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Review article

# Sudden Cardiac Death and Copy Number Variants: What Do We Know after 10 Years of Genetic Analysis?

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ABSTRACT

Over the last ten years, analysis of copy number variants has increasingly been applied to the study of arrhythmogenic pathologies associated with sudden death, mainly due to significant advances in the field of massive genetic sequencing. Nevertheless, few published reports have focused on the prevalence of copy number variants associated with sudden cardiac death. As a result, the frequency of these genetic alterations in arrhythmogenic diseases as well as their genetic interpretation and clinical translation has not been established. This review summarizes the current available data concerning copy number variants in sudden cardiac death-related diseases.

## 1. Introduction

Structural variants (SVs) are generally defined as a region of DNA approximately 1 kb and larger in size [1] and including deletions, duplications, novel insertions, inversions, translocations and mobile-element transpositions [2; 3]. SVs include inversions and balanced translocations or genomic imbalances (insertions and deletions), commonly referred to as copy number variants (CNVs). In 2006, Redon et al. defined a CNV as a DNA segment of one kilobase or larger with variable copy number in comparison with a reference genome [4] where the copies are > 90% identical [5]. If present in > 1% of a population, a CNV may be referred to as a copy number polymorphism (CNP). As is the case for other types of genetic variants, some CNVs are inherited whereas others spontaneously arise. Previous studies concluded that up to 12% of the genome may be variable in copy number [6; 7] and others that the average human genome contains > 1000 CNVs [8; 9], that occur at a rate of 0.07–0.12 per generation [10; 11; 12]. In 2015, an updated map of CNVs in the human genome not associated with adverse phenotypes was constructed; authors estimated that up to 9.5% of the genome contributes to CNV [13].

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### 1.1. CNV databases

Databases collecting clinical cases regarding genetic and phenotypic details were created to encompass all human CNVs and aid in interpreting their phenotypic effects. In 2004, the Database of Genomic Variants (DGV) (http://dgv.tcag.ca/dgv/app/home) was created to provide a publicly accessible, comprehensive curated catalogue of human CNVs and SVs from control individuals from worldwide populations [14]. Two years later, the European Cytogeneticists Association Register of Unbalanced Chromosome Aberrations (ECARUCA) was created [15] despite it is not currently available. In 2009, all known CNVs associated with any clinical condition were collected, using Ensembl Resources, in the Database of Chromosomal Imbalance and Phenotype in Humans (DECIPHER) (https://decipher.sanger.ac.uk/) [16]. In 2011, the International Standards for Cytogenomic Arrays (ISCA) -http://www.iscaconsortium.org/- were created [17]. To facilitate CNV analysis, same-species self-alignments and cross-species pairwise alignments were used to create a CNV Variant database (CNVVdb) cataloging putative CNVs among 16 vertebrate species, including humans (http://CNVVdb.genomics.sinica.edu.tw). In 2013, CNV data from healthy cohorts (DGV) and from disease-related databases (DECIPHER) were integrated into CNVinspector (http://www.cnvinspector.org), facilitating the daily work of clinical geneticists and accelerating delineation of new syndromes and gene functions. Also in 2013, the Clinical Genome Resource (GlinGen) (https://clinicalgenome.org/) was founded by the National Human Genome Research Institute. ClinGen is a National Institutes of Health (NIH)-funded central resource dedicated to defining the clinical relevance of genes and variants for use in precision medicine and research.

### 1.2. Methodological aspects

Technology for defining CNVs has improved over time. In 1992, Jacobs et al. identified large-scale CNVs with conventional karyotyping [18], but the rapid development in sequencing technology and analysis of the human genome has, over the last decade, transformed the use of genetics for clinical diagnosis. Multiplex Ligation-dependent Probe Amplification (MLPA) is one method currently used for genotyping CNV. Numerous commercial MLPA assays for CNV-related human diseases have been created, but there are currently no commercial kits for all genes. Therefore, to analyze additional genes, research laboratories have created self-designed synthetic probes that have not been approved for clinical diagnoses. Real-time quantitative PCR (RT-qPCR) has also been utilized for analysis of CNVs, especially if no commercial MLPA kits were available. MLPA has been shown to be superior to RT-qPCR for CNV quantification [19; 20]. However, the main limitation of both methods is that neither can screen the whole genome. Therefore, for diagnosis purposes, MLPA is the best approach nowadays if kit available. Concerning SCD cases with none gene suspicious of decease, neither MLPA nor RT-PCR is recommended due to economic cost as well as low rate yield.

Chromosome microarray analysis (CMA) techniques, which include array comparative genomic hybridization (aCGH) and SNP microarrays, have revolutionized the detection and discovery of CNVs. Both hybridization-based technologies infer copy number gains or losses compared to a reference sample or population, but differ in the details and application of the molecular assays. Microarrays are limited to detecting copy-number differences of sequences present in the reference assembly used to design the probes, thus they cannot identify balanced structural variants. Moreover, microarrays provide no information on the location of duplicated copies and are generally unable to resolve breakpoints at the single-base-pair level [2]. Use of aCGH should not be recommended in SCD cases due to high cost and no conclusive result.

Currently, rapidly developing massive sequencing technologies (called next generation sequencing or NGS) provide affordable high quality data and are fast, sensitive, and accurate. NGS is already a popular method for identifying single nucleotide variants and small insertions/deletions, but NGS data can also be used for the detection of SVs (both balanced and unbalanced). Although NGS technologies promise to further revolutionize studies of SVs, current experimental methods and computational analyses of NGS data sets show low levels of overlap, and false positives and false negatives are relatively common [2; 21; 22; 23]. Consequently, other techniques (usually MLPA or RT-qPCR) should be used to confirm or discard NGS- identified alterations. The most important benefit of NGS technologies is their potential to discover a multitude of variant classes with a single sequencing experiment. In addition, the sequence data is largely unbiased and may provide insight into the complete spectrum of genetic variation. The first NGS attempts at CNV analyses have recently been published and suggest that CNV calling from whole exome [24] and whole genome sequencing is robust and can benefit patients with a suspected genetic disease [25]. Use of NGS technology (mainly whole exome/genome sequencing) may discover CNVs unrelated to the primary indication but which may have potential health or reproductive importance. Therefore, the ACMG had recently stated that *"reporting some incidental [a.k.a. secondary] findings would likely have medical benefit for the patients and families of patients undergoing clinical sequencing"* Currently, the detection of CNVs by NGS is still challenging; thus, methods for the identification of SVs are still not routinely implemented in most NGS pipelines, especially in the field of clinical diagnosis. Despite this fact, use of NGS is the more appropriate approach to unravel cause of death in SCD. In addition, NGS is also the best first approach to identify any CNV, with posterior confirmation using MLPA and/or RT-PCR.

## 2. Sudden Cardiac Death

Sudden cardiac death (SCD) is a major contributor to morbidity and mortality in the Western world. Epidemiological studies have confirmed that genetic background plays an important role in SCD, mainly in populations less than 35-years old, making inherited arrhythmogenic cardiac diseases the most common cause of SCD in this population. In populations > 35 years old, the most common cause of SCD is coronary artery disease [26; 27].

SCD may be caused by pathogenic rare variants in genes encoding ion channels or associated proteins, which lead to channelopathies, with four main diseases: Long QT Syndrome (LQTS), Brugada Syndrome (BrS), Short QT Syndrome (SQTS), and Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT) [28]. In addition, SCD may be caused by pathogenic rare variants in genes encoding structural proteins (sarcomeric, desmosomal, and/or cytoskeletal), usually associated with cardiomyopathies, mainly hypertrophic (HCM), dilated (DCM), and arrhythmogenic (ACM) [29].

Both channelopathies and cardiomyopathies are inherited diseases; therefore, current guidelines recommend genetic analysis in families with at least two individuals suffering any above-mentioned arrhythmogenic disease, even if discovered post-mortem (molecular autopsy) with a non-conclusive cause of death after a comprehensive autopsy [30]. Despite progressive increases in the number of genes associated with SCD, definite pathogenic single nucleotide variants (SNVs) or small insertions and deletions (*indels*) are currently identified at low percentages in SCD-related diseases. Following current ACMG/AMP recommendations most variants remain of ambiguous significance [31]. Hence, a portion of SCD affected families remain without a conclusive genetic diagnosis because they lack certain variant pathogenic classification, have genetic alterations in regulatory regions, splice sites, epigenetic sites, or genes lacking known SCD association, or involve SVs, such as CNVs. These alterations are not deeply studied nowadays and no conclusive role concerning pathogenicity can be adopted. Main limitation is lack of real frequencies of these alterations in global population.

The last ten years have seen the initiation and progression of studies on the clinical role of CNVs in hereditary SCD-associated cardiac

arrhythmias, despite the few studies published to date. These few studies have been proof-of-concept reports limited to single families or small cohorts using mainly MLPA kits as the method of analysis [32–39]. This sole use of kits has impeded the current understanding of the prevalence of rearrangements, and the real contribution of CNVs to SCD is most likely under-recognized. The discovery of new CNVs would provide fresh insight into the pathophysiology of diseases associated with SCD and be crucial for clinical management of these patients and their families. One of main challenges is interpretation of CNVs before clinical translation. Main impediment is state the real frequency of CNV in healthy population. In next years, screening of CNV in large population of healthy population as well as patients will help to clarify real frequencies of CNV. In the present work, we consider all available studies in this field and discuss several issues that should be carefully considered when screening for CNVs in patients with cardiomyopathies and channelopathies.

## 3. Role of CNV in channelopathies

Few reports are available on CNVs in inherited cardiac channelopathy. In global terms, these studies state that no more than 5% of families suffering any SCD-associated channelopathy carry a deleterious CNV as a cause of any arrhythmogenic disease. It is important to remark that these percentages in channelopathies could be erroneous due to lack of enough CNV studies in patient's cohorts as well as healthy population. Currently, no precise data concerning frequencies of CNVs in global population is available.

### 3.1. Long QT Syndrome

This rare arrhythmogenic disease (1/5.000-1/20.000) is characterized by a prolonged QTc interval in the 12-lead ECG in the absence of any specific condition known to lengthen it (with QTc values > 480 ms) [30]. Patients may remain asymptomatic or experience syncope —the most common symptom but one mirrored in malignant ventricular arrhythmias—, and even SCD. Indeed, while rare, SCD may appear as the first manifestation of the disease. This lethal event usually occurs in healthy children/adolescents typically during exercise and emotional stress, less frequently during sleep, and usually without warning [40]. In the forensic field, LQTS has been reported as the main causative factor of sudden infant death syndrome (SIDS) accounting for almost 12% of SIDS cases [41].

Nineteen different genes encoding proteins related to sodium, potassium, or calcium currents (*AKAP9, ANK2, CACNA1C, CALM1, CALM2, CALM3, CAV3, KCNE1, KNCE2, KCNH2, KCNJ2, KCNJ5, KCNQ1, RYR2, SCN1B, SCN4B, SCN5A, SNTA1,* and *TRDN*) are known causes of LQTS [42; 43]. A comprehensive genetic analysis of SNV and small indels identifies the cause of the disease in 80-85% of patients, with the most commonly affected genes being *KCNQ1* (35%), *KCNH2* (20-25%), and *SCN5A* (10-15%). Studies published on the role of CNVs in LQTS have mostly screened only *KCNQ1* and *KCNH2*. Considering all published studies, the frequency of CNVs classified as pathogenic among LQTS patients is relatively higher than that found for other pathologies, ranging from 1% to 11.5% of all diagnosed cases [32; 34–36; 44–46]. To date, only pathogenic CNVs in *KCNQ1, KCNH2,* and *KCNE1* have been reported, and a single published study, which screened a wide spectrum of SCD-related genes in 145 LQTS patients, reported that pathogenic or probably pathogenic CNVs were only detected in these three abovementioned genes (3.4% of all diagnosed cases) [45]. Of note, the deletion of exons 7 and 8 of *KCNQ1* has been identified in four unrelated patients, all with severe phenotypes [36; 45] (Table 1; Fig. 1). Therefore, an exhaustive genetic analysis including SNV, small indels and CNV may unravel the potential cause of the disease in nearly 90% of cases.

**Table 1**
Frequency of CNVs in SCD-related diseases. ACM, Arrhythmogenic Cardiomyopathy; BrS, Brugada Syndrome; CPVT, Catecholaminergic Polymorphic Ventricular Tachycardia; DCM, Dilated Cardiomyopathy; HCM, Hypertrophic Cardiomyopathy; LQTS, Long QT Syndrome; SQTS, Short QT Syndrome.

| Disease | Genes with CNV | Maximum CNV frequency per gene | Maximum CNV frequency |
|---|---|---|---|
| BrS | *SCN5A* | 2.9% | 2.9% |
| LQTS | *KCNH2* | 7.7% | 13.7% |
| | *KCNQ1* | 4.8% | |
| | *KCNE1* | 0.7% | |
| | *KCNE2* | 0.5% | |
| CPVT | *RyR2* | 8.3% | 8.3% |
| SQTS | - | - | - |
| HCM | *MYBPC3* | 1.4% | 1.9% |
| | *PLN* | 0.3% | |
| | *PDLIM3* | 0.2% | |
| DCM | *LMNA* | 4% | 7.7% |
| | *DSP* | 1.5% | |
| | *BAG3* | 1.5% | |
| | *TTN* | 0.5% | |
| | *LAMP2* | 0.2% | |
| ACM | *PKP2* | 3.4% | 4.2% |
| | *DSP* | 0.8% | |

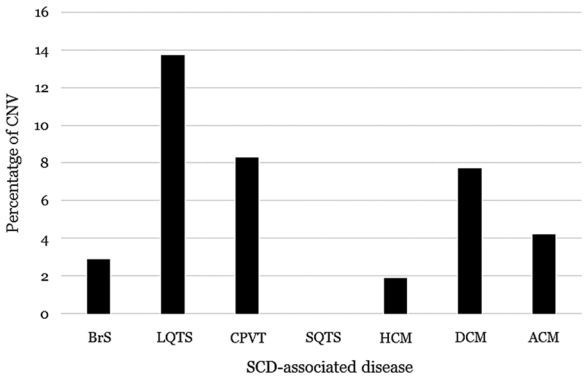


**Fig. 1.** Maximum published frequencies of CNV in SCD-related diseases. ACM, Arrhythmogenic Cardiomyopathy; BrS, Brugada Syndrome; CPVT, Catecholaminergic Polymorphic Ventricular Tachycardia; DCM, Dilated Cardiomyopathy; HCM, Hypertrophic Cardiomyopathy; LQTS, Long QT Syndrome; SQTS, Short QT Syndrome.

### 3.2. Brugada Syndrome

BrS is a rare inherited arrhythmogenic disease (1/2000 to 1/5.000) [28]. Patients with BrS have no structural heart disease, and clinical diagnosis requires a characteristic ST segment elevation > 2 mm followed by a T-wave in at least one of the right precordial leads (at baseline or after sodium blocker induction) [30] indicating ionic current impairments leading to depolarization and/or repolarization abnormalities result in ventricular arrhythmias (VAs) causing SCD. The clinical spectrum of BrS varies from completely asymptomatic to symptoms such as polymorphic ventricular tachycardia or even SCD. Non-vasovagal syncope or SCD can be the first manifestation of the disease, and lethality usually occurs at night or at rest and mainly in males [47].

BrS presents an autosomal dominant pattern of inheritance, and 24 genes are currently associated with the disease (*ABCC9, CACNA1C, CACNA2D1, CACNB2, FGF12, GPD1L, HCN4, HEY2, KCND2, KCND3, KCNE3, KCNE5, KCNH2, KCNJ8, PKP2, RANGRF, SCN10A, SCN1B, SCN2B, SCN3B, SCN5A, SEMA3A, SLMAP,* and *TRPM4*). However, a comprehensive genetic analysis including SNV and small indels identifies a cause in only 30-35% of patients. The main affected gene is *SCN5A*, responsible for 25-30% of BrS patients. Regarding CNVs, few

series have been published in BrS, most of them focused on the *SCN5A* gene. Therefore, no CNVs in BrS patients were identified in two studies performed so far [33; 39] although both only analyzed the *SCN5A* gene. To date, the only CNV identified as a cause of the disease was published in 2011 [37] (Table 1; Fig. 1).

### 3.3. Catecholaminergic Polymorphic Ventricular Tachycardia

CPVT is a lethal arrhythmogenic rare condition (1/10.000) [48]. The basal ECG of patients with CPVT tends to be normal, but during intense physical exercise or acute emotional stress, bigeminy and non-sustained ventricular tachyarrhythmia may appear. This condition may lead to syncope and even SCD, which is sometimes the first expression of the disease. Earlier onset of clinical symptoms and a significantly higher risk of cardiac events at a young age are observed during the course of the disease, which is more severe in males [30].

Currently, eight genes may be associated with CPVT (*ANK2, CASQ2, CALM1, CALM2, CALM3, KCNJ2, RYR2,* and *TRDN*). The pattern of inheritance (autosomal or recessive) depends on the mutated gene. A comprehensive genetic analysis of SNV and small indels identifies the cause in nearly 65% of patients, with *RYR2* being the main gene (50-55%) [49; 50].

The screening for CNVs in CPVT patients has mainly focused on *RYR2*. Two case reports have described deletions of *RYR2* exon 3 [51–53]. Three further studies exclusively screened this exon for CNVs and reported a deletion frequency of 1.9%-8.3% of diagnosed cases [54–56]. Of note, ten index cases with a deletion of *RYR2* exon 3 have been reported to date. In most cases, the phenotype is severe and complex, combining CPVT characteristics with other cardiomyopathies [51–56]. The single work screening a panel of multiple SCD-related genes in 19 CPVT patients detected no CNVs [45] (Table 1; Fig. 1). Therefore, an exhaustive genetic analysis including SNV, small indels and CNV may unravel the potential cause of the disease in nearly 70% of cases.

### 3.4. Short QT Syndrome

SQTS is the most lethal rare inherited disease (1/10.000) reported so far [28]. It is characterized by a short QTc interval (< 360 ms) without evident structural heart disease [30]. SQTS has been published in a small number of families worldwide. In up to 40% of patients, cardiac arrest is the most frequent symptom, followed by palpitations (30%), syncope (25%), and atrial fibrillation (20%) [57]. SCD may be the first manifestation of the disease and may occur in loud noise situations or during rest, exercise, or daily activity. SQTS has been diagnosed by pathology studies in fetuses of patients suffering multiple intra-utero deaths in consecutive pregnancies [58].

Currently, six genes have been associated with SQTS (*KCNQ1, KCNH2, KCNJ2, CACNA1C, CACNB2,* and *CACNA2D1),* following an autosomal dominant pattern of inheritance. A complete analysis of SNV and small indels identifies the cause of the disease in nearly 40% of patients [59]. No CNVs in SQTS patients have been reported so far. However, a pathogenic role for CNVs cannot be discarded, considering the small number of published SQTS families worldwide and that analysis of CNVs has not been routinely performed. Our group recently performed a comprehensive analysis of multiple SCD-related genes in seven SQTS patients, and no CNVs were identified [45]. Despite this, we recommend screening this genomic alteration in families diagnosed with this disease (Table 1; Fig. 1).

## 4. Role of CNV in cardiomyopathies

CNVs have been reported in various genes associated with cardiomyopathies, playing a pathogenic role in no more than 10% of patients. It is important to remark that these percentages in cardiomyopathies could be erroneous due to lack of enough CNV studies in patient's cohorts as well as healthy population. Currently, no precise data concerning frequencies of CNVs in global population is available. Therefore, their real contribution has not been systematically studied so far.

### 4.1. Hypertrophic Cardiomyopathy

HCM is defined as a cardiac inheritable condition (1/500) characterized by left ventricular hypertrophy with histological features of cellular hypertrophy, myofibrillar disarray, and interstitial fibrosis [60]. HCM is the most frequently inherited cardiomyopathy, with a prevalence of 0.2%, and the most relevant cause of SCD in young people [61]. Moreover, HCM is an important cause of ventricular tachycardia, syncope, and even SCD, particularly in young males [30].

HCM is inherited as an autosomal dominant trait and presents remarkable phenotypic variability and incomplete penetrance. A comprehensive genetic analysis of families suffering HCM identified SNV and small indels in numerous associated genes, of which 20 are main factors: *ACTC1, ALPK3, CAV3, CSRP3, FHL1, JPH2, MYBPC3, MYH6, MYH7, MYL2, MYL3, MYOZ2, PLN, TCAP, TNNC1, TNNI3, TNNT2, TPM1, TRIM63,* and *TTN*. The positive genetic yield is nearly 85% after an exhaustive analysis, and currently two main genes, *MYBPC3* and *MYH7*, account for 30% and 30% of cases, respectively. The role of CNVs in HCM has been analyzed in a limited number of studies. In 1992, the first CNV in a patient with HCM was reported in the *MYH7* gene [62]. Since then, other studies have reported CNVs in genes associated with HCM, mainly *MYBPC3* and *TNNT2* but also *PLN* and *PDLIM3* and detected CNV in less than 2% of cases [63–70]. The first published series of papers focused on *MYBPC3, TNNT2*, and/or *MYH7*, while more recent studies screened panels of genes (≥18 genes associated with HCM) by NGS. Lopes et al. analyzed 19 HCM-related or candidate genes by NGS in 505 patients and detected 4 CNVs (0.8%): 1 deletion in *MYBPC3*, 1 deletion in *PDLIM3*, an entire duplication of *TNNT2*, and duplication in *LMNA*. Later, Ceyhan-Birsoy assessed 708 HCM patients and identified CNVs in 4 of them (0.56%), but only one CNV, a deletion of *MYBPC3*, was classified as pathogenic [71]. Our group screened 303 HCM patients for CNVs in 25 HCM-associated or candidate genes and detected 4 CNVs (1.3% of our patients). Two CNVs were novel deletions in *MYBPC3*, and both were classified as pathogenic variants. Strikingly, a later study identified an identical *MYBPC3* large deletion involving several exons [66]. Further studies identified several CNVs that were classified as variants of unknown significance [64–68; 72; 73]. Our group performed two comprehensive analyses of the sarcomere genes most frequently affected in HCM (*MYBPC3, MYH7, TNNI3, TNNT2,* and *TPM1*) in a cohort of HCM-affected families. In both studies, we found a CNV prevalence of nearly 1,5% of diagnosed cases [45; 74] a value similar to those reported in two prior studies (see above) [68; 73; 75; 76] (Table 1; Fig. 1). Therefore, an exhaustive genetic analysis including SNV, small indels and CNV may unravel the potential cause of the disease in nearly 90% of cases.

### 4.2. Dilated Cardiomyopathy

DCM is a rare inherited condition (1/2.500) characterized by left ventricular or biventricular dilatation and systolic dysfunction, which cannot be explained by abnormal loading conditions or coronary artery disease, sufficient to cause global systolic impairment leading to heart failure and SCD [77]. There are several reported etiologies, but a large number of patients remain classified as idiopathic. Currently, molecular pathophysiology of this entity remains unknown. DCM has high rates of morbidity and mortality and is the most frequent cause of heart failure in the young, particularly young males. Penetrance is age-dependent and expressivity is variable even between family members [30].

DCM mainly follows an autosomal dominant pattern of inheritance, but X-linked and mitochondrial patterns have also been reported. To date, more than 60 genes have been associated with DCM, and the most

commonly affected ones are as follows: *ACTC1, BAG3, DSP, LMNA, MYBPC3, MYH6, MYH7, RBM20, SCN5A, TNNT2, TPM1,* and *TTN*. A complete genetic analysis of SNV and small indels identifies the potential cause of the disease in 60-70% of families [78]. Two major genes currently associated with DCM are *TTN* (25-30%), and *LMNA* (10-15%) [79]. CNV is associated with DCM but at a low frequency (< 5%) [73], and mainly in the *LMNA* gene [80]. However, CNVs have also been reported in the *BAG3* gene [81]. Our group performed an exhaustive CNV analysis of all main genes associated with DCM in a cohort of patients with the condition and found CNV in nearly 4,5% of diagnosed cases [45]. In contrast, Ceyhan-Birsoy et al. found a lower frequency of 0,6% [73] (Table 1; Fig. 1). Therefore, an exhaustive genetic analysis including SNV, small indels and CNV may unravel the potential cause of the disease in nearly 80% of cases.

### 4.3. Arrhythmogenic Cardiomyopathy

This rare inherited cardiac entity (1/2.500-1/5.000) encompasses any form of non-hypertrophic progressive cardiomyopathy characterized by right ventricular, or biventricular, fibrofatty replacement of myocardium from epicardium to endocardium in whether right or left ventricle [82]. Progressive myocyte loss and fibrofatty infiltration may lead to ventricular arrhythmias, impaired ventricular systolic function, heart failure, and SCD [83]. Lethal episodes mainly occur in young males during exercise. Clinical diagnosis is established using a set of criteria proposed by an international task force in 1994 and revised in 2010, which includes structural, histological, electrocardiographic, and genetic factors [30; 84].

The ACM inheritance pattern is autosomal dominant, and the disease presents incomplete penetrance and variable expressivity. An exhaustive analysis of all genes currently associated with ACM (*CTNNA3, DES, DSP, DSC2, DSG2, JUP, LMNA, PKP2, PLN, SCN5A, TMEM43, TGFB3,* and *TTN*) identifies SNV and small indels as cause of the disease in 65% of families. *PKP2*, accounting for 40% of cases, is the principal gene responsible for ACM, followed by *DSP* (10%-15%) [85]. Recently, other genetic alterations such as CNVs in *PKP2* have been associated with the disease but at lower frequencies (< 2%) [86; 87]. To our knowledge, the first study of CNV in ACM was published in 2011 [88]. In the past 5 years, published studies have reported pathogenic CNV in 1.1% to 4.2% of ACM patients [73; 86; 87; 89]. In a recent study, we identified a CNV rate of 5.1% in a cohort of families diagnosed with ACM after a comprehensive analysis of all genes currently associated with this disease [45] (Table 1; Fig. 1). Therefore, an exhaustive genetic analysis including SNV, small indels and CNV may unravel the potential cause of the disease in nearly 70% of cases.

## 5. Clinical translation

CNVs are one of the most difficult genetic alterations to interpret from a phenotypic point of view. The molecular consequences of CNVs, as for other genetic alterations, can be intuited only in a low proportion of cases. For most cases, distinguishing pathogenic or high-risk CNVs from benign alterations is currently challenging for geneticists and clinicians [90]. This fact is partially due to low number of population analyzed concerning CNVs.

To date, there are more than 100.000 CNVs described in healthy control samples [91]. A large proportion of these SVs may underlie certain human phenotypic diversity and susceptibility to disease [92]. However, it is worth mentioning that not all CNVs are pathogenic, and only a low percentage have been definitively linked to diseases, mainly congenital malformations and cognitive disorders [93; 94]. This is due to lack of functional studies supporting the potential pathogenic role. Additionally, environmental factors and/or other genetic variants probably contribute to variable expressivity and penetrance of CNVs [3; 93], and it has recently been proposed that CNVs located in intragenic regions are potential gene expression modulators, playing a key role in human diseases [95]. Thus, for a large proportion of patients it remains difficult to predict the phenotypic consequences of CNVs, partially owing to our incomplete knowledge of the genome and potential CNV interactions. Additional studies including precise frequencies of CNV in healthy population as well as functional studies focused on deleterious CNVs should be performed to clarify role of each CNV, allowing a proper translation into clinics.

Current CNV interpretation guidelines focus on the interpretation of large genomic rearrangements, generally involving multiple contiguous genes. These guidelines recommend evaluating available CNV information in global databases, the size of the CNV, the genomic content of the involved region, gains or losses of DNA, *de novo* alterations, and family segregation [93; 96–98]. Detailed consensual recommendations for interpreting intragenic CNVs do not exist, although these CNVs require special considerations. ACMG is likely developing CNV interpretation guidelines together with the ClinGen Structural Variation Interpretation Working group. Therefore, to classify pathogenicity of CNVs, published studies use different items that should be careful analyzed: available CNVs information in global databases, size of CNV, genomic content in the region affected by CNV, gains or losses of DNA, *de novo*, and the most important point, at our point of view, family segregation [99; 100]. Comprehensive interpretation of each CNV should always be done in the context of a clinical phenotype. After classification, a small number will remain clearly pathogenic, a portion will be considered benign, and the majority will remain of unknown/uncertain significance. Recently, ClinTAD (www.clintad.com), an easy to use bioinformatic tool based on emerging research in chromatin architecture (topologically associated domains –TAD-) had been developed focused on CNV interpretation [101].

The role of CNVs in phenotyping SCD-related conditions remains an ongoing challenge. Ceyhan-Birsoy observed that limitations in detecting CNVs are unlikely to explain their low prevalence and that overall, CNVs have a negligible contribution to genetic cardiomyopathies [73]. Our group concluded it was useful to screen for CNVs during routine clinical assessment, although they only explain a small portion of cases for most SCD-related diseases. Of note, genetic testing for SNVs and *indels* using NGS technology requires only a small additional cost, and identifying a possible cause of a patient´s disease allows appropriate clinical management, genetic counseling, and vital preventive actions for at-risk familial groups [45]. Even in the same family, interpretation should be individualized due to variable expressivity/incomplete penetrance, hallmarks of diseases associated with SCD. Considering current complexities of CNV interpretation, any clinical translation should be done with caution and in consensus of a group of experts, including geneticists, genetic counselors, forensics/pathologists, and clinicians.

## 6. Conclusions

Over the last decade, analysis of CNV has been progressively incorporated into the assessment of SCD-related diseases. Currently, available data suggest that CNVs explain from 2% to 13% of patients for most of these diseases; however, this percentage could be miscalculated due to the small number of published studies. Lack of comprehensive genotype-phenotype studies make the genetic interpretation of CNV difficult and impede making conclusions on the definitive role of CNV in SCD. Therefore, clinical translation should be done with caution. Despite this, we believe that CNV analyses should be included as a routine part of genetic diagnosis/molecular autopsy to improve the diagnostic yield of families suffering SCD-related diseases.

## Disclosure statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of

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