC3 | intronic | c.2149-1G>A | N/A | Het |
| 897897 | F | 3 | P | MYBPC3 | intronic | c.3330+2T>G | N/A | Het |
| 9284365 | F | 10 | P | MYBPC3 | intron12 | c.926+1G>C | N/A | Het |
| 9286117 | F | 0 | P | MYBPC3 | exon7 | c.776delinsTT | p.A259fs | Het |
| 9276406 | F | 18 | P | MYBPC3 | intron9 | c.851+2T>C | N/A | Het |
| 9237050 | F | 0 | P | MYBPC3 | exon16 | c.1624G>C | p.E542Q | Het |
| 9104610 | F | 16 | P | MYBPC3 | intron22 | c.2149-1G>A | N/A | Het |
| 9028751 | F | 44 | P | MYBPC3 | exon29 | c.3192dupC | p.K1065Qfs*12 | Het |
| 9306432 | M | 2 | P | MYBPC3 | exon26 | c.2864_2865del | p.P955fs | Het |
| 9286647 | F | 3 | P | MYBPC3 | exon7 | c.776delinsTT | p.A259fs | Het |
| 9277883 | M | 5 | P | MYBPC3 | intron30 | c.3330+2T>G | N/A | Het |
| 9227540 | F | 9 | P | MYBPC3 | exon16 | c.1504C>T | p.R502W | Het |
| 9223012 | F | 46 | P | MYBPC3 | exon2 | c.237C>G | p.Y79X | Het |
| 9158948 | M | 1 | LP | MYBPC3 | exon28 | c.3065G>C | p.R1022P | Het |
| 9116248 | F | 59 | P | MYBPC3 | exon16 | c.1504C>T | p.R502W | Het |
| 9027708 | M | 6 | P | MYBPC3 | exon33 | c.3811C>T | p.R1271* | Het |
| 9214418 | M | 3 | P | MYH6 | intron29 | c.3979-2A>C | N/A | Het |
| 9275908 | F | 13 | P | MYH6 | exon31 | c.4525G>T | p.E1509X | Het |
| 9302277 | M | 8 | P | MYH6 | exon32 | c.4612G>T | p.E1538X | Het |
| 9265600 | F | 1 | P | MYH6 | exon38 | c.5688dupC | p.K1897fs | Het |
| 9318326 | F | 0 | P | MYH6 | intron14 | c.1410+1G>A | N/A | Het |
| 9302619 | F | 3 | P | MYH6 | exon29 | c.4047C>A | p.Y1349X | Het |
| 9300293 | F | 2 | P | MYH6 | exon25 | c.3251+2T>C | N/A | Het |
| 9249674 | U | 27 | P | MYH6 | exon27 | c.3849delG | p.T1284fs | Het |
| 9222893 | F | 40 | P | MYH6 | exon10 | c.864C>G | p.Y288X | Het |
| 8982569 | M | 3 | P | MYH6 | exon10 | c.864C>G | p.Y288* | Het |
| 9172313 | F | 2 | P | MYH6 | exon10 | c.864C>G | p.Y288X | Het |
| 9247816 | M | 2 | P | MYH7 | exon37 | c.5401delG | p.E1801fs | Het |
| Accession | Gender | Age | Chromosome | Location | Mutation | Phenotype |
|-----------|--------|-----|-------------|----------|----------|------------|
| 9214179   | M      | 1   | MYH7       | exon25   | c.3157C>T | p.R1053W   | Het        |
| 9252616   | F      | 75  | MYH7       | intron11 | c.896-2A>G | N/A        | Het        |
| 9163168   | M      | 15  | MYH7       | exon37   | c.5401G>A  | p.E1801K   | Het        |
| 9265366   | M      | 2   | MYH7       | exon15   | c.1573G>A  | p.E525K    | Het        |
| 9281932   | F      | 0   | MYH7       | exon33   | c.4588C>T  | p.R1530X   | Het        |
| 9261619   | M      | 0   | MYH7       | intron31 | c.4170-2A>G | N/A        | Het        |
| 9081964   | M      | 31  | MYH7       | exon23   | c.2717A>G  | p.D906G    | Het        |
| 9238370   | M      | 0   | MYH7       | exon39   | c.5740G>A  | p.E1914K   | Het        |
| 9221589   | M      | 65  | MYH7       | exon27   | c.3592delG | p.D1198fs  | Het        |
| 9200127   | F      | 7   | MYH7       | exon18   | c.1988G>A  | p.R663H    | Het        |
| 9213408   | F      | 7   | MYH7       | exon21   | c.2389G>A  | p.A797T    | Het        |
| 9279293   | M      | 0   | MYH7       | exon7    | c.602T>C   | p.I201T    | Het        |
| 9255251   | F      | 5   | MYH7       | exon35   | c.4954G>T  | p.D1652Y   | Het        |
| 9188647   | M      | 22  | MYH7       | exon33   | c.4588C>T  | p.R1530X   | Het        |
| 9318391   | F      | 5   | NEXN       | exon13   | c.2023_2026del | 675_676del | Het        |
| 9224787   | M      | 1   | NEXN       | intron4  | c.299-1G>C | N/A        | Het        |
| 9199955   | F      | 41  | NEXN       | exon13   | c.1949_1951del | G650del   | Het        |
| 9269379   | F      | 6   | PKP2       | exon1    | c.144_165del | P49fs    | Het        |
| 9348227   | F      | 12  | PKP2       | exon3    | c.419C>T   | p.S140F   | Het        |
| 9294279   | M      | 4   | PKP2       | intron12 | c.2299+1G>A | N/A        | Het        |
| 9357592   | M      | 1   | PKP2       | exon3    | c.C419T   | p.S140F   | Het        |
| 9285538   | M      | 6   | PKP2       | exon10   | c.2013del  | p.K672fs  | Het        |
| 9355326   | F      | 0   | PKP2       | exon3    | c.C419T   | p.S140F   | Het        |
| 9324064   | M      | 1   | PKP2       | exon3    | c.663C>A   | p.Y221X   | Het        |
| 9257386   | M      | 1   | PKP2       | intron11 | c.2146-1G>C | N/A        | Het        |
| 9217471   | M      | 9   | PKP2       | exon7    | c.1677dupT | p.G560fs  | Het        |
| 8989765   | M      | 0   | PKP2       | exon1    | c.181C>T   | p.Q61*    | Het        |
| 9321113   | M      | 50  | PKP2       | exon12   | c.2484C>T  | p.G828G   | Het        |
| 9240662   | F      | 18  | PKP2       | intron8  | c.1689-1G>C | N/A        | Het        |
| 9305468   | F      | 1   | PKP2       | intron11 | c.2146-1G>C | N/A        | Het        |
| 9109983   | M      | 0   | PKP2       | exon3    | c.772A>T   | p.K258X   | Het        |
| 9033892   | M      | 21  | PKP2       | intronic | c.1379-1G>A | N/A        | Het        |
| 9162448   | M      | 1   | PLN        | exon2    | c.39_41del | p.R14del  | Het        |
| 9263671   | F      | 2   | RBM20      | exon2    | c.862C>T   | p.Q288X   | Het        |
| 9152004  | F  | 6 | P | RBM20     | exon11 | c.3261delC | p.S1087fs | Het  |
| 9275792  | F  | 1 | P | RBM20     | exon11 | c.3260_3261insC | p.S1087fs | Het  |
| 9234011  | F  | 3 | P | SCN5A     | exon22 | c.3911C>T | p.T1304M | Het  |
| 9312833  | M  | 2 | P | SCN5A     | exon22 | c.3911C>T | p.T1304M | Het  |
| 9070239  | M  | 0 | P | SCN5A     | exon28 | c.5350G>A | p.E1784K | Het  |
| 9287024  | M  | 3 | P | SCN5A     | exon2  | c.80G>A  | p.R27H  | Het  |
| 9348328  | M  | 10| P | SCN5A     | exon7  | c.892G>A | p.G298S | Het  |
| 9247808  | M  | 10| P | SCN5A     | exon14 | c.2204C>T | p.A735V | Het  |
| 9200921  | M  | 5 | P | SCN5A     | exon22 | c.3911C>T | p.T1304M | Het  |
| 9120136  | M  | 5 | P | SCN5A     | exon28 | c.5825_5826delCT | p.P1942fs | Het  |
| 9304686  | F  | 16| P | SCN5A     | exon23 | c.4018G>A | p.V1340I | Het  |
| 9274526  | F  | 5 | P | SCN5A     | exon14 | c.2091G>A | p.W697X | Het  |
| 9223555  | F  | 11| P | SCN5A     | exon28 | c.5830C>T | p.R1944X | Het  |
| 9221609  | F  | 7 | P | SCN5A     | exon12 | c.1567C>T | p.R523C | Het  |
| 9242182  | M  | 5 | P | SCN5A     | exon28 | c.5227G>A | p.G1743R | Het  |
| 9311069  | F  | 0 | P | SCN5A     | exon28 | c.5830C>T | p.R1944X | Het  |
| 9304464  | F  | 1 | P | SCN5A     | exon28 | c.5464_5467del | p.E1823fs | Het  |
| 9214806  | M  | 10| P | SCN5A     | exon22 | c.3911C>T | p.T1304M | Het  |
| 9204408  | M  | 0 | P | SCN5A     | exon21 | c.3823G>A | p.D1275N | Het  |
| 9294484  | M  | 15| P | SCN5A     | exon2  | c.80G>A  | p.R27H  | Het  |
| 9326783  | M  | 4 | P | SCN5A     | exon22 | c.3911C>T | p.T1304M | Het  |
| 9306175  | M  | 2 | P | SCN5A     | exon28 | c.5851G>A | p.V1951M | Het  |
| 9337616  | M  | 10| P | SCN5A     | exon22 | c.3911C>T | p.T1304M | Het  |
| 9284225  | M  | 9 | P | SCN5A     | exon2  | c.80G>A  | p.R27H  | Het  |
| 9284404  | M  | 0 | P | SCN5A     | intron22 | c.3840+1G>A | N/A | Het  |
| 9281823  | F  | 16| P | SCN5A     | exon28 | c.5851G>A | p.V1951M | Het  |
| 9146820  | F  | 8 | P | SCN5A     | exon22 | c.3911C>T | p.T1304M | Het  |
| 9108724  | M  | 1 | LP| SCN5A     | exon28 | c.5872C>T | p.R1958X | Het  |
| 9267024  | F  | 13| P | SGCD      | exon6  | c.493C>T  | p.R165X | Het  |
| 9252421  | M  | 15| P | SYNE1     | intron72 | c.11734-1G>A | N/A | Het  |
| 9295982  | M  | 7 | P | SYNE1     | exon126 | c.22918C>T | p.Q7640X | Het  |
| 9198126  | M  | 9 | P | SYNE1     | exon94  | c.17680C>T | p.Q5894X | Het  |
| 9259834  | F  | 8 | P | SYNE1     | intron40 | c.5288+1G>A | N/A | Het  |
| 9339052  | F  | 0 | P | SYNE1     | exon61  | c.9777_9778del | p.3259_3260del | Het |
| Document ID | Genotype | Gene | Exon/Intron | variant | Mutation Type | Parental Origin |
|-------------|----------|------|-------------|---------|---------------|-----------------|
| 9333397     | M        | SYNE1| exon16      | c.1583C>G | p.S528X       | Het             |
| 9260379     | F        | SYNE1| exon83      | c.15898C>T | p.R5300X      | Het             |
| 9219968     | F        | SYNE1| exon114     | c.20935C>T | p.R6979X      | Het             |
| 9149696     | M        | SYNE1| intron64    | c.10166+1G>A | N/A           | Het             |
| 9123665     | F        | SYNE1| exon106     | c.19626delG | p.K6543fs     | Het             |
| 9319382     | F        | SYNE1| exon77      | c.14077C>T | p.R4693X      |                 |
| 9301351     | M        | SYNE1| exon56      | c.8978_8979insC | p.L2993fs |                 |
| 9275946     | F        | SYNE1| intron119   | c.21648+1G>A | N/A           | Het             |
| 9275786     | M        | SYNE1| intron102   | c.18891+2T>C | N/A           | Het             |
| 9193228     | F        | SYNE1| exon15      | c.1390delG  | p.D464fs      | Hom             |
| 9192256     | F        | SYNE1| exon77      | c.13117C>T | p.R4373X      |                 |
| 9105888     | F        | SYNE1| exon43      | c.6264_6268del | p.2088_2090del |                 |
| 9102431     | M        | SYNE1| exon35      | c.4582C>T  | p.R1528X      |                 |
| 9232304     | M        | TAZ  | intron3     | c.284+1G>T  | N/A           | Hem             |
| 9224694     | F        | TCP  | exon2       | c.260G>A    | p.R87Q        |                 |
| 9276987     | M        | TNNC1| exon3       | c.162delT   | p.P54fs       |                 |
| 9314798     | M        | TNNI3| exon7       | c.484C>T    | p.R162W       |                 |
| 8987475     | M        | TNNI3| exon5       | c.258delC   | p.L88Wfs*27   |                 |
| 9203650     | F        | TNNI3| exon7       | c.485G>A    | p.R162Q       |                 |
| 9206448     | F        | TNNI3| exon7       | c.544G>A    | p.E182K       |                 |
| 9202021     | M        | TNNI3| exon7       | c.470C>T    | p.A157V       |                 |
| 8972543     | M        | TNNI3| exon5       | c.204delG   | p.R69Afs*8    |                 |
| 9171174     | F        | TNNI3| exon8       | N/A         | N/A           | Hom             |
| 9211395     | M        | TNNT2| exon11      | c.472C>T    | p.R158X       |                 |
| 9331832     | M        | TNNT2| exon14      | c.732G>T    | p.E244D       |                 |
| 9187512     | M        | TNNT2| exon11      | c.475C>T    | p.R159X       |                 |
| 8970130     | M        | TNNT2| exon10      | c.421delC   | p.R141Gfs*41  |                 |
| 9259795     | F        | TNNT2| exon10      | c.391C>T    | p.R131W       |                 |
| 9017877     | F        | TPM1 | exon4       | c.475G>A    | p.D159N       |                 |
| 9319947     | M        | TPM1 | exon7       | c.688G>A    | p.D230N       |                 |
| 9248907     | M        | TPM1 | exon1       | c.58C>T     | p.Q20X        | Hom             |
| 9017876     | F        | TPM1 | exon4       | c.475G>A    | p.D159N       |                 |
| 9261702     | F        | VCL  | exon19      | c.2828_2829del | p.943_943del |                 |
| 9329913     | M        | VCL  | exon1       | c.57delG    | p.I20fs       |                 |
| ID     | Sex | Age | Gene | Exon | Mutation          | Phenotype | Haplotype |
|--------|-----|-----|------|------|-------------------|-----------|-----------|
| 9267878| F   | 14  | VCL  | exon19 | c.2828_2829del   | p.943_943del | Het       |
| 9226564| M   | 14  | VCL  | exon19 | c.2949delG       | p.K983fs   | Het       |
| 9245159| F   | 0   | VCL  | exon20 | c.3111_3112insGGCC | p.K1038fs | Het       |

P-Pathogenic LP-Likely Pathogenic
**Figure S1:** Gene-specific yields from dilated cardiomyopathy (DCM) pathologic variants identified in the literature and DCM genetic testing referrals from Invitae. Bar graph showing yield of likely pathogenic or pathogenic (LP/P) variants among the literature cohort (orange fill) and LP/P variants identified in the Invitae cohort (blue fill). Error bars represent 95% confidence interval.
Figure S2: Bar graphs of the pathogenicity-specific yield by cohort in the set of genes with definitive association with cardiomyopathy based on ClinGen\textsuperscript{30}. Graph of the likely pathogenic or pathogenic (LP/P) variant yield for the dilated cardiomyopathy (DCM), exome sequencing (ES), and gnomAD cohorts (A). Graph of variant of unknown significance (VUS) variant yield for the ES and gnomAD cohorts (B).
Figure S3: Signal-to-noise (S:N) analysis of variant yield of the dilated cardiomyopathy (DCM) cohort (A) and exome sequencing (ES) cohort likely pathogenic or pathogenic (LP/P) (B), respectively, normalized to gnomAD variant yield labeled LP/P in ClinVar by gene, as well as positive predictive value (PPV) of an LP/P variant in the general population by gene using a population prevalence of familial DCM of 1:2,500 (C). Error bars represent 95% confidence intervals of divided proportions.
Figure S4: Comparison of variant yield by non-TTN, dilated cardiomyopathy (DCM)-associated genes in the DCM and exome sequencing (ES) cohorts, by pathogenicity, with the gnomAD cohort overall yield. Displayed comparisons include variant yields in the DCM vs ES cohort (likely pathogenic or pathogenic (LP/P)) (A), the DCM vs gnomAD cohort (B), the ES (LP/P) vs gnomAD cohort (C), and the ES (variant of unknown significance (VUS)) vs gnomAD cohort (D). All variants in the DCM cohort are LP/P. $R^2$ values represent the square of the Pearson’s correlation coefficient. Each point represents a single gene, labeled when possible. SYNE1 is a clear outlier in panels B, C,
and D, and is therefore excluded from calculation of the best fit lines and the $R^2$ values and represented with a red “X”.

**Figure S5**: Comparison of variant yield by non-**TTN**, dilated cardiomyopathy (DCM)-associated genes in the DCM and exome sequencing (ES) cohorts, by pathogenicity, with the protein size (amino acid length). Displayed comparisons include variant yields in the DCM cohort (A), the ES cohort (likely pathogenic or pathogenic (LP/P)) (B), the exome sequencing (ES) cohort (variant of unknown significance (VUS)) (C), and the gnomAD cohort (D). All variants in the DCM cohort are LP/P. $R^2$ values represent the square of the Pearson’s correlation coefficient. Each point represents a
single gene, labeled when possible. *SYNE1* is a clear outlier in all panels and is therefore excluded from calculation of the best fit lines and the $R^2$ values and represented with a red “X”.