



RESEARCH ARTICLE

Critical assessment of secondary findings in genes linked to primary arrhythmia syndromes

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Abstract

As comprehensive sequencing technologies gain widespread use, questions about so-called secondary findings (SF) require urgent consideration. The American College of Medical Genetics and Genomics has recommended to report SF in 59 genes (ACMG SF v2.0) including four actionable genes associated with inherited primary arrhythmia syndromes (IPAS) such as catecholaminergic polymorphic ventricular tachycardia, long QT syndrome, and Brugada syndrome. Databases provide conflicting results for the purpose of identifying pathogenic variants in SF associated with IPAS at a level of sufficient evidence for clinical return. As IPAS account for a significant proportion of sudden cardiac deaths (SCD) in young and apparently healthy individuals, variant interpretation has a great impact on diagnosis and prevention of disease. Of 6381 individuals, 0.4% carry pathogenic variants in one of the four actionable genes related to IPAS: RYR2, KCNQ1, KCNH2, and SCN5A. Comparison of the databases ClinVar, Leiden Open-source Variant Database, and Human Gene Mutation Database showed impactful differences (0.2% to 1.3%) in variant interpretation improvable by expert-curation depending on database and classification system used. These data further highlight the need for international consensus regarding the variant interpretation, and subsequently management of SF in particular with regard to treatable arrhythmic disorders with increased risk of SCD.

KEYWORDS

actionable genes, cardiac channelopathy genes, primary arrhythmia syndromes, secondary findings, variant classification, variant interpretation

1 | BACKGROUND

The use of wide-scale sequencing in clinical medicine is increasing the need to define guidelines on the management of so-called secondary findings (SF), that is variants in genes unrelated to the primary disease conditions. As SF can be of great value in early disease prevention and intervention (Lawrence et al., 2014), recommendations for laboratories to report those findings have stimulated interest (Amendola et al., 2015;

Dorschner et al., 2013). The American College of Medical Genetics and Genomics (ACMG) has recommended return of SF from a minimum set of 59 actionable genes when comprehensive NGS analysis was performed (ACMG SF v2.0; Green et al., 2013; Kalia et al., 2017) to manage risks for selected genetic disorders through interventions aimed at preventing or reducing morbidity and mortality. However, the interpretation of identified sequence variants across these selected genetic disorders is still challenging.

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Interpretation of variants requires evaluation of data from diverse sources, including computational data, data derived from the literature and clinical and functional observations. Lack of consensus criteria for pathogenicity assessment of variants is an ongoing issue in genomic medicine. Importantly, 26 of 59 actionable genes on the ACMG SF v2.0 list are associated with cardiovascular diseases such as aortopathies, vascular connective tissue disease, cardiomyopathies or inherited primary arrhythmia syndromes (IPAS), namely catecholaminergic polymorphic ventricular tachycardia (CPVT), long QT syndrome (LQTS), and Brugada syndrome (BrS). IPAS are also called “cardiac ion channelopathies” as a majority of the primary arrhythmias are caused by pathogenic variants in the genes encoding the ion channels of the heart, namely Na⁺, K⁺, and Ca⁺⁺ channels. IPASs account for a significant proportion of sudden cardiac deaths (SCD) in young and apparently healthy individuals (Gray, Ackerman, Semsarian, & Behr, 2019). The four core genes causing IPAS are *KCNQ1* associated with long QT syndrome type 1 (LQTS1), *KCNH2* associated with long QT syndrome type 2 (LQTS2), *SCN5A* associated with long QT syndrome type 3 (LQTS3) or BrS and *RYR2* associated with CPVT. As the early awareness of genetic risk (identification of a pathogenic variant in one of the main genes associated with IPAS) could be of great importance for disease prevention and intervention, reporting these SF can be an opportunity to significantly reduce the risk of SCD. On the other hand, reports of genetic variants related to certain severe diseases before the occurrence of any phenotype may cause an unnecessary psychological burden on individuals. Thus, a major controversy has developed regarding the return of secondary findings to patients, particularly in a disease with reduced penetrance. The cardiovascular genetic community has developed recommendations for the detection and management of genotype-positive, phenotype-negative patients in the context of directed familial cascade screening (Al-Khatib et al., 2018; Priori et al., 2013). As genetic results have a great impact on the clinical treatment strategies, only variants of known or expected pathogenicity (class 4 or 5) should be considered for evaluation (Richards et al., 2015). However, standardization of pathogenicity assessment of those variants needs to be improved by correct variant interpretation in certified laboratories with the appropriate expertise. Here we examined the frequency of SF in the four actionable genes causing IPAS in 6,381 NGS analyses, evaluated the pathogenicity assessment of those variants by common databases, such as public archive of interpretations of clinically relevant variants (ClinVar), Leiden Open-source Variant Database (LOVD), and Human Gene Mutation Database (HGMD) and compared the results with our variant classification. We curated variants with conflicting data and demonstrated the complexity of variant interpretation in particular in genes associated with IPAS. Our data analysis and variant interpretation provide a general view of SF associated with the risk of SCD and thus could be used for the management of patients with SF.

2 | METHOD

2.1 | High throughput sequencing and bioinformatics pipeline

Next-generation sequencing analysis (NGS) was carried out on an Illumina NextSeq 500 system (Illumina, San Diego, CA) as 150 bp paired-end sequencing runs using v2.0 SBS chemistry. Sequencing reads were aligned to the human reference genome (GRCh37/hg19) using BWA (v0.7.13-r1126) with standard parameters. Statistics on coverage and sequencing depth on the clinically targeted regions (i.e., RefSeq coding exons and ± 5 intronic region) was calculated with a custom script. SNV and INDEL calling on the genes were conducted using SAMtools (v1.3.1) with subsequent coverage and quality dependent filter steps. Variant annotation was performed with snpEff (v4.2) and Alamut-Batch (v1.4.4). Variants (SNVs/small INDELS) in the coding and flanking intronic regions (± 50 bp) were only evaluated.

2.2 | Development of the gene list for the screening of secondary findings

About 6,381 next-generation sequencing (NGS) data (individuals unrelated to arrhythmic or cardiovascular disorders) were analyzed for variants in the four actionable genes of the ACMG secondary findings (SF) v2.0 list (Biesecker, 2017; Kalia et al., 2017) associated with inherited primary arrhythmia syndromes (IPAS) such as catecholaminergic polymorphic ventricular tachycardia (CPVT), long QT syndrome (LQTS), and Brugada syndrome (BrS). The four selected genes related to IPAS are *KCNH2* (NM_000238.3), *KCNQ1* (NM_000218.2), *RYR2* (NM_001035.2), and *SCN5A* (NM_198056.2). Informed consent was obtained from all individuals and approved by local institutions (2019-091). According to the German data protection and gene diagnostic law, we reported the pathogenic variants in actionable genes listed by ACMG. Variants of unknown significance, whose involvement in disease at the current time was unclear, were not reported.

2.3 | Nomenclature, interpretation, and classification of genetic variants

The nomenclature guidelines of the Human Genome Variation Society (HGVS) were used to describe DNA sequence variants (den Dunnen & Antonarakis, 2000). The missense variants were interpreted with amino acid (AA) substitution effect prediction methods: Sorting Invariant from Tolerated (SIFT), PolyPhen-2, Mutation Taster, and MAPP. Splice-sites were predicted with MES and SSF. Population databases were used to assess the allele frequencies of the variants: Database of all known single nucleotide polymorphisms (dbSNP), Exome Aggregation Consortium (ExAC), and Genome Aggregation Database (gnomAD). The variants were considered benign for rare autosomal dominant disorders when the minor allele frequency (MAF) was >0.01 .

The variants were classified according to the ACMG guidelines with the 5-tier classification system: class 5 (pathogenic), class 4 (likely pathogenic), class 3 (variants of unknown significance, VUS), class 2 (likely benign), and class 1 (benign; Matthijs et al., 2016). In addition, Cardio Classifier (Whiffin et al., 2018), a semi-automated decision-support tool for inherited cardiac conditions (ICCs) was used for variant interpretation. Cardio Classifier integrates data retrieved from multiple sources with user-input case-specific information, to support variant interpretation if possible. The literature cited by HGMD, PubMed, and Mastermind (Genomenon) were reviewed. Variants classified as likely pathogenic (class 4), pathogenic (class 5), and disease-causing mutation (DM) are named uniformly as pathogenic in the following study. The variant classification was compared by common databases such as HGMD, LOVD, and ClinVar. HGMD represents an attempt to collate all published variants in genes responsible for the human inherited disease (Stenson et al., 2017). LOVD is a web-based open-source database developed at the Leiden University Medical Center in the Netherlands. LOVD is designed as a tool for gene-centered collection and display of DNA variants (Fokkema et al., 2011). ClinVar is a freely accessible, public archive of reports of the relationship among human variations and phenotypes, with supporting evidence (Landrum et al., 2016). ClinVar uses standard terms for clinical significance recommended by an authoritative source when available. Differences in interpretation among submitters within those five levels are reported as a conflict

using the phrase “conflicting interpretations of pathogenicity”. Our variant interpretation was done by certified molecular geneticists specialized in data analysis and annotation with significant relevant expertise and clinical geneticists with more than 10 years of experience (MGZ-curators).

3 | RESULTS

3.1 | 6,381 next-generation sequencing analyses were screened for pathogenic secondary findings associated with inherited primary arrhythmia syndrome

In the first step, we investigated the presence of SF in the four actionable IPAS-related genes: *RYR2*, *KCNQ1*, *KCNH2*, and *SCN5A* in 6,381 NGS analyses of individuals unrelated to arrhythmic or cardiovascular disorders.

Taken together, 1,217 sequence variants (class 1–5) in *RYR2* associated with CPVT, 1,216 variants in *KCNQ1* associated with LQTS1, 540 variants in *KCNH2* associated with LQTS2, and 758 variants in *SCN5A* associated with LQTS3 or BrS were identified in 6,381 NGS analyses (Figure 1). Variants with a frequency of 1% or higher in the population (MAF > 0.01), variants that are listed as

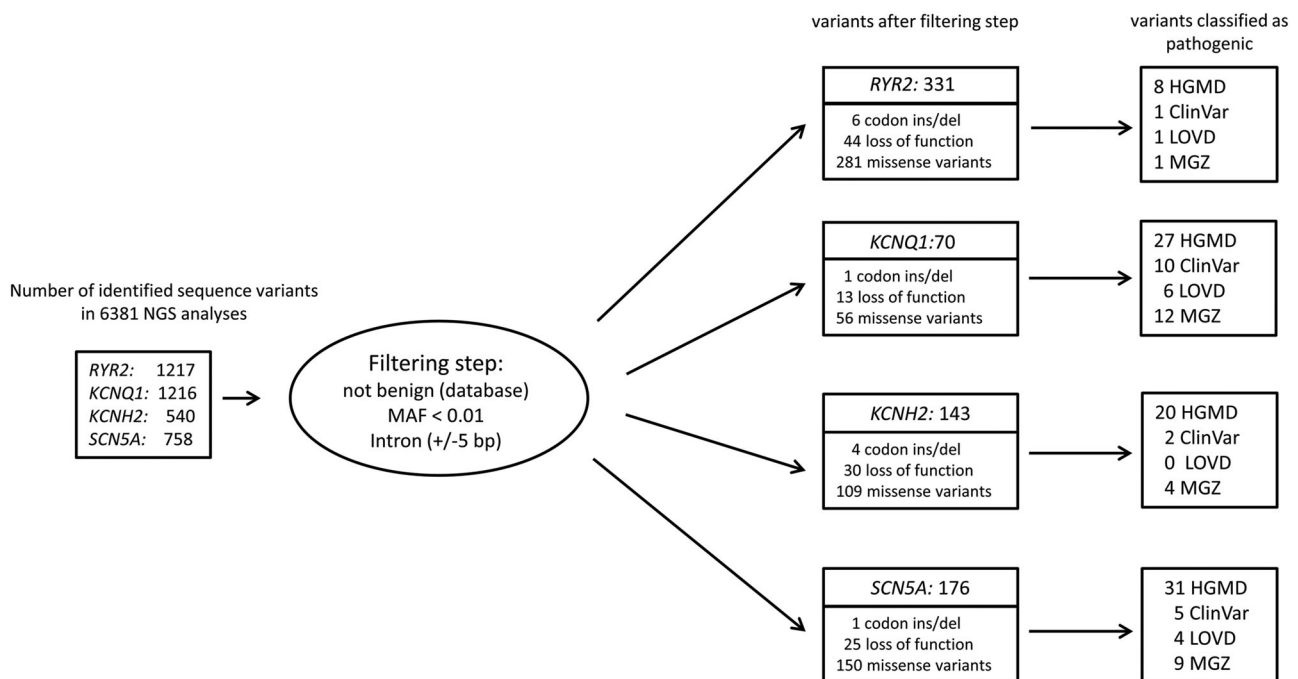


FIGURE 1 Flow chart of variant filtering steps for identification of pathogenic variants in the actionable genes *RYR2*, *KCNQ1*, *KCNH2*, and *SCN5A*. 6,381 next-generation sequencing (NGS) data were analyzed for pathogenic variants in *RYR2*, *KCNQ1*, *KCNH2*, and *SCN5A*. Variants were filtered based on genotype quality, coverage and allele frequency. The variants were considered benign when the minor allele frequency (MAF) was >0.01. Number and type of variants after the filtering step was listed as codon insertion/deletion (codon ins/del), loss of function (LoF) variants and missense variants. LoF variants include frameshift, stop-gain, start-loss, and splice-site variants. Number of variants classified as pathogenic (class 4, class 5 according to the ACMG guidelines and disease causing mutations (DM)) in at least one of the databases Human Gene Mutation Database (HGMD), public archive of interpretations of clinically relevant variants (ClinVar), Leiden Open-source Variant Database (LOVD), or by Medical Genetics Center (MGZ)-curators were listed

benign in databases and intronic variants that are located more than five bases upstream or downstream of an exon were excluded. After that, 331 variants in *RYR2*, 70 variants in *KCNQ1*, 143 variants in *KCNH2*, and 176 variants in *SCN5A* were identified and retained for further classification (Figure 1). Only variants classified as likely pathogenic (class 4), pathogenic (class 5) or “disease-causing mutation” (DM) in at least one database were considered for further evaluation (Figure 1 and Table S1).

The 331 variants in *RYR2* were grouped into six codon insertion/deletion (ins/del) variants, 44 loss-of-function (LoF) variants (frameshift, stop-gained/loss, splice-site), and 281 missense variants. Of these variants eight variants were listed as DM by HGMD, one was classified as pathogenic by ClinVar, one variant was listed as pathogenic by LOVD and one variant was classified as pathogenic by us. The 70 variants identified in *KCNQ1* include 1 single codon deletion, 13 LoF, and 56 missense variants. Twenty-seven variants were listed as DM in HGMD, 10 variants were classified as pathogenic by ClinVar, six variants were listed as pathogenic by LOVD. We classified 12 of these variants in the *KCNQ1* gene as pathogenic. From all 143 sequence variants identified in the *KCNH2* gene after the first filtering step, four codon ins/del, 30 LoF, and 109 missense variants were documented. Twenty of these variants were classified as DM by HGMD, two variants were classified as pathogenic by ClinVar, no variant was listed as pathogenic by LOVD and four of these variants were classified as pathogenic by our review. In *SCN5A*, 1 codon del/ins, 25 LoF, and 150 missense variants were present in all NGS analyses. Thirty-one variants were listed as disease-causing in HGMD, five of these identified variants were classified as pathogenic by ClinVar, four were listed as pathogenic by LOVD, and nine by our review (Figure 1).

Overall, in 6,381 NGS analyses, 86 (1.3%) pathogenic variants were identified by HGMD, 18 (0.3%) by ClinVar, 11 (0.2%) by LOVD, and 25 (0.4%) by our review in *RYR2*, *KCNQ1*, *KCNH2*, and *SCN5A*.

3.2 | Comparison of variant classification between different databases in IPAS-related actionable genes

As interpretation of the variants in *RYR2*, *KCNQ1*, *KCNH2*, and *SCN5A* has a great impact in providing diagnosis and subsequently prevention of SCD, we focused on the interpretation of the identified pathogenic variant in the four genes and compared the number of pathogenic variants listed in HGMD, ClinVar, and LOVD with our curated data (Table S1 and Figure 2). Data were evaluated to determine the potential pathogenicity of each variant, as described above.

In *RYR2* (CPVT), eight variants were classified as pathogenic in at least one of the databases or by our curators. Only one of the eight pathogenic variants listed by HGMD was classified as pathogenic by ClinVar, LOVD, and by our curators (Table S1). One variant of *RYR2* was classified with conflicting interpretations (class 1–3) by ClinVar and two by LOVD.

In *KCNQ1* (LQTS1), 28 variants were listed as pathogenic in at least one of the databases or classified by our curators (27 in HGMD, 10 in ClinVar, 6 in LOVD, and 12 by MGZ). Overall, 11 of 28 variants

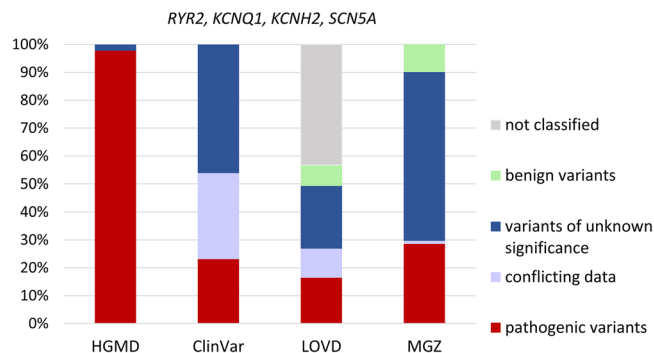


FIGURE 2 Variant classification of secondary findings in *RYR2*, *KCNQ1*, *KCNH2*, and *SCN5A*. Data from 6381 next-generation sequencing (NGS) runs were analyzed for pathogenic variants in *RYR2*, *KCNQ1*, *KCNH2*, and *SCN5A*. Variants were selected for further interpretation if they were classified as pathogenic in at least one of the databases Human Gene Mutation Database (HGMD), ClinVar (public archive of interpretations of clinically relevant variants), Leiden Open-source Variant Database (LOVD), or by Medical Genetics Center (MGZ)-curators (MGZ; class 4 and class 5 according to the ACMG guidelines or disease causing mutations, DM). Red bars indicate the percentage of pathogenic variants, blue bars indicate the percentage of variants of unknown significance (class 3 according to the ACMG guidelines or disease causing? (DM?)), green bars indicate benign variants (class 1 and class 2 according to the ACMG guidelines), purple bars indicate conflicting data of variant interpretation by different submitters. HGMD listed 86 variants (95%), ClinVar 18 variants (20%), LOVD 11 variants (12%), and MGZ classified 26 variants (29%) as pathogenic. ClinVar listed 29% conflicting data

were listed in LOVD but not classified (n.c.). Five variants of the 28 (18%) were classified as pathogenic by all four databases (HGMD, ClinVar, LOVD, and MGZ). Five variants were documented as pathogenic in HGMD, ClinVar and by us. All these variants were listed but not classified by LOVD. Four variants were classified with conflicting interpretations by ClinVar (class 1–5). None of those four conflicting variants was classified as pathogenic by us (Table S1). Two variants were listed by LOVD with conflicting interpretations (class 1–3). One of those variants was classified as benign and one as VUS (class 3) by us. The majority (21 of 28) of the listed pathogenic variants in *KCNQ1* were missense variants, seven were LoF variants (three frameshift, two codons del/ins, and two splice site variants). One frameshift variant c.270del (p.Val91Serfs*146; class 5) identified by us in *KCNQ1* was not listed in any of the databases.

In *KCNH2* (LQTS2), 21 variants were listed as pathogenic in at least one of the databases or classified by our curators (20 in HGMD, two in ClinVar, and four by our curators). None of those variants were listed as pathogenic by LOVD. Three variants of 21 (14%) were listed in HGMD, ClinVar and MGZ as pathogenic variants but were not classified in LOVD. Overall, nine variants were listed but not classified in LOVD. Nineteen of 21 identified pathogenic variants were missense variants, only two were frameshift variants. The missense variants were classified with low concordance between HGMD, ClinVar, LOVD, and our team. None of the missense variants

was classified as pathogenic by HGMD, ClinVar, LOVD, and MGZ. Moreover, nine of the potentially pathogenic variants identified in *KCNH2* were classified with conflicting interpretations (class 2–5) by ClinVar. We classified only one of those conflicting variants as pathogenic.

In *SCN5A* (LQTS3 and BrS), 34 variants were classified as pathogenic in at least one of the databases or by our curators (31 in HGMD, 5 in ClinVar, 4 in LOVD, and 9 by our review). Eight variants listed by LOVD were not classified. Two of the 34 identified variants (6%) were classified as pathogenic in all databases. Two of the identified variants were listed as pathogenic in HGMD, ClinVar, and in our database but not in LOVD. One pathogenic variant c.3141_3142dup (p.Pro1048Argfs*98) in *SCN5A* found in this study has not been reported in any of the databases before. Ten of all 34 potentially pathogenic missense variants were classified with conflicting interpretations (class 1–5) by ClinVar, indicating that variant interpretation of *SCN5A* is most challenging.

Overall, 97 pathogenic variants in *KCNQ1*, *KCNH2*, *SCN5A*, and *RYR2* found in 6,381 NGS analysis were listed in at least one of the databases or classified by our curators. HGMD classified 86 variants (95%), ClinVar 18 variants (20%), and LOVD 11 variants (12%) and we classified 26 variants (29%) as pathogenic (Figure 2). About 26% of those variants showed conflicting data in ClinVar. Recently submitted variants showed higher concordance between the classification compared to the variants submitted earlier. Our curators classified 25% of the conflicting variants listed by ClinVar as benign and 12.5% as pathogenic variants (Figure 2) and the remaining variants were classified as a variant of VUS.

4 | DISCUSSION

Here, we reported that 0.4% of the 6,381 individuals carry pathogenic variants in one of the four IPAS-related genes (*RYR2*, *KCNQ1*, *KCNH2*, *SCN5A*) listed as actionable genes in the ACMG v2.0 list. Comparison of the databases ClinVar, LOVD, and HGMD showed impactful differences (0.2% to 1.3%) in variant interpretation. The frequency of pathogenic variants identified was dependent on the database and the classification system. As IPAS accounts for a significant proportion of SCD in young and apparently healthy individuals, the classification of variants in IPAS related genes has a great impact to provide diagnosis and prevention of disease. Thus, we compared the classification of the identified variants in these genes in the most relevant reference databases, HGMD, ClinVar, and LOVD and compared our in-house classification with these data. Our study shows that the rate of medically significant discrepancies in variant interpretation between ClinVar, LOVD, and MGZ was low, indicating a good concordance especially for pathogenic variants between those sources. The highest concordance of the variant interpretation was found in *KCNQ1*. Overall, the lowest agreement in the classification of pathogenic variants was found in *SCN5A*. The data rates demonstrate the need for an improved classification system, curation of

variants by expert teams and further highlights the need of a disease- and gene-specific decision support tool.

The ACMG guidelines for the interpretation of sequence variants were a major step towards establishing a shared framework for variant classification. Nevertheless, in addition to our study, it has been recently shown that even when following the ACMG guidelines, variant interpretation can differ between laboratories, with a discordance greater than 10% (Harrison et al., 2017). Initiatives such as the Clinical Genome Resource (ClinGen; Rehm et al., 2015) are working to define disease- and gene-specific thresholds, although these are currently limited to pilot phases for specific gene-disease pairs (Whiffin et al., 2018). Whiffin et al. (2018) developed the Cardio Classifier, which is a decision-support tool that assists in the disease-specific interpretation of genetic variants in genes associated with inherited cardiac conditions (ICCs). Cardio Classifier utilizes the framework outlined by the ACMG guidelines to automatically annotate variants across different criteria, which have been individually parameterized for gene and disease-specific knowledge. As Cardio Classifier is an important tool in the variant interpretation of cardiogenetic genes, this classification program should be extended in the future with additional genes and further gene-specific evidence.

Nevertheless, the interpretation of genetic variants has not kept pace with the expansion of data generation using high-throughput DNA sequencing (Walsh et al., 2017). A large number of VUS has been identified by high-throughput DNA sequencing. Interpretation of identified sequence variants is an ongoing challenge and reclassification of variants has become more important. Wong et al. (2019) recently reclassified identified sequence variants based on revised criteria and new population data of patients with inherited cardiac disease. Reclassification of variants changed the medical management, further indicating the need for expert teams for the interpretation of variants in actionable genes. Moreover, functional studies are important to clarify VUS. The recommendation that VUS in SF should not be reported is derived from the low prior probability that a participant has a pathogenic variant when SF is considered. This is in contrast to an individual who presents clinically with a relevant disorder. For example, an unclear variant in *KCNQ1* is more likely to be pathogenic in a patient with a corrected long QT (QTc) interval >500 ms than in a person without a personal or family history of LQTS or SCD of young family members.

Interestingly, a current study of a patient population without a history of cardiac disease revealed that genetic variants putatively associated with a risk of SCD were not linked with arrhythmia phenotypes (Van Driest et al., 2016). Van Driest et al. (2016) attempted to elucidate the clinical phenotypes associated with the two well-recognized target arrhythmia genes, *KCNH2* and *SCN5A*. After manual review, 22 of 63 participants (35%) with designated variants had an ECG or arrhythmia phenotype, and only two had QTc interval longer than 500 ms, which strongly supports a diagnosis of LQTS (Piori et al., 2015). Thus, the study demonstrated that in an unselected population, the putatively pathogenic genetic variants were not associated with an abnormal phenotype. On the other hand,

there are several potential explanations for the paucity of clinical manifestations among participants with variants in arrhythmia genes. Variants may have low penetrance or cause subclinical disease. Some individuals with familial LQTS or BrS may have normal ECG or medication use may unmask the latent syndrome (Vincent, Timothy, Leppert, & Keating, 1992).

The issue of SF has been studied extensively over the last couple of years. Some clinical laboratories return a smaller or a broader set of SF (Amendola et al., 2015; Dewey et al., 2016; Dorschner et al., 2013). A recently published study aimed to evaluate the frequency of actionable genes from a longer list than the ACMG list that included a variety of different types of genes causing diseases accessible to treatment or prevention (late-onset diseases, genetic counseling, and pharmacogenetics) in a series of 700 exomes (Thauvin-Robinet et al., 2019).

Overall, there are limited data available on the frequency of SF in the population. A recently published study by Hart et al. (2019) reported that 74 of 6,240 (1.2%) participants who underwent genome or exome sequencing through the Clinical Sequencing Exploratory Research (CSER) Consortium received one or more SF from the original ACMG recommended 56 genes (Hart et al., 2019). Additionally, Dorschner and coworkers classified pathogenic variants of 1,000 individuals (Dorschner et al., 2013) in a defined group of actionable genes. Data show a frequency of 3.4% for European and 1.2% for African descent.

Importantly, there are limited data available on the frequency of SF associated with abnormal heart rhythm, heart muscle disease, and vascular and connective tissue disease. A previous study performed exome sequencing on 870 individuals not selected for arrhythmia, cardiomyopathy, or a family history of SCD. Overall, 0.5% of participants in this study had pathogenic variants in known cardiomyopathy or arrhythmia genes (Ng et al., 2013).

Walsh et al. (2017) analyzed sequencing data from 7,855 clinical cardiomyopathy cases and 60,706 ExAC reference samples to obtain a better understanding of genetic variation in an autosomal dominant disorder and found that in some genes previously reported as an important cause of cardiomyopathy, rare variation is not clinically informative. On the other hand, diagnostic laboratories may be overly conservative in the interpretation of a variant as disease-causing (Walsh et al., 2017). Cardiomyopathy genes feature prominently in the ACMG list of proposed genes to be routinely analyzed in all exome or genome sequencing (Green et al., 2013).

Due to the fact that most of the identified variants in cardiac channelopathies are missense variants, the consistency of the pathogenicity assessment is poor. Here, the lowest agreement in the classification of pathogenic variants was found in *SCN5A*. The reason, therefore, could be that different variants in *SCN5A* can cause a variety of diseases. Differences in phenotypes are probably due to differences in the electrophysiological abnormalities induced by the specific variant (Abdelsayed et al., 2017; Clancy & Rudy, 2002; Deschenes et al., 2000). LoF variants in *SCN5A* cause BrS rather than LQTS3. Depending on the variant and environmental triggers,

variants in *SCN5A* may result in a gain of function (LQTS3), loss of function (BrS), or both (mixed syndromes; Abdelsayed et al., 2017). As β -blockers can be useful as pharmacological therapy for individuals with LQTS3 but not for individuals with BrS, variant interpretation in *SCN5A*, in particular, has a great impact on the therapy options. Moreover, exercise may be therapeutic or maybe an arrhythmogenic trigger in some patients with pathogenic variants in *SCN5A* (Abdelsayed et al., 2017).

Overall, variant interpretation discrepancies depend on the source and the weight of each database in the variant interpretation process. HGMD collects all known variants associated with inherited diseases published in the literature. Identification of the relevant literature is carried out via a combination of manual journal screening and automated text mining. Classifications excerpted from published literature or imported from research efforts were frequently discordant with formal classifications produced by clinical laboratories. ClinVar provides a central platform for analyses of the reproducibility of variant classification between different laboratories. ClinVar does not curate submitted interpretations to determine if they are correct. Compared with recent classifications, older classifications were much more likely to be in conflict. Thus, it is recommended that laboratories submit reclassifications to databases. The purpose of LOVD is to assemble molecular variants in a standardized format. LOVD curates the variants listed in the database by disease-specific experts. Thus, the amount of conflicting data is lower compared to other databases.

Here, we highlight the importance of a careful variant interpretation in actionable genes recommended for return as SF. We suggest that the variants should be classified according to the ACMG guidelines with information gathered from different sources and disease- and gene-specific classification programs, if available.

5 | CONCLUSION

As SF can allow early detection and prevention of disease and SCD, consistent criteria should be developed for reporting pathogenic variants, and these criteria should be more stringent for genes linked to inherited primary arrhythmia syndromes. Incorrect assignment of pathogenicity of rare variants will devalue their potential utility. Each rare variant must undergo rigorous clinical and laboratory evaluation before it can be described as pathogenic and returned to patients. As our knowledge expands, a system needs to be in place for the review of genetic findings and VUS should be upgraded to disease-causing or downgraded to benign in particular with regard to treatable arrhythmic disorders with the risk of SCD.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

DATA AVAILABILITY STATEMENT

The variants classified during the current study are available at <https://databases.lovd.nl/shared/variants/RYR2>, <https://databases.lovd.nl/shared/genes/KCNQ1>, <https://databases.lovd.nl/shared/variants/KCNH2>, and <https://databases.lovd.nl/shared/variants/SCN5A>

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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