Forensic Science International: Reports 6 (2022) 100300

Contents lists available at ScienceDirect

Forensic Science International: Reports

journal homepage: www.sciencedirect.com/journal/forensic-science-international-reports

# Next-generation sequencing of postmortem molecular markers to support for medicolegal autopsy

Joseph Alape Ariza [a,b,e,*], Arbey Hernán Medina Rocha [c], Rodrigo Cabrera Pérez [d,e], Clara Isabel Bermudez-Santana [f,g]

[a] Forensic Sciences Research Group, National Institute of Legal Medicine and Forensic Sciences, Calle 7A No. 12A 51, Bogotá, D.C., Colombia
[b] Forensic Genetics Research Group, National Institute of Legal Medicine and Forensic Sciences, Calle 7A No. 12A 51, Bogotá, D.C., Colombia
[c] Forensic Pathology Group, Bogotá Regional Directorate, National Institute of Legal Medicine and Forensic Sciences, Calle 7A No. 12A 51, Bogotá, D.C., Colombia
[d] Center for Research in Genetics and Genomics (CIGGUR), Institute of Translational Medicine (IMT), Universidad del Rosario, Bogotá, D.C., Colombia
[e] Molecular Biology Laboratory, Fundación Cardio Infantil – Instituto de Cardiología, Calle 163A No. 13 B 80, Bogotá, D.C., Colombia
[f] Theoretical and Computational RNomics group, Department of Biology, Faculty of Sciences, National University of Colombia, Carrera 45, Bogotá, D.C., Colombia
[g] Center of Excellence in Scientific Computing, National University of Colombia, Carrera 45, Bogotá, D.C., Colombia

ARTICLE INFO

*Keywords:*
SUD
Cardiac Channelopathies
LQTs
NGS

ABSTRACT

In most cases, sudden unexplained death (SUD) is caused by hereditary cardiac arrhythmias that standard forensic autopsy procedures cannot prove. For this reason, forensics analysis must apply other methodologies to uncover related factors. For example, postmortem molecular analysis (molecular autopsy) based on next-generation sequencing represents a promising and effective tool for diagnosing SUD. This analysis allows scientists to detect well-known, new, and rare pathogenic exonic variants or those with unknown significance that could be related to the cause of sudden death. Using exome sequencing, we identified rare exon variants in MYBPC3, KCND3, TTN, and ANK3 in a fifteen-year-old male SUD case with negative toxicology analysis and autopsy showing microscopic abnormality of heart fiber disarray. Our findings suggested that this case might be associated with cardiac channelopathy long QT syndrome, type 2, as a potential causative factor.

## 1. Introduction

Cardiovascular diseases are one of the leading causes of sudden cardiac death (SCD), with an estimated incidence of around 20% in industrialized countries. Nowadays, it is becoming a public health problem worldwide [1,2]. Uncovering inherited cardiomyopathies and channelopathies is challenging in postmortem examinations. Nevertheless, they are known to be causally linked to sudden unexplained death (SUD). Cardiomyopathies such as hypertrophic, dilated, and arrhythmogenic, can present with minimal structural changes in the heart in SCD [3,4] and channelopathies are not associated with anatomical differences but affect heart rate and cardiac electrical conduction, triggering sudden cardiac arrest [3,5].

In recent years, genetic studies to establish the genetic basis of SCD include the analysis of gene mutations from four main families of proteins: sarcomeric (associated with hypertrophic cardiomyopathy) [6], cytoskeletal (associated with dilated cardiomyopathy) [7], desmosomal (associated with arrhythmogenic right ventricular dysplasia) [8] and ion channels (associated with hereditary arrhythmias such as long and short QT syndrome) [9,10], Brudaga syndrome (BrS) [11], Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT) [12], Early Repolarization Syndrome and Idiopathic Ventricular Fibrillation (PRSyLVF) [13]. However, the genetic basis for SCD has not been fully deciphered, leading to it being classified among conditions with complex inheritance [14].

Additionally, SCD has become a public health problem given its high incidence in young people worldwide [15]; therefore, its study is of the utmost importance given its genetic heterogeneity and pleiotropy.

Standard protocols to determine the correct cause of death are 1. analysis of antemortem clinical data, 2. an inspection of the scene where the death occurred, 3. autopsy with dissection of the major organs (brain, heart, lungs, liver, and kidneys) 4. toxicology assays, 5. microbiologic cultures, and 6. metabolic screening in newborns [16]. After many efforts, the cause of death is still unsuccessful in some cases. As a

* Corresponding author at: Forensic Sciences Research Group, National Institute of Legal Medicine and Forensic Sciences, Calle 7A No. 12A 51, Bogotá, D.C., Colombia.
*E-mail address:* jalape@medicinalegal.gov.co (J.A. Ariza).

https://doi.org/10.1016/j.fsir.2022.100300
Received 6 September 2022; Received in revised form 10 October 2022; Accepted 11 November 2022
Available online 14 November 2022

result, those deaths are suggested to have a high probability of being associated with channelopathy-type cardiac arrhythmias [17,18], making the diagnosis problematic since they cannot be detected during an autopsy. Besides, for further genetic counseling, an accurate post-mortem identification of the disease-causing mutation could be used to assess if there is a risk in other family members to the onset of SCD [19].

Channelopathies are known to be caused by mutations in ion channels. They present a Mendelian inheritance pattern and variable expression with incomplete penetrance [20]. These discoveries have changed how pathologists approach sudden death in autopsies. Molecular diagnoses might become a practical methodology to enrich the diagnosis of the causes of SCD [21,22]. Researchers are encouraged to incorporate molecular tests to understand the breadth and depth of heart disease.

Our findings are a specific example of the role and advantages of using molecular analysis in cases of sudden unexplained death. It has turned out to be very important for studying complex diseases such as diabetes, cancer, and conditions of cardiac origin (channelopathies) associated with SCD, among others [7]. For that, the development of massively parallel or next-generation sequencing (NGS) allows the analysis of a patient's complete genome/exome sequence and, as a result, permits the analysis of a bigger pool of genes. In this study, clinical exome sequencing was applied to support the potential diagnosis of SUD as the probable causative of the death of a fifteen-year-old male. The teenager died in 2016, and his results were negative for toxicology and virology. Furthermore, there were negative forensic autopsy results, i.e., no structural heart damage. Finally, our approach enriched the markers suggested to be associated with this SUD.

## 2. Materials and methods

### 2.1. Subject

A fifteen-year-old male showed microscopic abnormality of heart fiber disarray and negative toxicological tests after an autopsy without family records of heart disease. He died in the dormitory while sleeping.

### 2.2. Autopsy and pathology testing

A forensic scientist performed the autopsy. The heart, brain, liver, kidney, lung, and other primary organ tissues were collected for histopathological examination. Blood from the heart was collected for toxicological analyses.

#### 2.2.1. DNA sampling

DNA extraction was performed from blood taken from a male fifteen-year-old who died in 2016, with the QIAamp®DNA Blood Midi/Maxi kit, following the manufacturer's recommendation. The blood was stored and refrigerated in the Evidence Center of the National Institute of Legal Medicine and Forensic Sciences of the Bogotá Regional Directorate.

#### 2.2.2. Construction of libraries and NGS sequencing

Sample library preparation was performed using the TruSight One Sequencing Panel Series (Illumina), which includes 4834 clinically relevant genes, using the Nextera XT Kit (Illumina). The libraries were quantified using the Quantitating dsDNA kit in the Quantus™ Fluorometer instrument and following the manufacturer's recommendations. Subsequently, the libraries were sequenced as paired reads of 2 × 150 bp with the MiSeq kit (Illumina Inc., San Diego, CA. USA) using MiSeq Reagent V3 (150 cycles), following the manufacturer's recommendations.

#### 2.2.3. Bioinformatic analysis

The quality of the sequences was performed using the FASTQC [23] and FASTP [24] software, the alignment algorithms BWA-Backtrack (MiSeq Reporter-Illumina), Bowtie2 [25], BWA-MEM [26], GSNAP [27] and NovoAlign [28], HaplotypeCaller-GATK-HC [29], Samtools mpileup [30] and Freebayes [31] were used for variant calling. DNA read alignment was performed against the reference genome GRCH37/hg19.

#### 2.2.4. Variant annotation

The annotation of the exome variants was carried out through the integration of the SnpEff [32] and ANNOVAR [33,34] bioinformatic tools that integrate the population databases: dbSNP [35], (the 1000 Genomes Project Consortium), NHLBI Exome Sequencing Project (ESP) [36], database [37], and the gnomAD and exomAD databases (http://gnomad.broadinstitute.org). Information related to the association between human phenotype and causative genes was added from ClinVar [38,39].

#### 2.2.5. Pathogenicity association of variants

Twelve types of in silico predictive algorithms were included: SIFT [40], PolyPhen-2 [41], MutationTaster [42], LRT [43], Mutation Assesor [44], FATHMM (Functional Analysis Through Hidden Markov Models) [45], MetaSVM [46], RadialSVM, LR, CADD, GERP+ + [47], phyloP [48], SiPhy [49], to assess the pathogenicity of identified variants. Filtering considered exome variants with a MAF $< 0.01$. The norms and guidelines for interpreting sequence variants suggested by the American College of Medical Genetics and Genomics (ACMG) were followed to classify the causality of each of the variants [50].

#### 2.2.6. Prediction of SNP impact on protein stability

I-Mutant3.0 tools were used to predict the stability of a protein based on the presence and.

type of microvariant, available at (http://gpcr.biocomp.unibo.it/cgi/predictors/). For MuPro, structural analysis allowing calculation of protein stability variations at arbitrary SNPs is available at (http://mupro.proteomics. ics.uci.edu/).

The HOPE server was used to analyze the effect of the SNP on the 3D structure of proteins and to calculate physicochemical properties. This tool searches for 3D structures of proteins by collecting structural information from a series of sources, including calculations in the 3D coordinates of the protein. UniProt base sequence annotations and predictions from DAS services are available at http://www.cmbi.ru.nl/hope/.

Additionally, we analyzed protein-protein interaction and functional networks to investigate the direct physical and functional relationships between identified genes, using the (STRING) database available at http://string.embl.de. The GeneMANIA server, available at http://www.genemania.org/, was also used; it is an approach to recognizing the protein's function by integrating multiple genomics and proteomics sources to make inferences about the role of unknown proteins. The input was the keywords MYBkeywords3, TTN, ANK3, KCNH2, and CTDSP2. The output was an image showing the biological network interaction between those genes and their correlated genes.

## 3. Results

### 3.1. Case features

A male teenager, fifteen years old, passes away in his room while sleeping. His toxicology results were negative for ethyl alcohol, cocaine, derivatives, opiates, carboxyhemoglobin, and cyanide. The histopathology report established the brain without specific alterations, a lung with edema, and sections of the heart show foci of fiber disarray, without fibrosis or inflammatory changes or lesions from a previous infarction, and some myocytes with contraction bands indicating recent ischemia. The forensic analysis and conclusion state: "In the absence of other findings, concluding that death is due to cardiovascular causes is not possible. It is considered that the cause of death is undetermined and

probably natural.".

After recovering the necropsy findings, a genetic study was carried out using NGS to determine whether the cause of death could probably be a cardiac channelopathy.

From the molecular autopsy performed by next-generation sequencing (NGS) on clinical exome analysis, a total of 8851 sequence variants were identified. Bioinformatic filtering was performed with the quality of the variants, population frequency, the information provided by the various databases, and in silico prediction, and following the recommendations of the ACMG/AMP group [51]. Table 1 shows the relevant variants found during the study where the variants were selected for further analysis according to the MAF $\leq$ 0.1 calculated for four different population sources ExAC, sources AD, gnomAD, and 1000 genome project. Five genetic variants were identified: C2992G, G1712A, C49424A, G275C, and G6955A present in the MYBPC3, KCND3, TTN, KCNH2, and ANK3 genes, respectively.

### 3.2. Functional prediction of pathogenic variant

Using in silico pathogenic predictors, pathogenic (D), probably pathogenic (Pp), and benign (T) variants were determined. A cut-off point of 20 was used for the CADD algorithm. Prediction of conservation of the sequence used a cut-off point greater than 4.4. Results are shown in Table 2.

### 3.3. Protein stability prediction

Protein stability analysis was performed with I. Mutant 3.0 and MUPro software. The change in free energy caused by the pathogenic variant in the protein was calculated by I.Mutant. For MUPro, values $<$ 0 mean stability decreases, and scores $>$ 0 indicate increased protein stability. The results of these predictions are shown in Table 3. MuPro predictor results indicate reductions in stability for all the mutations. Nevertheless, this trend changed in ANK3 and MYBPC3 when the predictor, the I-Mutator was used.

### 3.4. Server-based 3D structural modeling project hope

The MYBPC3 gene variant (rs11570112: NM_000256.3: Q998E) lies in a UniProt domain as Ig-like C2-type 6 and introduces a glutamic acid at this position. The mutant residue is positively charged, and the wild-type residue is neutrally charged. The wild-type residue is highly conserved, but other residue types have also been observed at this position. Neither the mutant nor any other kind of residue with similar properties was observed in different homologous sequences at this position. Based on conservation scores, this pathogenic variant likely damages the protein. In Fig. 1, a close-up of the pathogenic variant is shown. Position 998 with the change Q by E is highlighted.

The KCND3 gene variant (rs186194682: NM_172198: R571H) introduces histidine at this position. The mutant residue is minor and neutrally charged, while the wild-type residue is positively charged. The wild-type residue is highly conserved, but other residue types have also been observed at this position. The mutant residue was not found among different residues observed at this position in other homologous proteins.

The TTN gene variant (rs541464855: NM_003319: P16475Q) is within one domain, annotated in UniProt as Ig-120. The pathogenic variant will cause the loss of hydrophobic interactions in the core of the protein. The mutant residue is larger and less hydrophobic. In Fig. 2, a close-up of the pathogenic variant is shown where the change P by Q is highlighted in the 16,475 positions.

The ANK3 gene variant (rs140463162: NM_020987: D2319N) introduces asparagine at this position. The mutant residue is charge neutral, while the wild-type residue is negative; there is a loss of charge from the wild-type residue. The mutant residue was not found among the other residue types observed at this position in different homologous sequences. No structural information was found on the HOPE server.

KCNH2 gene variant (NM_000238.4) (exon 2: c.G275C: R92P) introduces proline at this position. The mutated residue is located on the Surface of a domain with an unknown function. The mutant residue is smaller, more hydrophobic than the wild type, and neutral in charge. The wild-type residue forms a hydrogen bond with glutamine at position 84. It forms a salt bridge with glutamic acid at position 90. 3D modeling is shown in Fig. 3, a close-up of the pathogenic variant. The pathogenic variant introduces proline at this position. Position 92 with the change of arginine to proline is highlighted.

### 3.5. Protein-protein interaction analysis

The Functional networks of the six proteins of interest were built by STRING and covered some known functional networks. In Fig. 4, a hub represents a cardiac channelopathy caused by the KCNH2 where other network proteins converged. Interesting, the networks vertices correspond gene associated with Voltage-gated Potassium channels, Phase 0 – rapid depolarization, Phase 1 – inactivation of fast Na+ channels, Potassium channel, voltage dependent, Kv3.1, Voltage gated Potassium channels, Phase 3 – rapid repolarization, originating Long QT syndrome 2 and in addition to other related pathologies. Proteins have more interactions with each other than expected from a random set of proteins of the same size and degree of distribution drawn from the genome. Such enrichment indicates that the proteins are partially biologically connected as a group.

### 3.6. Gene-gene interaction

The analysis by GeneMANIA showed that MYBPC3, KCND3, TTN, ANK3, and KCNH2 have many vital functions and interact with a network of genes- Any alteration in one or more of them can alter the gene interaction and cause an illness. The genes were co-expressed, shared a similar protein domain, or contributed to similar functions, as shown in Fig. 5.

## 4. Discussion

Of the 8851 variants identified by NGS on a clinical exome, five relevant SNPs, in the MYBPC3, KCND3, TTN, ANK3, and KCNH2 genes, were found after applying the different bioinformatic filters based on recommendations from the ACMG/AMP group [51]. All five rare potentially pathogenic exome variants were classified as poorly tolerated by at least two variant prediction algorithms. Therefore, it was

**Table 1**
Variants selected for analysis according to population frequencies with a MAF $\leq$ 0.1.

| rsID | Chr | Gen | Func. Ref Gene | Genetic variant | Change protein level | zygosity | MAF | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | ExAC | exome_AD | gnomAD | 1000 g |
| rs11570112 | chr11 | MYBPC3 | exon27 | c.C2992G | p.Q998E | het | 0,0052 | 0,0072 | 0,0021 | 0,0061901 |
| rs186194682 | chr1 | KCND3 | exon7 | c.G1712A | p.R571H | het | 0,04701 | 0,04917 | 0,03232 | 0,00039936 |
| rs541464855 | chr2 | TTN | exon154 | c.C49424A | p.P16475Q | het | Absent | Absent | 0,03236 | 0,00019968 |
| . | chr7 | KCNH2 | exon2 | c.G275C | p.R92P | hom | Absent | Absent | Absent | Absent |
| rs140463162 | chr10 | ANK3 | exon37 | c.G6955A | p.D2319N | het | 0,0028 | 0,0033 | 0,0033 | 0,00139776 |

**Table 2**
Prediction of pathogenicity of SNPs in silico for SIFT, PolyPhen2, Mutation Taster, LRT, Mutation Assesor, FATHMM, MetaSVM, RadialSVM, LR, CADD, GERP+ +, PhyloP, and SiPhy.

| Gene | AA change | rsID | SIFT | Polyphen2 | LRT | Mutation Taster | Mutation Assessor | FATHMM | RadialSVM |
|---|---|---|---|---|---|---|---|---|---|
| KCND3 | R571H | rs186194682 | **D** | Pp | D | Pp | Medium | D | D |
| TTN | P16475Q | rs541464855 | **D** | Pp | . | Pp | High | T | D |
| KCNH2 | R92P | . | **T** | Pp | T | Pp | Medium | D | D |
| ANK3 | D2319N | rs140463162 | **T** | Pp | D | Pp | Medium | T | T |
| MYBPC | Q998E | rs11570112 | **D** | Pp | . | Pp | Medium | T | T |
| **Gene** | **AA change** | **rsID** | **LR** | **CADD** | **GERP+ +RS** | **phyloP** | **SiPhy** | **IMPACT** | |
| KCND3 | R571H | rs186194682 | D | 28.7 | 5.63 | 2814 | 19639 | Moderate | |
| TTN | P16475Q | rs541464855 | D | 12.28 | 5.56 | 2608 | 19497 | Moderate | |
| KCNH2 | R92P | . | D | 20.7 | 3.49 | 811 | 11994 | High | |
| ANK3 | D2319N | rs140463162 | T | 23.8 | 5.94 | 2816 | 20359 | Moderate | |
| MYBPC | Q998E | rs11570112 | T | 21.2 | 4.1 | 1311 | 14449 | Moderate | |

**Table 3**
Protein stability of the SNPs by I-Mutant 3.0 and Mupro.

| Gen | SNP ID | Change AA | I-Mutant 3.0 Prediction | RI | DDG valor predicción | MuPro Prediction | MuPro Score |
|---|---|---|---|---|---|---|---|
| KCNH2 | . | R92P | Decrease | 6 | -0.52 | Decreases | -0.5125869 |
| ANK3 | rs140463162 | D2319N | Increase | 2 | -0.22 | Decreases | -0.60154872 |
| TTN | rs541464855 | P16475Q | Decrease | 8 | -1.29 | Decreases | -1.0325811 |
| KCND3 | rs186194682 | R571H | Decrease | 6 | -1.20 | Decreases | -0.7320396 |
| MYBPC3 | rs11570112 | Q998E | Increase | 4 | -0.16 | Decreases | -0.73155586 |

DDG: free energy change value; RI: reliability index

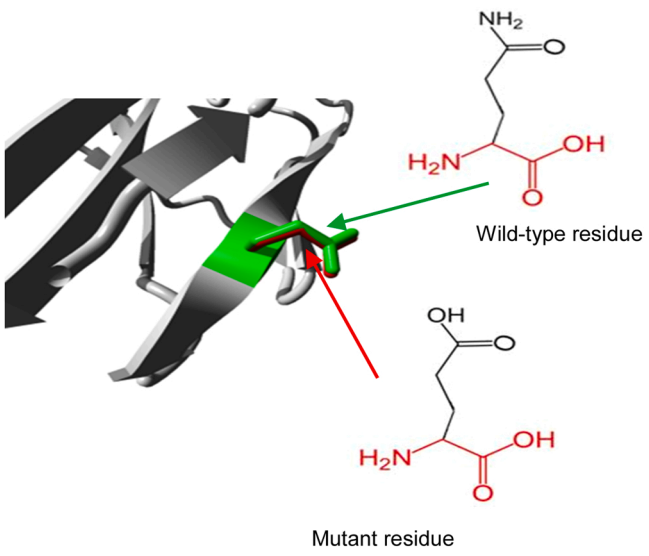


**Fig. 1.** Close-up of the pathogenic variant. The protein is shown in gray, and the side chains of the wild type and mutant residues are shown and colored green and red, respectively. MYBPC3 variant: rs11570112: Q998E, protein position 998 change from glutamine to glutamic acid.

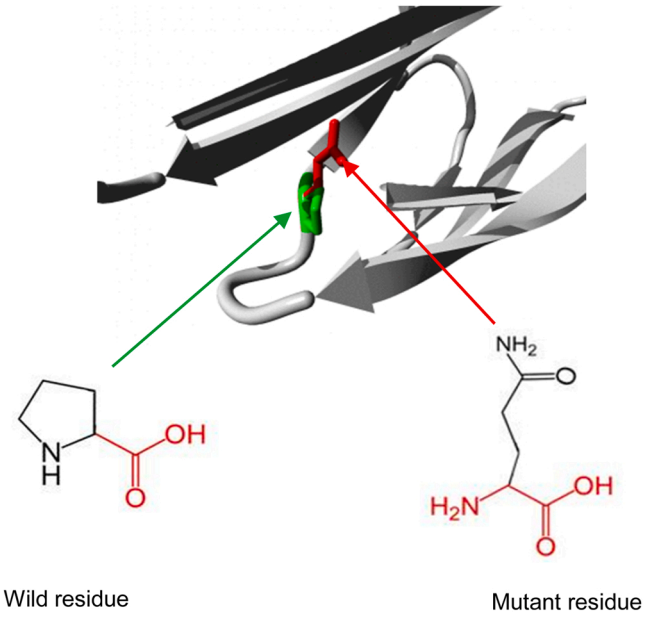


**Fig. 2.** Close-up of the pathogenic variant. The protein is shown in gray, and the side chains of the wild type and mutant residues are shown and colored green and red, respectively. TTN variant: rs541464855: P16475Q, protein position 92 changed from proline to glutamine.

decided to perform further analyses. Predicted decrease or increase in protein stability caused by the pathogenic variants was shown in Table 3. Those results are consistent with the physicochemical characteristics of each variant.

- MYBPC3: rs11570112 is a missense and splicing region variant associated with primary dilated cardiomyopathy, hypertrophic cardiomyopathy, left ventricular non-compaction cardiomyopathy, primary familial hypertrophic cardiomyopathy, familial hypertrophic cardiomyopathy 4, cardiovascular phenotype, cardiomyopathy dilated and dominant diabetic myopathy [52].
- KCND3: rs186194682 is a variant with a conflicting interpretation of pathogenicity. The KCND3 gene (Potassium Voltage-Gated Channel Subfamily D Member 3) is associated with diseases such as Brugada syndrome, a cardiac channelopathy with high genetic heterogeneity that has a high incidence of sudden death in patients with a structurally normal heart [53].
- TTN: rs541464855 is a missense variant. The TTN gene encodes the sarcomere titin protein found in cardiac and skeletal muscle, and its mutations are associated with hypertrophic and dilated cardiomyopathy and various skeletal muscle diseases [54].
- ANK3: rs140463162 may participate in the maintenance/direction of ion channels and cell adhesion molecules at the nodes of Ranvier and initial axon segments. It regulates the activity of the KCNA1

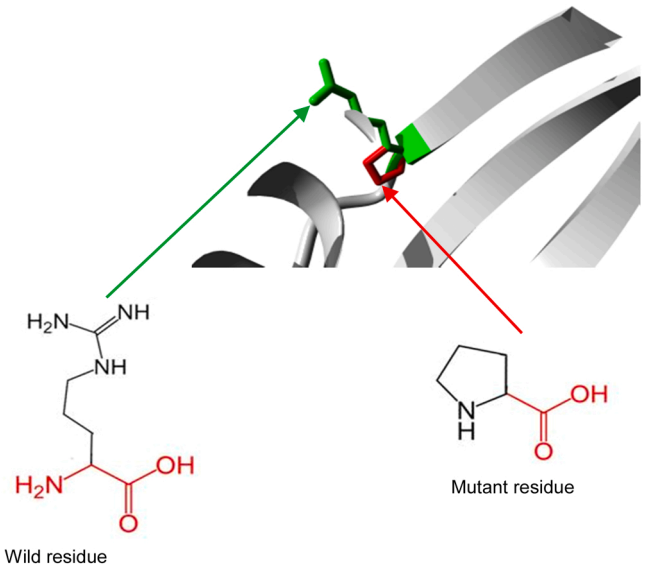


**Fig. 3.** Close-up of the pathogenic variant. The protein is shown in gray, and the side chains of the wild type and mutant residues are shown and colored green and red, respectively. Variant (KCNH2: NM_000238.4: R92P), protein position 92 changed from an arginine to a proline.

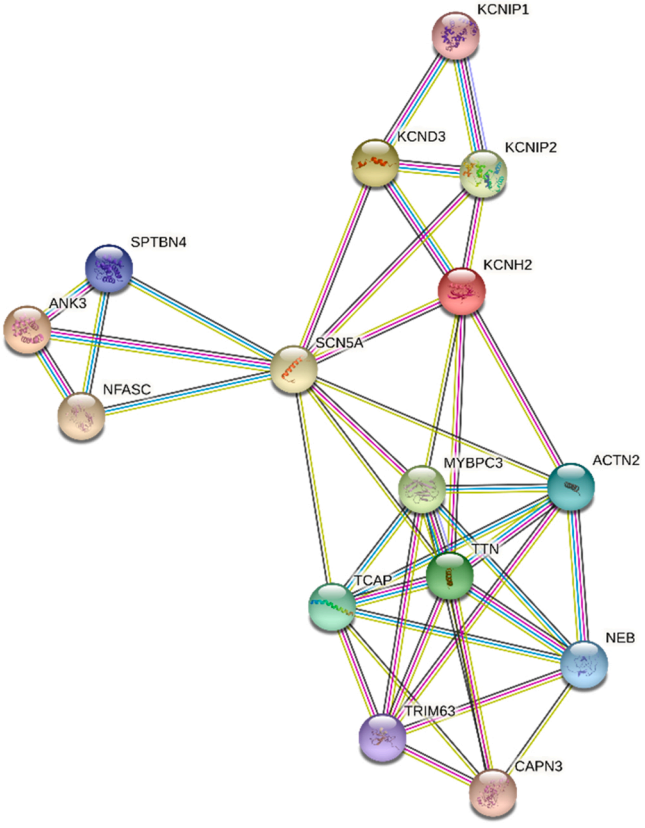


**Fig. 4.** Functional interaction between MYBPC3, KCND3, TTN, ANK3, and KCNH2 genes and related genes.

channel depending on the levels of Mg (2 +) in the diet and, therefore, contributes to the regulation of renal reabsorption of Mg (2 +). ANK3 (ANKG) is required for the localization of NaV1.5 and CaMKII in the intercalated disc of cardiomyocytes [57]. A variant of the SCN5A gene in the ANKG-binding motif of NaV1.5 has been associated with Brugada syndrome and arrhythmia [58]. This same variant is a loss-of-function variant when expressed in primary cardiomyocytes. AnkG variants have been associated with Brugada syndrome and, more recently, dilated cardiomyopathy [59].

- KCNH2 (exon 2: c.G275C: R92P): Analysis of this variant reveals that the mutated residue is found in a MilD_Hotspot region for type 2 LQT syndrome that contains highly lethal variants [60]. Variants in exon 7 of the KCNH2 gene have previously been reported to cause sudden death [61], which is consistent with the structural effects of the mutations in the protein. The mutation in this gene could be responsible for the cause of death.

Since conformational changes are vital to the function of many proteins, conformational flexibility and stiffness must be very well balanced [62]. SNPs rs11570112, rs186194682, rs541464855, rs140463162, and R92P were validated as highly conserved by the HOPE algorithm, suggesting that they have deleterious effects on protein structure and therefore likely to be pathogenic. This is consistent with the observation that harmful SNPs are more common in conserved sites [63]. Furthermore, if mutated residues are found in internal domains of a protein's structure, incorrect folding may decrease protein stability, leading to effects mimicking the presence of deleterious nonsense variants [64].

The R92, P16475, R571, and Q998 residues, found in essential domains for each protein structure, will cause a loss of hydrophobic interaction and changes in size, which will not allow for correct molecular interactions, affecting the function of protein complexes [65].

Although functional and clinical studies are required for definitive classification of deleterious variants, it can take a long time to obtain data on all variants. Different approaches with a certain degree of reliability predict highly dangerous SNPs [66]. The methods used in our present study offer evidence of the applicability of these approaches to infer variant pathogenicity.

Protein-protein interaction calculated with STRING (Fig. 4) showed that KCNH2 has strong interactions with KCND3, ACTN2, MYBPC3, and TTN. Furthermore, the variants found in the genes MYBPC3, TTN, and ACTN2 have been associated with hypertrophic cardiomyopathy, channelopathies such as the Brugada syndrome, and other arrhythmias [67–69]. If they are simultaneously presented, those pathogenic variants might be strong candidates to trigger sudden cardiac death. Although ANK3 is not included in the network prediction, it is known that it is associated with the NaV1.5 cardiac sodium channel of the SCNA5 gene, which is expressed in the ventricular intercalated disc and the membranes of cardiomyocyte T tubules. As described, alterations in the Nav1.5 protein that block ANK3 binding and surface expression of Nav1.5 in cardiomyocytes can lead to the cardiac arrhythmia known as Brudaga syndrome [67].

Finally, the gene interaction network built by GeneMANIA shown in Fig. 5 shows a robust physical interaction and a relationship between domains with some co-expressed genes. These findings might indicate that alterations in the proteins of the genes KCNH2, KCND3, ACTN2, MYBPC3, and TTN together could be potential factors associated with sudden cardiac death. Our results recommend further molecular dynamics studies among these genes [68,69].

We claim that other experiments should be performed to assess if the protein localization triggers SUD and other electrophysiologist assays. While our survey shows that it is possible to detect variants with NGS of exomes, functional analysis are required to determine their pathogenicity using mice and other model organisms. Finally, the complexity of the variants, such as their heterogeneity and the variable expression, must also be considered in further analysis. In this context, substantial challenges to complete the SUD diagnosis remain. Hence, our study gives a set of candidates to further validate their pathogenic role in SUD. Our screening demonstrates the potential that NGS technology has to be applied to increase the number of markers to be analyzed in SUDs [69] and forensic science [70,71].

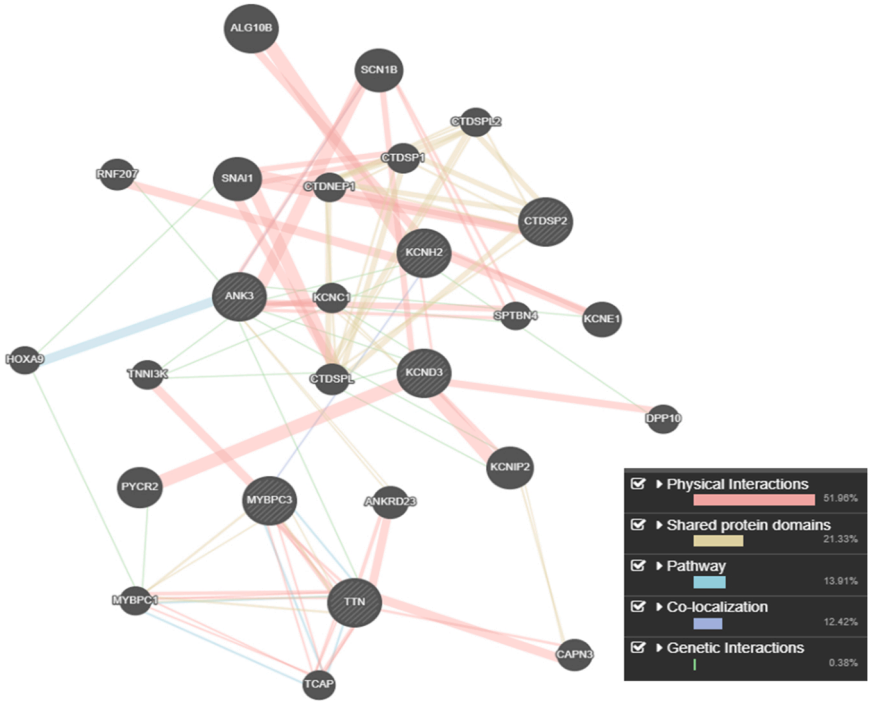


**Fig. 5.** Biological network of interaction between MYBPC3, KCND3, TTN, ANK3, and KCNH2 genes and other related genes, predicted by GeneMANIA.

## 5. Conclusion

We present a molecular autopsy supported by next-generation sequencing of the clinical exome that includes 4834 clinically relevant genes for a suspected case of SCD. Genetic analysis using NGS was performed on the blood sample recovered during an autopsy to identify the causal variants of the phenotype and the reason for sudden death.

The molecular analysis supported by NGS allowed us to identify variants in the KCND3, CTDSP2, MYBP3, TTN, and ACTN2 genes possibly related to SUD in a fifteen-year-old male. Additionally, the homozygous variant in the KCNH2 gene associated with long QT syndrome type 2 suggests the patient's death was due to a cardiac channelopathy. Our results support the diagnosis of this death as potentially associated with SCD due to genetic variants detected that affect the structure and function of the proteins and mild disarray found in the individual's cardiomyocyte fibers. Finally, to better assess the prevalence of these variants in sporadic SUD, molecular studies need to be performed on a more significant number of cases.

## Ethical approval

Ethical approval for this study was granted by the local committee of the Science Faculty of the National University of Colombia. Principles contained in the updated Declaration of Helsinki were followed, as well as Laws, Decrees, and Resolutions related to viscerotomies and the use of forensic samples for research and teaching at the National Institute of Legal Medicine and Forensic Sciences.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgments

The authors thank the University of Magdalena and the University of Applied and Environmental Sciences UDCA, for their financial support, within their agreement with the National Institute of Legal Medicine and Forensic Sciences as well as the National University of Colombia Faculty of Science and the Center of Excellence in Scientific Computing at the National University of Colombia.

## References

[1] C. Basso, M. Burke, P. Fornes, et al., Guidelines for autopsy investigation of sudden cardiac death, Virchows Arch. 452 (2008) 11–18, https://doi.org/10.1007/s00428-007-0505-5.
[2] A.S. Adabag, R.V. Luepker, V.L. Roger, B.J. Gersh, Sudden cardiac death: epidemiology and risk factors, Nat. Rev. Cardiol. 7 (2010) 216–225, https://doi.org/10.1038/nrcardio.2010.3.
[3] S.S. Chugh, K. Reinier, C. Teodorescu, et al., Epidemiology of sudden cardiac death: clinical and research implications, Prog. Cardiovasc. Dis. 51 (2008) 213–228, https://doi.org/10.1016/j.pcad.2008.06.003.
[4] J.R. Kaltman, P.D. Thompson, J. Lantos, et al., Screening for sudden cardiac death in the young: Report from a national heart, lung, and blood institute working group, Circulation 123 (2011) 1911–1918, https://doi.org/10.1161/CIRCULATIONAHA.110.017228.
[5] J. Tester David, Argelia Medeiros-Domingo, Melissa L. Will, Carla M. Haglund, Michael J. Ackerman, Cardiac channel molecular autopsy: insights from 173 consecutive cases of autopsy-negative sudden unexplained death referred for postmortem genetic testing, Mayo Clin. Proc. 87 (6) (2012) 524–539, https://doi.org/10.1016/j.mayocp.2012.02.017.
[6] M.J. Ackerman, S.G. Priori, S. Willems, C. Berul, R. Brugada, H. Calkins, et al., HRS/EHRA expert consensus statement on the state of genetic testing for the channelopathies and cardiomyopathies: this document was developed as a partnership between the Heart Rhythm Society (HRS) and the European Heart Rhythm Association (EHRA), Heart Rhythm 8 (8) (2011) 1308–1339, https://doi.org/10.1016/j.hrthm.2011.05.020.
[7] N. Narula, D.J. Tester, A. Paulmichl, J.J. Maleszewski, M.J. Ackerman, Postmortem whole exome sequencing with gene-specific analysis for autopsy-negative sudden unexplained death in the young: a case series, Pediatr. Cardiol. 36 (2015) 768–778, https://doi.org/10.1007/s00246-014-1082-4.
[8] J. Neubauer, C. Haas, C. Bartsch, A. Medeiros-Domingo, W. Berger, Postmortem whole-exome sequencing (WES) with a focus on cardiac disease-associated genes in five young sudden unexplained death (SUD) cases, Int. J. Leg. Med. 130 (2016) 1011–1021, https://doi.org/10.1007/s00414-016-1317-4.
[9] A.A.T. Geisterfer-Lowrance, S. Kass, G. Tanigawa, H.P. Vospert, W. McKenna, J. G. Seidman, et al., A molecular basis for familial hypertrophic cardiomyopathy: a beta cardiac myosin heavy chain missence mutation, Cell 62 (1999) 999–1006, https://doi.org/10.1016/0092-8674(90)90274-i.
[10] E.L. Burkett, R.E. Hershberger, Clinical and genetic issues in familial dilated cardiomyopathy, J. Am. Coll. Cardiol. 45 (2005) 969–981, https://doi.org/10.1016/j.jacc.2004.11.066.

[11] M. Tomé Esteban, J. Garcia Pinilla, W.J. McKenna, Update in arrhythmogenic right ventricular cardiomyopathy: genetic, clinical presentation and risk stratification, Rev. Esp. Cardiol. 57 (8) (2004) 757–767, https://doi.org/10.1016/S1885-5857(06)60310-1.

[12] P.Zipes Douglas, et al., ACC/AHA/ESC 2006 guidelines for management of patients with ventricular arrhythmias and the prevention of sudden cardiac death: a report of the American College of Cardiology/American Heart Association Task Force and the European society of cardiology committee for practice guidelines (Writing committee to develop guidelines for management of patients with ventricular arrhythmias and the prevention of sudden cardiac death), J. Am. Coll. Cardiol. 5 48 (5) (2006) e247–e346, https://doi.org/10.1016/j.jacc.2006.07.010 (Heart Rhythm Society, European Heart Rhythm Association).

[13] S.G. Priori, S.V. Pandit, I. Rivolta, O. Berenfeld, E. Ronchetti, A. Dhamoon, C. Napolitano, J. Anumonwo, M.R. di Barletta, S. Gudapakkam, G. Bosi, M. Stramba-Badiale, J. Jalife, A novel form of short QT syndrome (SQT3) is caused by a mutation in the KCNJ2 gene, Circ. Res. 96 (7) (2005) 800–807, https://doi.org/10.1161/01.RES.0000162101.76263.8c.

[14] O. Campuzano, C. Allegue, S. Partemi, A. Iglesias, A. Oliva, R. Brugada, Negative autopsy and sudden cardiac death, Int. J. Leg. Med. 128 (4) (2014) 599–606, https://doi.org/10.1007/s00414-014-0966-4.

[15] Go A.S., Mozaffarian D., Roger V.L., Benjamin E.J., Berry J.D., Borden W.B., Bravata D.M., Dai S., Ford E.S., Fox C.S., Franco S., Fullerton H.J., Gillespie C., Hailpern S.M., Heit J.A., Howard V.J., Huffman M.D., Kissela B.M., Kittner S.J., Lackland D.T., Lichtman J.H., Lisabeth L.D., Magid D., Marcus G.M., Marelli A., Matchar D.B., McGuire D.K., Mohler E.R., Moy C.S., Mussolino M.E., Nichol G., Paynter N.P., Schreiner P.J., Sorlie P.D., Stein J., Turan T.N., Virani S.S., Wong N. D., Woo D., Turner M.B.; American Heart Association Statistics Committee and Stroke Statistics Subcommittee (2013). Heart disease and stroke statistics - -2013 update: a report from the American Heart Association. Circulation. 1;127(1): e6-e245. DOI: 10.1161/CIR.0b013e31828124ad.

[16] C.R. Bezzina, N. Lahrouchi, S.G. Priori, Genetics of sudden cardiac death, Circ. Res. 116 (2015) 1919–1936, https://doi.org/10.1161/CIRCRESAHA.116.304030.

[17] Yingying Tang, Jay Stahl-Herz, Barbara A. Sampson, Molecular diagnostics of cardiovascular diseases in sudden unexplained death, Cardiovasc. Pathol. 23 (1) (2014) 1–4, https://doi.org/10.1016/j.carpath.2013.09.002.

[18] M.J. Ackerman, et al., HRS/EHRA expert consensus statement on the state of genetic testing for the channelopathies and cardiomyopathies this document was developed as a partnership between the heart rhythm society (HRS) and the European heart rhythm association (EHRA), Heart Rhythm 8 (2011) 1308–1339, https://doi.org/10.1016/j.hrthm.2011.05.020.

[19] C. Semsarian, R.M. Hamilton, Key role of the molecular autopsy in sudden unexpected death, Heart Rhythm 9 (2012) 145–150, https://doi.org/10.1016/j.hrthm.2011.07.034.

[20] J.R. Giudicessi, M.J. Ackerman, Determinants of incomplete penetrance and variable expressivity in heritable cardiac arrhythmia syndromes, Transl. Res. 161 (1) (2013) 1–14, https://doi.org/10.1016/j.trsl.2012.08.005.

[21] C. Semsarian, J. Ingles, A.A. Wilde, Sudden cardiac death in the young: the molecular autopsy and a practical approach to surviving relatives, Eur. Heart J. 36 (2015) 1290–1296, https://doi.org/10.1093/eurheartj/ehv063.

[22] S.G. Priori, A.A. Wilde, M. Horie, et al., HRS/EHRA/APHRS expert consensus statement on the diagnosis and management of patients with inherited primary arrhythmia syndromes: expert consensus statement on inherited primary arrhythmia syndromes: document endorsed by HRS, EHRA, and APHRS in May 2013 and by ACCF, AHA, PACES, and AEPC in June 2013, Heart Rhythm 10 (12) (2013) 1932–1963, https://doi.org/10.1016/j.hrthm.2013.05.014.

[23] Andrews, S. , 2010. FastQC: A quality control tool for high throughput sequence data [Online]. Available online at: ⟨http://www.bioinformatics.babraham.ac.uk/projects/fastqc/⟩.

[24] S. Chen, Y. Zhou, Y. Chen, J. Gu, fastp: an ultra-fast all-in-one FASTQ preprocessor, Bioinformatics 34 (17) (2018) i884–i890, https://doi.org/10.1093/bioinformatics/bty560.

[25] B. Langmead, S. Salzberg, Fast gapped-read alignment with Bowtie 2, Nat. Methods 9 (357–359) (2012) 2012, https://doi.org/10.1038/nmeth.1923.

[26] Li H. , 2013. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. arXiv:1303.3997v2 [q-bio.GN]. (if you use the BWA-MEM algorithm or the fastmap command or want to cite the whole BWA package). DOI: https://doi.org/10.48550/arXiv.1303.3997.

[27] T.D. Wu, S. Nacu, Fast and SNP-tolerant detection of complex variants and splicing in short reads, Bioinformatics 26 (7) (2010) 873–881, https://doi.org/10.1093/bioinformatics/btq057.

[28] Novocraft. , 2017. Novoalign & NovoalignCS Reference Manual. Release 3.07.00, 9th January. https://www.novocraft.com/userfiles/file/Novocraft.pdf

[29] J.E. Lunshof, et al., Personal genomes in progress: from the human genoma project to the personal genome project, Dialog-. Clin. Neurosci. 12 (2010) 47–60, https://doi.org/10.31887/DCNS.2010.12.1/jlunshof.

[30] H. Li, B. Handsaker, A. Wysoker, T. Fennell, J. Ruan, N. Homer, G. Marth, G. Abecasis, R. Durbin, The sequence alignment/map (SAM) format and SAMtools, Bioinformatics 25 (16) (2009) 2078–2079, https://doi.org/10.1093/bioinformatics/btp352 (1000 Genome Project Data Processing Subgroup).

[31] Garrison, E. & Marth, G. , 2012. Haplotype-based variant detection from short-read sequencing. arXiv preprint, ArXiv:1207.3907 [q-bio.GN]: https://doi.org/10.48550/arXiv.1207.3907.

[32] P. Cingolani, A. Platts, L.L. Wang, M. Coon, T. Nguyen, L. Wang, et al., A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of Drosophila melanogaster strain w 1118; iso-2; iso-3, Landes Biosci. 2 (2012) 1–13, https://doi.org/10.4161/fly.19695.

[33] K. Wang, M. Li, H. Hakonarson, ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data, Nucleic Acids Res. 38 (2010), e164, https://doi.org/10.1093/nar/gkq603.

[34] H. Yang, K. Wang, Genomic variant annotation and prioritization with ANNOVAR and wANNOVAR, Nat. Protoc. 10 (2015) 1556–1566, https://doi.org/10.1038/nprot.2015.105.

[35] S.T. Sherry, M.H. Ward, M. Kholodov, J. Baker, L. Phan, E.M. Smigielski, K. Sirotkin, dbSNP: the NCBI database of genetic variation, Nucleic Acids Res. 29 (1) (2001) 308–311, https://doi.org/10.1093/nar/29.1.308.

[36] W. Fu, T.D. O'Connor, G. Jun, H.M. Kang, G. Abecasis, S.M. Leal, S. Gabriel, M. J. Rieder, D. Altshuler, J. Shendure, D.A. Nickerson, M.J. Bamshad, Analysis of 6,515 exomes reveals the recent origin of most human protein-coding variants, Nature 493 (7431) (2013) 216–220, https://doi.org/10.1038/nature11690.

[37] Erratum in: Nature. 2013 Mar 14;495(7440):270. Rieder, Mark J [added]. PMID: 23201682; PMCID: PMC3676746.

[38] Konrad J. Karczewski, Ben Weisburd, Brett Thomas, Matthew Solomonson, Douglas M. Ruderfer, David Kavanagh, Tymor Hamamsy, Monkol Lek, Kaitlin E. Samocha, Beryl B. Cummings, Daniel Birnbaum, The Exome Aggregation Consortium, Mark J. Daly, Daniel G. MacArthur, The ExAC browser: displaying reference data information from over 60 000 exomes, Nucleic Acids Res. 45 (D1) (2017) D840–D845, https://doi.org/10.1093/nar/gkw971.

[39] M.J. Landrum, J.M. Lee, M. Benson, G.R. Brown, C. Chao, S. Chitipiralla, B. Gu, J. Hart, D. Hoffman, W. Jang, K. Karapetyan, K. Katz, C. Liu, Z. Maddipatla, A. Malheiro, K. McDaniel, M. Ovetsky, G. Riley, G. Zhou, J.B. Holmes, B. L. Kattman, D.R. Maglott, ClinVar: improving access to variant interpretations and supporting evidence, Nucleic Acids Res. 46 (D1) (2018) D1062–D1067, https://doi.org/10.1093/nar/gkx1153. PMID: 29165669; PMCID: PMC5753237.

[40] P.C. Ng, S. Henikoff, SIFT: predicting amino acid changes that affect protein function, Nucleic Acids Res. 31 (13) (2003) 3812–3814, https://doi.org/10.1093/nar/gkg509. PMID: 12824425; PMCID: PMC168916.

[41] I.A. Adzhubei, S. Schmidt, L. Peshkin, V.E. Ramensky, A. Gerasimova, P. Bork, A. S. Kondrashov, S.R. Sunyaev, A method and server for predicting damaging missense mutations, Nat. Methods 7 (4) (2010) 248–249, https://doi.org/10.1038/nmeth0410-248. PMID: 20354512; PMCID: PMC2855889.

[42] J. Schwarz, D. Cooper, M. Schuelke, et al., MutationTaster2: mutation prediction for the deep-sequencing age, Nat. Methods 11 (2014) 361–362, https://doi.org/10.1038/nmeth.2890.

[43] S. Chun, J.C. Fay, Identification of deleterious mutations within three human genomes, Genome Res. 19 (9) (2009) 1553–1561, https://doi.org/10.1101/gr.092619.109.

[44] B. Reva, Y. Antipin, C. Sander, Predicting the functional impact of protein mutations: application to cancer genomics, Nucleic Acids Res. 39 (2011), e118, https://doi.org/10.1093/nar/gkr407.

[45] H.A. Shihab, J. Gough, D.N. Cooper, et al., Predicting the functional, molecular, and phenotypic consequences of amino acid substitutions using hidden Markov models, Hum. Mutat. 34 (1) (2013) 57–65, https://doi.org/10.1002/humu.22225.

[46] C. Dong, P. Wei, X. Jian, R. Gibbs, E. Boerwinkle, K. Wang, X. Liu, Comparison and integration of deleteriousness prediction methods for nonsynonymous SNVs in whole exome sequencing studies, Hum. Mol. Genet. 24 (8) (2015) 2125–2137, https://doi.org/10.1093/hmg/ddu733. Epub 2014 Dec 30. PMID: 25552646; PMCID: PMC4375422.

[47] G.M. Cooper, D.L. Goode, S.B. Ng, A. Sidow, M.J. Bamshad, J. Shendure, D. A. Nickerson, Single-nucleotide evolutionary constraint scores highlight disease-causing mutations, Nat. Methods 7 (4) (2010) 250–251, https://doi.org/10.1038/nmeth0410-250. PMID: 20354513; PMCID: PMC3145250.

[48] Siepel A., Pollard K., Haussler D., 2006. New methods for detecting lineage-specific selection. In: Proceedings of the 10th Int'l Conference on Research in Computational Molecular Biology. Berlin: Springer-Verlag, pp.190–205. DOI: 10.1007/11732990_17.

[49] M. Garber, M. Guttman, M. Clamp, M.C. Zody, N. Friedman, X. Xie, Identifying novel constrained elements by exploiting biased substitution patterns, Bioinformatics 25 (12) (2009) i54–i62, https://doi.org/10.1093/bioinformatics/btp190. PMID: 19478016; PMCID: PMC2687944.

[50] S. Richards, N. Aziz, S. Bale, D. Bick, S. Das, J. Gastier-Foster, W.W. Grody, M. Hegde, E. Lyon, E. Spector, K. Voelkerding, H.L. Rehm, Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American college of medical genetics and genomics and the association for molecular pathology, Genet. Med. 17 (5) (2015) 405–424, https://doi.org/10.1038/gim.2015.30 (ACMG Laboratory Quality Assurance Committee. , Epub 2015 Mar 5. PMID: 25741868; PMCID: PMC4544753).

[51] A.N. Abou Tayoun, T. Pesaran, M.T. DiStefano, A. Oza, H.L. Rehm, L.G. Biesecker, Recommendations for interpreting the loss of function PVS1 ACMG/AMP variant criterion, Hum. Mutat. 39 (11) (2018) 1517–1524, https://doi.org/10.1002/humu.23626 (ClinGen Sequence Variant Interpretation Working Group).

[52] H. Duzkale1, J. Shen1, H. McLaughlin, A. Alfares, M.A. Kelly, T.J. Pugh, B. H. Funke, H.L. Rehm, M.S. Lebo, A systematic approach to assessing the clinical significance of genetic variants, Clin. Genet. 84 (5) (2013) 453–463, https://doi.org/10.1111/cge.12257.

[53] Jennifer J. David Ng, Jamie K. Johnston, Larry N. Teer, Lindsey C. Singh, Jamila S. Peller, Katie L. Wynter, David N. Lewis, Peter D. Cooper, James C. Stenson, Mullikin, G. Leslie, Biesecker, Interpreting secondary cardiac disease variants in an exome cohort, Circ. Cardiovasc Genet. 6 (4) (2013), https://doi.org/10.1161/CIRCGENETICS.113.000039.

[54] M. Savarese, J. Sarparanta, A. Vihola, B. Udd, P. Hackman, Increasing role of titin mutations in neuromuscular disorders, J. Neuromuscul. Dis. 3 (3) (2016) 293–308, https://doi.org/10.3233/JND-160158. PMID: 27854229; PMCID: PMC5123623.

[57] M.A. Makara, J. Curran, E.R. Lubbers, N.P. Murphy, S.C. Little, H. Musa, S. A. Smith, S.D. Unudurthi, M.V.S. Rajaram, P.M.L. Janssen, P.A. Boyden, E. A. Bradley, T.J. Hund, P.J. Mohler, Novel mechanistic roles for ankyrin-G in cardiac remodeling and heart failure, JACC Basic Transl. Sci. 5 (2018) 675–689, https://doi.org/10.1016/j.jacbts.2018.07.008. PMID: 30456339; PMCID: PMC6234521.

[58] B.M. Kroncke, T. Yang, P. Kannankeril, M.B. Shoemaker, D.M. Roden, Exploiting ion channel structure to assess rare variant pathogenicity, Heart Rhythm 15 (6) (2018) 890–894, https://doi.org/10.1016/j.hrthm.2018.01.021.

[59] K. Hong, P. Bjerregaard, I. Gussak, R. Brugada, Short QT syndrome and atrial fibrillation caused by mutation in KCNH2, J. Cardiovasc. Electrophysiol. 16 (2005) 394–396, https://doi.org/10.1046/j.1540-8167.2005.40621.x.

[60] B.M. Kroncke, J. Mendenhall, D.K. Smith, C.R. Sanders, J.A. Capra, A.L. George, J. D. Blume, J. Meiler, D.M. Roden, Protein structure aids predicting functional perturbation of missense variants in *SCN5A* and *KCNQ1*, Comput. Struct. Biotechnol. J. 17 (2019) 206–214, https://doi.org/10.1016/j.csbj.2019.01.008. PMID: 30828412; PMCID: PMC6383132.

[61] P.L. Jia, Y.B. Wang, H. Fu, W.L. Huang, S.R. Zhong, L. Ma, Y.H. Li, Y. Dong, Z. C. Sun, L. Yang, P.F. Qu, S. Zhao, Y.Q. Qu, Y.M. Xi, S.W. Wang, X. Tang, P.P. Lei, Postmortem analysis of 4 mutation hotspots of KCNQ1, KCNH2, and SCN5A genes in sudden unexplained death in Southwest of China, Am. J. Forensic Med Pathol. 39 (3) (2018) 218–222, https://doi.org/10.1097/PAF.0000000000000411. PMID: 29851656.

[62] R. Walsh, K.L. Thomson, J.S. Ware, B.H. Funke, J. Woodley, K.J. McGuire, F. Mazzarotto, E. Blair, A. Seller, J.C. Taylor, E.V. Minikel, Consortium Exome Aggregation, D.G. MacArthur, M. Farrall, S.A. Cook, H. Watkins, Reassessment of Mendelian gene pathogenicity using 7,855 cardiomyopathy cases and 60,706 reference samples, Genet. Med. 19 (2) (2017) 192–203, https://doi.org/10.1038/gim.2016.90. Epub 2016 Aug 17. PMID: 27532257; PMCID: PMC5116235.

[63] F. Robert, J. Pelletier, Exploring the impact of single-nucleotide polymorphisms on translation, Front. Genet. 9 (2018) 507, https://doi.org/10.3389/fgene.2018.00507. PMID: 30425729; PMCID: PMC6218417.

[64] P.J. Wijnker, F.W. Friedrich, A. Dutsch, S. Reischmann, A. Eder, I. Mannhardt, G. Mearini, T. Eschenhagen, J. van der Velden, L. Carrier, Comparison of the effects of a truncating and a missense MYBPC3 mutation on contractile parameters of engineered heart tissue, J. Mol. Cell Cardiol. 97 (2016) 82–92, https://doi.org/10.1016/j.yjmcc.2016.03.003.

[65] MaksymilianProndzynskiMarc D. LemoineAntonia T.L. ZechAndrás HorváthVittoria Di MauroJussi T. KoivumäkiNico KresinJosefine BuschTobias KrauseElisabeth KrämerSaskia SchlossarekMichael SpohnFelix W. FriedrichJulia MünchSandra D. LauferCharles RedwoodAlexander E. VolkArne HansenGiulia MeariniDaniele CatalucciChristian MeyerTorsten ChristMonica PattenThomas EschenhagenLucie Carrier (2019). Disease modeling of a mutation in α-actinin 2 guides clinical therapy in hypertrophic cardiomyopathy. EMBO Mol Med 1: e11115. https://doi.org/10.15252/emmm.201911115.

[66] P.J. Mohler, I. Rivolta, C. Napolitano, G. LeMaillet, S. Lambert, S.G. Priori, V. Bennett, Nav1.5 E1053K mutation causing Brugada syndrome blocks binding to ankyrin-G and expression of Nav1.5 on the surface of cardiomyocytes, Proc. Natl. Acad. Sci. USA 101 (50) (2004) 17533–17538, https://doi.org/10.1073/pnas.0403711101.

[67] P.J. Wijnker, F.W. Friedrich, A. Dutsch, S. Reischmann, A. Eder, I. Mannhardt, G. Mearini, T. Eschenhagen, J. van der Velden, L. Carrier, Comparison of the effects of a truncating and a missense MYBPC3 mutation on contractile parameters of engineered heart tissue, J. Mol. Cell Cardiol. 97 (2016) 82–92, https://doi.org/10.1016/j.yjmcc.2016.03.003.

[68] Maksymilian Prondzynski,Marc D. Lemoine,Antonia T.L. ZechAndrás HorváthVittoria Di MauroJussi T. KoivumäkiNico KresinJosefine BuschTobias KrauseElisabeth KrämerSaskia SchlossarekMichael SpohnFelix W. FriedrichJulia MünchSandra D. LauferCharles RedwoodAlexander E. VolkArne HansenGiulia MeariniDaniele CatalucciChristian MeyerTorsten ChristMonica PattenThomas EschenhagenLucie Carrier (2019). Disease modeling of a mutation in α-actinin 2 guides clinical therapy in hypertrophic cardiomyopathy. EMBO Mol Med 1: e11115. https://doi.org/10.15252/emmm.201911115.

[69] Chun Wang, Shan Duan, Guoli Lv, Xiaoping Lai, Rui Chen, Hanguang Lin, Shengyuan Qiu, Jianpin Tang, Wenjian Kuang, Chuanchao Xu, Using whole exome sequencing and bioformatics in the molecular autopsy of a sudden unexplained death syndrome (SUDS) case, Forensic Sci. Int. 257 (2015) e20–e25, https://doi.org/10.1016/j.forsciint.2015.08.022.

[70] D. Ballard, J. Winkler-Galicki, J. Wesoły, Massive parallel sequencing in forensics: advantages, issues, technicalities, and prospects, Int. J. Leg. Med. 134 (2020) 1291–1303, https://doi.org/10.1007/s00414-020-02294-0.

[71] V. Castiglione, M. Modena, A. Aimo, E. Chiti, N. Botto, S. Vittorini, B. Guidi, G. Vergaro, A. Barison, A. Rossi, C. Passino, A. Giannoni, M. Di Paolo, M. Emdin, Molecular autopsy of sudden cardiac death in the genomics era, Diagnostics 11 (8) (2021) 1378, https://doi.org/10.3390/diagnostics11081378. PMID: 34441312; PMCID: PMC8394514.