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Broad-based molecular autopsy: a potential tool to investigate the involvement of subtle cardiac conditions in sudden unexpected death in infancy and early childhood

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ABSTRACT

Objectives Sudden unexplained death in children is a tragic and traumatic event, often worsened when the cause of death cannot be determined. This work aimed to investigate the presence of putative pathogenic genetic variants in a broad spectrum of cardiomyopathy, channelopathy and aortic disease associated genes that may have increased these children's vulnerability to sudden cardiac death.

Design We performed molecular autopsy of 41 cases of sudden unexplained death in infants and children through massive parallel sequencing of up to 86 sudden cardiac death-related genes. Multiple in silico analyses were conducted together with a thorough review of the literature in order to prioritise the putative pathogenic variants.

Results A total of 63 variants in 35 cases were validated. The largest proportion of these variants is located within cardiomyopathy genes although this would have been more expected of channelopathy gene variants. Subtle microscopic features of heart tissue may indicate the presence of an early onset cardiomyopathy as a predisposing condition to sudden unexpected death in some individuals.

Conclusions Next-generation sequencing technologies reveal the existence of a wide spectrum of rare and novel genetic variants in sarcomere genes, compared with that of cardiac ion channels, in sudden unexplained death in infants and children. Our findings encourage further investigation of the role of early onset inherited cardiomyopathies and other diseases involving myocardial dysfunction in these deaths. Early detection of variants in these individuals could help to unmask subtle forms of disease within their relatives, who would eventually benefit from better counselling about their genetic history.

What is already known on this topic

- Cardiac channelopathies are regarded as a major genetic contributor to sudden infant death syndrome and sudden arrhythmic death syndrome.
- Current guidelines state the potential usefulness of arrhythmia syndrome orientated molecular autopsy for all sudden unexplained infant deaths.
- There is very little knowledge about the involvement of other sudden cardiac death related diseases in these deaths.

What this study adds

- Next-generation molecular autopsy presents as a useful tool to detect genetic variants with a putative pathogenic role in the trigger of sudden unexplained infant deaths.
- The discovery of a remarkably high number of genetic variants within cardiomyopathy genes reinforces the involvement of sarcomeric dysfunction in some of these cases.

INTRODUCTION

Sudden unexpected death in infants (SUDI) and in children over 1 year of age are tragic events with a deep impact on families, often worsened if the cause of death remains unidentified after the autopsy as happens in some sudden infant death syndrome (SIDS) and sudden arrhythmic death syndrome (SADS) cases in children over 1 year of age.[1 2] SIDS is a worldwide major cause of death of children under 1 year of age that remains unexplained after a complete autopsy and a thorough investigation of the clinical history and a review of the circumstances of death.[3] In Spain, the rate of deaths attributable to SIDS is 0.12/1000 live births.[4] Because SIDS and SADS are diagnoses of exclusion, cardiac primary electric diseases (also known as channelopathies) have been one of the main targets of research, as any abnormality would only be evident at the molecular level.[5–7] However, only 10% of these deaths may be attributable to cardiac channelopathies.[8] It is still unclear whether other inherited sudden cardiac death (SCD)-related diseases, such as hypertrophic cardiomyopathy (HCM) or dilated cardiomyopathy (DCM), may be involved in some SIDS and/or SADS cases in the absence of a visible phenotype as in their most incipient forms. We have recently reported the presence of HCM-causing variants in a big cohort of SIDS cases, suggesting that disruption of the sarcomere activity may alter the Ca^{2+} homoeostasis and be responsible for arrhythmogenesis.[9] The great advances in next-generation sequencing

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(NGS) technologies have enormously widened the limits of research in a much more affordable way, providing huge amounts of information that were formerly barely accessible. The present work is aimed at exploring the burden of putative pathogenic variants in a series of SIDS and SADS cases in a broad panel of SCD-associated genes.

METHODS

Ethical issues

This study was developed according to the recommendations of the Helsinki Declaration.

Autopsy criteria and sample collection

All autopsy cases performed in the Institute of Legal Medicine of the University of Cologne, Germany, between a period of 6 years (2005–2010) were screened. A total of 41 cases, consisting of 38 SIDS and 3 SADS cases (table 1) of European, African and Asian ancestry were collected after a thorough external and internal examination, which included histological, toxicological and microbiological studies, as well as an extensive investigation of the family and clinical histories, and review of the death scene and its context.

Study design and massive-parallel sequencing

For identification of putative pathogenic variants we designed a custom gene panel comprising 81 genes previously associated to cardiovascular pathologies with an increased risk of SCD. As custom panels can be modified on demand, we subsequently added five new genes to the second version of the panel, for a total of 86 genes. The complete gene list is presented in online supplementary table S1. DNA templates were obtained from frozen blood or renal tissue; targeted sequences were enriched and subsequently sequenced in a 5500xl SOLiD System (Life Technologies) following a paired-end sequencing approach. Primary and secondary bioinformatics analyses (sequence alignment, base quality score recalibration, local realignment, variant calling and variant annotation) were similar to that recently described by Brion *et al*.[10] Tertiary analysis (variant prioritisation) was performed based on allele frequency estimates from Exome Variant Server, and functional impact on the protein (exonic missense, splicing and truncating variants were included). Prioritised variants were confirmed using conventional PCR amplification and Sanger sequencing. Full description of protocols and methods can be found in the online supplementary methods.

Table 1 Sample distribution and general information

	SIDS (38)	SADS (3)
Mean age at death	18 weeks	3 years
Gender, male/female (% male)	28/10 (74%)	2/1 (67%)
Ancestry		
European	33 (86.8%)	3 (100%)
African	3 (7.9%)	0
Asian	2 (5.3%)	0
SCD family history	2 (5%)	0
Surrounding abuse		
Smoking	10 (26%)	0
Cannabis	1 (3%)	0
Mother's abuse during pregnancy		
Smoking	6 (16%)	0
Heroin, methadone	1 (3%)	0
Medical history		
Preterm birth	5 (13%)	
Low weight/growth	6 (16%)	
Position found (sleep)		
Prone	11 (29%)	0
Supine	18 (47%)	2 (67%)
Not stated	9 (24%)	1 (33%)
Toxicology		
Negative	34 (89%)	3 (100%)
EtOH	1 (3%)	0
Caffeine	1 (3%)	0
EtOH and caffeine	1 (3%)	0
Benzodiazepine	1 (3%)	0

EtOH, ethanol; SADS, sudden arrhythmic death syndrome; SCD, sudden cardiac death; SIDS, sudden infant death syndrome.

RESULTS

A total of 41 SIDS and SADS cases of European, African and Asian ancestry were studied. On target average depth of coverage across samples was 610× and average per cent of target base-pairs covered at least 20× was 98.88% (see online supplementary table S2). As a result of the computational analysis, 63 variants in 35 individuals were prioritised as possibly pathogenic, and subsequently confirmed by Sanger sequencing. Variant distribution within disease categories was as follows: 51 (80.9%) variants out of the total were located within cardiomyopathy genes; 11 (17.5%) variants within channelopathy genes and 1 (1.6%) variant within genes related to aortic disorders. With regard to their occurrence, 37 (58.7%) out of the total were novel, and 26 (41.3%) were present at least in either National Center for Biotechnology Information's Single Nucleotide Polymorphism database (NCBI dbSNP) (http://www.ncbi.nlm.nih.gov/SNP/), or the Human Gene Mutation Database (http://www.biobase-international.com/product/hgmd). Altogether, we have found 20 individuals carrying one single putative pathogenic variant and 15 individuals carrying two or more putative pathogenic variants. The remaining six individuals were negative for any putative pathogenic variant (see online supplementary figure S1). According to Ng *et al*[11] criteria for gene variant assignment of pathogenicity, novel variants were assigned to Class 3 variants, also known as 'variants of uncertain significance'. Of the variants already present in any database, two were assigned to Class 4 (Likely Pathogenic) as a basis for the reported evidence for each one within the literature, and the remaining were assigned to Class 3, in the lack of any report of pathogenicity, or sufficient supporting evidence. None of the variants could be definitely assigned to Class 5 (Pathogenic) using the same criteria. Class 1 (Benign) and Class 2 (Likely Not-Pathogenic) variants were previously filtered out in the prioritisation process. Table 2 and online supplementary tables S3 and S4 gather a complete list of the 63 confirmed variants including detailed information concerning gene and transcript location, as well as bioinformatics scores and predictions from each tool, aimed to facilitate the classification as a basis for their putative pathogenicity.

DISCUSSION

To our knowledge, the present study provides the first high-throughput targeted resequencing analysis of SCD-related genes in a series of 38 SIDS and 3 SADS cases. It is currently believed that approximately 10% of the SUDI/SIDS cases may be due to underlying channelopathies,[8] but the involvement of other SCD-related disorders, such as cardiomyopathies, remains poorly understood. We set a precedent for this work by performing a large-scale variant screening within 16 adult-HCM

Table 2 Summary of putative pathogenic variants identified in the study

Sample	Gene	Transcript	Nucleotide	Protein	dbSNP ID	HGMD (Phenotype)
ID445	*MYBPC3*	NM_000256	c.1283T>C	p.Leu428Ser		
ID447	*MYH6*	NM_002471	c.5275A>G	p.Lys1759Glu		
ID450	*JUP*	NM_002230	c.1219G>A	p.Val407Ile	rs370913228	CM107149 (ARVC)
ID452	*LDB3*	NM_007078	c.881C>T	p.Ala294Val		
ID453	*DSC2*	NM_024422	c.1787C>T	p.Ala596Val	rs148185335	
ID454	*TTN*	NM_001256850	c.81422C>T	p.Pro27141Leu		
ID455	*MYLK2*	NM_033118	c.134C>T	p.Pro45Leu		
ID456	*AKAP9*	NM_005751	c.3449T>C	p.Leu1150Pro		
ID456	*TTN*	NM_001256850	c.95509T>G	p.Trp31837Gly	rs372304158	
ID456	*TTN*	NM_001256850	c.95524G>C	p.Glu31842Gln	rs368321767	
ID457	*TTN*	NM_001256850	c.68006C>T	p.Pro22669Leu		
ID458	*FBN1*	NM_000138	c.4150A>G	p.Met1384Val		
ID460	*AKAP9*	NM_005751	c.1396C>T	p.Arg466Trp	rs373876340	
ID460	*TTN*	NM_001256850	c.3835A>T	p.Met1279Leu	rs374497665	
ID461	*TTN*	NM_001256850	c.54058G>A	p.Gly18020Ser		
ID464	*MYH6*	NM_002471	c.3447C>G	p.Ser1149Arg		
ID465	*JUP*	NM_002230	c.568G>C	p.Val190Leu		
ID466	*TTN*	NM_001256850	c.39016G>A	p.Ala13006Thr		
ID468	*TTN*	NM_001256850	c.74991A>C	p.Gln24997His		
ID469	*SCN5A*	NM_198056	c.2890G>A	p.Ala964Thr		
ID470	*TTN*	NM_001256850	c.43075G>C	p.Asp14359His	rs201388509	
ID470	*MYH7*	NM_000257	c.5120T>C	p.Ile1707Thr		
ID470	*RYR2*	NM_001035	c.1046T>C	p.Met349Thr		
ID472	*DSG2*	NM_001943	c.880A>G	p.Lys294Glu		CM060960 (ARVC)
ID474	*AKAP9*	NM_005751	c.6195G>A	p.Met2065Ile	rs369212896	
ID474	*DES*	NM_001927	c.710C>T	p.Ala237Val	rs374144840	
ID474	*MYLK2*	NM_033118	c.688T>C	p.Phe230Leu		
ID474	*GLA*	NM_000169	c.416A>G	p.Asn139Ser	rs138886989	CM103681 (FD)
ID475	*KCNE1L*	NM_012282	c.79A>G	p.Ser27Gly		
ID475	*RYR2*	NM_001035	c.9619A>G	p.Asn3207Asp	rs372601642	
ID476	*MYL2*	NM_000432	c.37G>A	p.Ala13Thr	rs104894363	CM961004 (HCM)
ID477	*TTN*	NM_001256850	c.91069G>A	p.Glu30357Lys		
ID477	*TTN*	NM_001256850	c.97348C>T	p.Arg32450Trp	rs140319117	CM057411 (HMERF)
ID477	*TTN*	NM_001256850	c.5479G>T	p.Ala1827Ser	rs141213991	
ID478	*DES*	NM_001927	c.170C>T	p.Ser57Leu	rs372825868	
ID478	*TTN*	NM_001256850	c.74656T>C	p.Cys24886Arg		
ID479	*DSC2*	NM_024422	c.1892C>T	p.Thr631Ile		
ID479	*DSP*	NM_004415	c.7391G>A	p.Arg2464His		
ID479	*RANGRF*	NM_016492	c.124G>T	p.Val42Leu		
ID479	*TTN*	NM_001256850	c.40912G>A	p.Val13638Met		
ID480	*DMD*	NM_004006	c.5043G>A	p.Met1681Ile		
ID480	*MYH7*	NM_000257	c.1699C>T	p.Arg567Cys		
ID481	*TTN*	NM_001256850	c.76324T>C	p.Ser25442Pro	rs186273940	
ID482	*AKAP9*	NM_005751	c.7176A>G	p.Ile2392Met		
ID482	*MYH6*	NM_002471	c.1336G>A	p.Ala446Thr		
ID482	*TNNT2*	NM_000364	c.407G>A	p.Met105Ile		
ID483	*TTN*	NM_001256850	c.75893T>C	p.Ile25298Thr		
ID483	*TTN*	NM_001256850	c.65512C>T	p.Arg21838Trp		
ID483	*MYBPC3*	NM_000256	c.3470C>T	p.Pro1157Leu	rs373304680	
ID484	*DSC2*	NM_024422	c.1309G>C	p.Val437Leu		
ID484	*TTN*	NM_001256850	c.49217C>T	p.Ala16406Val	rs373815064	
ID484	*TTN*	NM_001256850	c.60323C>T	p.Thr20108Ile		
ID485	*BAG3*	NM_004281	c.212G>T	p.Arg71Leu		
ID485	*DSP*	NM_004415	c.1103T>C	p.Ile368Thr	rs141163578	
ID485	*SCN1B*	NM_199037	c.478G>A	p.Val160Ile	rs369058711	
ID488	*TTN*	NM_001256850	c.59623C>T	p.His19875Tyr		
ID488	*TTN*	NM_001256850	c.79952G>A	p.Arg26651Lys		
ID490	*TTN*	NM_001256850	c.90160G>A	p.Gly30054Arg	rs376403373	CM133389 (DCM)
ID490	*TTN*	NM_001256850	c.91603T>A	p.Tyr30535Asn	rs377291343	

Continued

Table 2 Continued

Sample	Gene	Transcript	Nucleotide	Protein	dbSNP ID	HGMD (Phenotype)
ID490	*TTN*	NM_001256850	c.91501G>A	p.Val30501Ile		
ID490	*RYR2*	NM_001035	c.13267A>C	p.Lys4423Gln	rs376908332	
ID491	*TTN*	NM_001256850	c.69490G>A	p.Val23164Ile	rs371306826	
ID492	*RBM20*	NM_001134363	c.2042A>G	p.Tyr681Cys	rs372048968	

ARVC, arrhythmogenic right ventricular cardiomyopathy; DCM, dilated cardiomyopathy; FD, Fabry disease; HCM, hypertrophic cardiomyopathy; HGMD, Human Gene Mutation Database; HMERF, hereditary myopathy with early respiration failure.

associated genes in 286 SIDS cases.[9] One of the identified variants in that study was found within the troponin I gene (*TNNI3*) leading to the amino acid substitution p.S166F. In a recent report, the variant p.S166F has been found to alter the ability of troponin I to directly change Ca^{2+} binding properties of the whole troponin complex.[12] These pathological stimuli at the molecular level may affect cardiac contractile properties contributing to disease phenotypes.[12] Furthermore, several other studies have provided in vitro and in vivo evidence that variants in sarcomere genes *MYH7*, *MYBPC3* and *TNNT2* alter cardiac energetic efficiency even before the development of HCM, and therefore, a sarcomeric variant itself may be a trigger for HCM pathogenesis.[13 14]

A number of interesting findings arise from this work. Individual ID476 was an 8-month-old boy who was found cyanotic in bed and died after 1.5 h of reanimation manoeuvres. Two days before, he had cough. The only histopathological lesion observed was a mild tracheitis. Review of the medical records revealed hypoglycaemia of the infant after birth and nicotine abuse of the mother during pregnancy. This individual carries the first of the Class 4 variants identified in the present study, *MYL2*c.37G>A, which leads to the amino acid substitution p.A13T in the human ventricular myosin regulatory light chain. This variant has been identified in several HCM-affected individuals from different European-American populations,[11 15–18] and has also been reported in the Human Gene Mutation Database as disease causing, in UniProt (http://www.uniprot.org/) as possibly pathogenic, in NCBI ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/) as pathogenic, and in Exome Variant Server with frequencies fitting the estimates of the disease prevalence. Several functional studies have demonstrated p.A13T-induced alterations in actomyosin interactions, which make an essential part of the molecular engine of muscle contraction.[19–21] The dominant effect observed for p.A13T-regulatory light chain proteins on myocardial function may result in higher-order effects at the fibre level, such as myocyte disarray or fibrosis, and this may contribute to the pathogenesis of HCM.[19] No other remarkable variants were found in this or any other gene of the panel, in this individual.

Individual ID477 was a 1-month old boy with a structural normal heart who carried the second Class 4 variant identified in our analysis, *TTN*c.97348 C>T, which leads to the amino acid substitution p.R25218 W in the M-line kinase domain (TK) of the protein titin. In vitro data supported the involvement of p.R32450 W (p.R279W in the cited report) in the disruption of a mechanochemical signalling pathway that caused hereditary myopathy with early respiratory failure (HMERF) in Swedish patients.[22 23] However, there is currently some controversy regarding the involvement of this variant in HMERF, as it has been subsequently found in healthy controls and also concomitant with variants in the fibronectin type III domain 119, which now appear more likely to be responsible for this phenotype.[24 25] A modifier role of this variant cannot be discarded, though. This individual carries, in total, three heterozygous variants in the *TTN* gene, all of which are in silico predicted to be damaging (see online supplementary table S4). We did not find any remarkable variants in other genes of the panel.

Individual ID445 was a 2-year-old boy found dead in bed. He had previously suffered febrile convulsions at the age of 18 months and was found with fever up to 38.7°C (101.7°F) within 5 h prior to death, although no seizure was witnessed in this occasion. An incipient bronchitis was found on the autopsy; however, no viral infections were identified. No remarkable histological alterations were found at the heart tissue level, either. Normally, the prognosis after a febrile seizure is excellent, although an increased risk of sudden unexpected death has been observed under special circumstances, such as respiratory insufficiency or underlying cardiac arrhythmias.[26 27] Genetic analysis of this individual revealed a novel variant in *MYBPC3*, which may have contributed to developing an arrhythmic background in the heart of the toddler.

Individual ID464 was an otherwise healthy male newborn with no remarkable personal history of disease. His mother, however, suffers from cardiovascular disease (not specified in the available medical records) for which she regularly follows drug therapy with metoprolol. Toxicological results for metoprolol were negative in the infant, but low levels of caffeine were found. Examination of myocardial sections with light microscopy revealed vacuolisation of the cardiomyocytes, while electron microscopy showed irregular distribution of the mitochondria and evidence of irregularities in the structure of the mitochondrial cristae. These microscopical findings are consistent with those observed in patients with DCM[28] although other incipient forms of cardiomyopathy should not be discharged. This individual is a carrier of a novel variant in the *MYH6* gene, which has been previously associated with DCM and HCM.

Individual ID469 was a 1.5-month-old boy found dead in bed whose autopsy and histological examination revealed a structural normal heart. A novel variant in the *SCN5A* gene was the only putative pathogenic variant found in this individual. Furthermore, family history reports the SCD of the infant's uncle at the age of 18 years. Although we do not know the genotype of the deceased relative, these findings are in agreement with the strong association between variants in *SCN5A* and sudden death in infants and young boys.[29 30]

It is unclear whether these findings could have led, separately, to infants' deaths; however, a combination of intrinsic and extrinsic factors is more likely to trigger a SIDS event, as this is considered a multifactorial entity,[7] and a similar mechanism may be acting in some cases of sudden death in young children.

The main outcomes of this study are three; first, the high efficiency of NGS technologies with—sometimes, partially degraded—autopsy samples, resulting in the identification of 63 putative pathogenic variants in 35 out of 41 samples; second,

the presence of a much higher number of variants within cardiomyopathy genes compared with channelopathy genes in a current scenario where cardiac ion channel variants are the best established genetic contributor to SIDS[7]; and third, the identification of two previously reported variants associated with sarcomere dysfunction at the molecular level that may have contributed to increasing the intrinsic vulnerability of the children to suffer a sudden unexpected death.

Approximately 59% of the genes included in our panels are associated to cardiomyopathies and hold 51 (80.9%) of the total number of putative pathogenic variants identified in our study (68.4%, if we exclude *TTN* variants). In the titin case, although several missense and truncating *TTN* variants have been associated with HMERF, HCM and most of all with DCM,[31 32] it has recently been estimated that approximately 1.96 *TTN* variants are expected to be predicted simply by chance.[33] Furthermore, a recent analysis of the 1000 Genomes cohort (http://www.1000genomes.org/) has reported a high prevalence of *TTN* variants, including a cumulative frequency of indels (9%) suggesting that the discovered variants may act as phenotype modifiers rather than causing themselves the disease.[34] On the other hand, there is evidence of the involvement of modifier genes, as well as digenic and oligogenic inheritance patterns, in disease penetrance in cases of HCM, long QT syndrome or Brugada syndrome (see Cooper *et al*,[35] for an extensive review). We have identified a significant number of individuals carrying multiple variants (table 2, see online supplementary figure S1, supplementary tables S3 and S4) but the combined contribution of these in increasing the intrinsic vulnerability of the infants remains to be elucidated.

Our study has some limitations as we have focused only in index cases and have not tested variant cosegregation within families. However, postmortem genetic testing is currently recommended in SUDI victims and future work will reinforce the usefulness of molecular autopsy in unmasking arrhythmia syndromes and subtle forms of cardiomyopathy within SUDI-affected families.[1]

Contributors MS was involved in the creation, development and maintenance of the local database, study design, sample processing and qualitative analyses, sequencing analysis and interpretation of data, and drafted the manuscript; AB-V and RG were involved in the design of the study, sample processing and qualitative analyses; PMS and KB contributed to case collection, data acquisition, design of the database and sample preparation at local site; JC performed histological examination of the hearts and review of the clinical histories; AC oversaw the study with MB, put all the technological equipment at our disposal, and contributed to data analysis; and MB designed, conceptualised and led the study, and contributed to data analysis. All the authors reviewed and approved the final manuscript.

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Ethics approval Comité de etica de Galicia.

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REFERENCES

1 Priori SG, Wilde AA, Horie M, *et al*. HRS/EHRA/APHRS expert consensus statement on the diagnosis and management of patients with 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* 2013;10:1932–63.
2 Wong LCH, Behr ER. Sudden unexplained death in infants and children: the role of undiagnosed inherited cardiac conditions. *Europace* 2014;16:1706–13.
3 Krous HF, Beckwith JB, Byard RW, *et al*. Sudden infant death syndrome and unclassified sudden infant deaths: a definitional and diagnostic approach. *Pediatrics* 2004;114:234–8.
4 Instituto Nacional de Estadística. http://www.ine.es/ (accessed 14 January 2015).
5 Schwartz PJ, Stramba-Badiale M, Segantini A, *et al*. Prolongation of the QT interval and the sudden infant death syndrome. *New Engl J Med* 1998;338:1709–14.
6 Brugada R, Hong K, Dumaine R, *et al*. Sudden death associated with short-QT syndrome linked to mutations in HERG. *Circulation* 2004;109:30–5.
7 Wilders R. Cardiac ion channelopathies and the sudden infant death syndrome. *ISRN Cardiol* 2012;2012:846171.
8 Tester DJ, Ackerman MJ. Cardiac channelopathies and the molecular autopsy. In: Collins K, Byard R, eds. *Forensic pathology of infancy and childhood*. Springer, 2014:899–942.
9 Brion M, Allegue C, Santori M, *et al*. Sarcomeric gene mutations in sudden infant death syndrome (SIDS). *Forensic Sci Int* 2012;219:278–81.
10 Brion M, Blanco-Verea A, Sobrino B, *et al*. Next generation sequencing challenges in the analysis of cardiac sudden death due to arrhythmogenic disorders. *Electrophoresis* 2014;35:3111–16.
11 Ng D, Johnston JJ, Teer JK, *et al*. Interpreting secondary cardiac disease variants in an exome cohort. *Circ Cardiovasc Genet* 2013;6:337–46.
12 Liu B, Tikunova SB, Kline KP, *et al*. Disease-related cardiac troponins alter thin filament Ca^{2+} association and dissociation rates. *PLoS ONE* 2012;7:e38259.
13 Crilley JG, Boehm EA, Blair E, *et al*. Hypertrophic cardiomyopathy due to sarcomeric gene mutations is characterized by impaired energy metabolism irrespective of the degree of hypertrophy. *J Am Coll Cardiol* 2003;41:1776–82.
14 Witjas-Paalberends ER, Güçlü A, Germans T, *et al*. Gene-specific increase in the energetic cost of contraction in hypertrophic cardiomyopathy caused by thick filament mutations. *Cardiovasc Res* 2014;103:248–57.
15 Poetter K, Jiang H, Hassanzadeh S, *et al*. Mutations in either the essential or regulatory light chains of myosin are associated with a rare myopathy in human heart and skeletal muscle. *Nat Genet* 1996;13:63–9.
16 Andersen PS, Havndrup O, Bundgaard H, *et al*. Myosin light chain mutations in familial hypertrophic cardiomyopathy: phenotypic presentation and frequency in Danish and South African populations. *J Med Genet* 2001;38:e43.
17 Lopes LR, Zekavati A, Syrris P, *et al*. Genetic complexity in hypertrophic cardiomyopathy revealed by high-throughput sequencing. *J Med Genet* 2013;50:228–39.
18 Mook OR, Haagmans MA, Soucy JF, *et al*. Targeted sequence capture and GS-FLX Titanium sequencing of 23 hypertrophic and dilated cardiomyopathy genes: implementation into diagnostics. *J Med Genet* 2013;50:614–26.
19 Farman GP, Muthu P, Kazmierczak K, *et al*. *Impact of familial hypertrophic cardiomyopathy-linked mutations in the NH2 terminus of the RLC on β-myosin cross-bridge mechanics*. *J Appl Physiol (1985)* 2014;117:1471–7.
20 Szczesna D, Ghosh D, Li Q, *et al*. Familial hypertrophic cardiomyopathy mutations in the regulatory light chains of myosin affect their structure, Ca^{2+} binding, and phosphorylation. *J Biol Chem* 2001;276:7086–92.
21 Kazmierczak K, Muthu P, Huang W, *et al*. Myosin regulatory light chain mutation found in hypertrophic cardiomyopathy patients increases isometric force production in transgenic mice. *Biochem J* 2012;442:95–103.
22 Lange S, Xiang F, Yakovenko A, *et al*. The kinase domain of titin controls muscle gene expression and protein turnover. *Science* 2005;308:1599–603.
23 Chauveau C, Bonnemann CG, Julien C, *et al*. Recessive TTN truncating mutations define novel forms of core myopathy with heart disease. *Hum Mol Genet* 2013;23:980–91.
24 Pfeffer G, Barresi R, Wilson IJ, *et al*. Titin founder mutation is a common cause of myofibrillar myopathy with early respiratory failure. *J Neurol Neurosurg Psychiatry* 2014;85:331–8.
25 Ohlsson M, Hedberg C, Brådvik B, *et al*. Hereditary myopathy with early respiratory failure associated with a mutation in A-band titin. *Brain* 2012;135:1682–94.
26 Camfield P, Camfield C. Sudden unexpected death in people with epilepsy: a pediatric perspective. *Semin Pediatr Neurol* 2005;12:10–14.
27 Vestergaard M, Pedersen MG, Østergaard JR, *et al*. Death in children with febrile seizures: a population-based cohort study. *Lancet* 2008;372:457–63.
28 Knieriem HJ. Electron-microscopic findings in congestive cardiomyopathy. In: Kaltenbach M, Loogen F, Olsen EGJ, eds. *Cardiomyopathy and myocardial biopsy*. Berlin Heidelberg: Springer, 1978:71–86.
29 Remme CA. Cardiac sodium channelopathy associated with SCN5A mutations: electrophysiological, molecular and genetic aspects. *J Physiol* 2013;591(Pt 17): 4099–116.
30 Wang DW, Desai RR, Crotti L, *et al*. Cardiac sodium channel dysfunction in sudden infant death syndrome. *Circulation* 2007;115:368–76.
31 Linke WA, Hamdani N. Gigantic business titin properties and function through thick and thin. *Circul Res* 2014;114:1052–68.
32 Herman DS, Lam L, Taylor MRG, *et al*. Truncations of titin causing dilated cardiomyopathy. *N Engl J Med* 2012;366:619–28.
33 MacArthur DG, Manolio TA, Dimmock DP, *et al*. Guidelines for investigating causality of sequence variants in human disease. *Nature* 2014;508:469–76.
34 Golbus JR, Puckelwartz MJ, Fahrenbach JP, *et al*. Population-based variation in cardiomyopathy genes. *Circ Cardiovasc Genet* 2012;5:391–9.
35 Cooper DN, Krawczak M, Polychronakos C, *et al*. Where genotype is not predictive of phenotype: towards an understanding of the molecular basis of reduced penetrance in human inherited disease. *Hum Genet* 2013;132:1077–130.

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