

ORIGINAL ARTICLE

Postmortem genetic analysis of sudden unexpected death in infancy: neonatal genetic screening may enable the prevention of sudden infant death

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Tandem mass screening has recently been started in Japan, but genetic screening has yet to be widely performed in neonates and many unexpected deaths are still being reported. We previously reported two cases of sudden infant death that may have been prevented had newborn screening been performed. In this study, we retrospectively reviewed 71 cases of sudden infant death for 66 arrhythmia- and 63 metabolic disease-related genes to identify how many cases of sudden infant death may have been prevented had mass screening been performed. Next-generation sequencing revealed that six cases had arrhythmia-related gene variants and five cases had metabolic disease-related gene variants. Had genetic screening been performed in addition to biochemical and physiological screening during the neonatal period to identify those at risk of arrhythmia or metabolic disease, these infants could have been diagnosed and treated, preventing their deaths. As such, screening of newborns may prevent sudden infant death.

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INTRODUCTION

Sudden unexpected death (SUD) in infancy is described as the sudden death of healthy infants within their first year of life, the cause of which is unknown. It includes deaths involving accidents, abuse, congenital diseases, inflammatory diseases and so on. If a complete examination, including an autopsy, cannot explain the exact cause of death, the case is diagnosed as sudden infant death syndrome. Although the number of sudden infant death syndrome cases has gradually decreased in Japan due to the 'back to sleep' or 'safe to sleep' campaign,^{1,2} annually several hundreds of children still die suddenly and unexpectedly before or around the age of one in Japan.

Congenital diseases, including metabolic diseases related to fatty acid oxidation and ketone body metabolism, and genetic arrhythmia, are linked to SUD.^{3–10} These diseases are generally asymptomatic in daily life, but may become symptomatic if left unnoticed and untreated. Some fatty acid oxidation disorders can be treated by avoiding fasting and providing glucose supplementation. Delaying diagnosis by even a few days can lead to permanent mental retardation, coma or sudden death. Genetic arrhythmia is also preventable with medical intervention. The diagnosis of these diseases before they become symptomatic may enable intervention that can

prevent disease onset. As such, the early detection, diagnosis and treatment of these diseases at the asymptomatic phase are essential; this highlights the importance of newborn screening.

Tandem mass screening started in Japan in the 1990s, and is now available nationwide.^{11,12} It can detect more than 20 congenital metabolic diseases in newborns within the first few days of life. Neonatal electrocardiographic screening has also been started and it can identify asymptomatic long QT patients.¹³ We previously reported two cases of SUD in infancy, whose causes of death were determined to be carnitine palmitoyltransferase (CPT) II deficiency.^{14,15} These patients appeared healthy and the CPT II deficiency was not noticed before their deaths. If screening had been performed before their deaths, they could have been treated and saved.

In this study, we retrospectively reviewed 71 cases of SUD from a genetic viewpoint for arrhythmia and metabolic disease to reveal how many cases of sudden infant death may have been prevented had genetic screening been performed.

MATERIALS AND METHODS

Case collection

According to the selection criteria (1: died before or around the age of 1 year; 2: informed consent and/or approval from an ethics committee was obtained; 3:

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DNA samples were available; 4: no involvement of a congenital abnormality, infection, trauma, murder, or abuse.), a total of 71 cases of SUD occurring in infants aged under or around 1 year (35 males, 36 females; age range: 1 day to 1 year and 5 months) were selected (Supplementary Table 1). A comprehensive forensic investigation, including a thorough examination of the death scene, a review of the clinical history, and performance of an autopsy that included macroscopic and microscopic examinations and a toxicology examination, were performed in all cases. Phenylketonuria, maple syrup urine disease, homocystinuria, galactosemia, congenital adrenal hyperplasia or congenital hypothyroidism had not been detected in these cases using conventional Guthrie mass screening.

Extraction of genomic DNA and genetic analysis

Genomic DNA was isolated from blood leukocytes with the QIAamp DNA Blood Mini Kit (Qiagen, Tokyo, Japan) in accordance with the manufacturer's standard methods. A TruSight One sequencing panel (Illumina, San Diego, CA, USA) was used and the sequencing was performed on an Illumina MiSeq (Illumina).

Targeted genes and filtering steps

The sequencing reads were mapped to the hg19 human reference genome sequence using Variant Studio (version 2.2.3) software (Illumina). From the 4813 TruSight One panel targeting genes, we extracted sequence information in the 129 genes for the inherited arrhythmia, arrhythmogenic cardiomyopathy and metabolic diseases; 19 BrS genes, 15 LQTS genes, 6 SQTS genes, 6 PCCD genes, 5 CPVT genes, 5 ARVC genes, other 24 cardiac genes and 63 inherited metabolic disease genes such as fatty acid oxidation, amino acid and organic acid disorders. Gene list and the criteria of gene selection are provided in Tables 1 and 2.

Single-nucleotide variations causing non-synonymous substitutions, non-sense substitutions or located at the splice site, and insertions/deletions occurring in the coding regions were retrieved. The variants with a low Q30 score and a read depth below 30 were excluded. Copy number variation was not analyzed in this study. To identify putatively pathogenic variants, variants with allele frequencies equal to or <1% in East Asian ethnic subgroups were retained and listed using data from the dbSNP (<http://www.ncbi.nlm.nih.gov/projects/SNP>), the 1000 Genomes Project (<http://www.1000genomes.org>), the NHLBI Exome Sequencing Project (ESP) (<http://evs.gs.washington.edu/EVS>), the Exome Aggregation Consortium (ExAC) (<http://exac.broadinstitute.org>)

and the Genome Cohort Study of Tohoku Medical Megabank Organization (ToMMo) (<http://ijgvd.megabank.tohoku.ac.jp>). Fastq files generated by MiSeq Control Software were mapped on the hg19 human reference genome sequence, sorted and duplication-removed by using NovoAlign (version 3.03.02) and NovoSort (version 1.04.03) software packages (Novocraft, Petaling Jaya, Malaysia). To prepare target regions for read depth counting, coding regions of target genes, including multiple transcripts, stored in the GENCODE basic version 19 table were extracted from the UCSC genome browser database using an in-house script and merged by using the BEDTools package (version 2.22.1).^{16–19} Depth counting was performed by using the DepthOfCoverage tool of the Genome Analysis Toolkit version 3.7.²⁰ *In silico* algorithms, SIFT (<http://sift.jcvi.org>) and PolyPhen-2 (<http://genetics.bwh.harvard.edu/pph2>), were used to predict whether the detected variants would affect the function of each protein.

Sanger sequencing

Sanger sequencing was performed to confirm the detected pathogenic variants, as previously described.¹⁴ The PCR primers were designed using Primer3 version 0.4.0 (<http://bioinfo.ut.ee/primer3-0.4.0>).

Ethics

Written informed consent was obtained from the parents or the use of the samples in this study was approved by the ethics committees of Nagasaki University Graduate School of Medicine and Fukuoka University. This study was performed in accordance with the Declaration of Helsinki.

RESULTS

Target sequence of sudden death cases

On average, ~18 million total reads were produced and ~13 million reads mapped to the targeted region in each sample. The mean coverage of the coding sequence was 115.3 ± 41 reads, with an overall average gene level coverage at ≥ 20 reads of $94.3 \pm 0.04\%$.

Detected variants aligned to the target 129 genes

After the filtering steps, the total number of detected variants that were aligned to the 129 target genes was 185. On average, each case had 1.4 ± 1 variants of the 66 arrhythmia-related genes and 1.2 ± 1.2

Table 1 Arrhythmia-related genes investigated in this study⁷⁰

<i>Brugada</i> ⁷¹	<i>Long QT</i> ⁷²	<i>Short QT</i> ⁷³	<i>PCCD</i> ^{74–78}	<i>CPVT</i> ⁷⁹	<i>ARVC</i> ^{80,81}	<i>Others</i>	
<i>SCN5A</i>	<i>KCNQ1</i>	<i>KCNH2</i>	<i>SCN5A</i>	<i>RYR2</i>	<i>PKP2</i>	<i>CAMK2D</i>	<i>SLC8A1</i>
<i>GPD1L</i>	<i>KCNH2</i>	<i>KCNQ1</i>	<i>LMNA</i>	<i>CASQ2</i>	<i>DSC2</i>	<i>CALM3</i>	<i>TAZ</i>
<i>CACNA1C</i>	<i>SCN5A</i>	<i>KCNJ2</i>	<i>EMD</i>	<i>TRDN</i>	<i>DSG2</i>	<i>DES</i>	<i>TBX3</i>
<i>CACNB2</i>	<i>ANK2</i>	<i>CACNA1C</i>	<i>SCN1B</i>	<i>CALM1</i>	<i>JUP</i>	<i>DPP6</i>	<i>TBX5</i>
<i>SCN1B</i>	<i>KCNE1</i>	<i>CACNB2</i>	<i>TRPM4</i>	<i>CALM2</i>	<i>DSP</i>	<i>GJA1</i>	<i>TCAP</i>
<i>KCNE3</i>	<i>KCNE2</i>	<i>CACNA2D1</i>	<i>GJA5</i>			<i>GJC1</i>	
<i>SCN3B</i>	<i>KCNJ2</i>					<i>KCNA5</i>	
<i>KCNH2</i>	<i>CACNA1C</i>					<i>KCNE4</i>	
<i>KCNJ8</i>	<i>CAV3</i>					<i>KCNIP2</i>	
<i>CACNA2D1</i>	<i>SCN4B</i>					<i>KCNJ3</i>	
<i>RANGRF</i>	<i>AKAP9</i>					<i>KCNK17</i>	
<i>KCNE5</i>	<i>SNTA1</i>					<i>KCNN2</i>	
<i>KCND3</i>	<i>KCNJ5</i>					<i>MYBPC3</i>	
<i>HCN4</i>	<i>CALM1</i>					<i>MYH6</i>	
<i>SLMAP</i>	<i>CALM2</i>					<i>MYH7</i>	
<i>TRPM4</i>						<i>NCS1</i>	
<i>SCN2B</i>						<i>NKX2-5</i>	
<i>FGF12</i>						<i>NPPA</i>	
<i>SCN10A</i>						<i>PLN</i>	

Table 2 Inherited metabolic disease-related genes investigated in this study⁸²

Amino acid	Organic acid	Fatty acid oxidation	Carbohydrate	Others	
ASL	ACAT1	MUT	ACADM	GALE	ABCD4
ASS1	AUH	OPA3	ACADVL	GALK1	BTD
BCKDHA	ETFA	PCCA	CPT1A	GALT	DNAJC19
BCKDHB	ETFB	PCCB	CPT2		GCH1
BCKDK	ETFDH		HADHA		HCFC1
CBS	GCDH		HADHB		HSD17B10
CPS1	HLCS		SLC22A5		MTHFR
DLD	HMGCL		SLC25A20		MTR
DBT	HMGCS2				MTRR
MAT1A	IVD				SLC52A1
NAGS	LMBRD1				SLC52A2
OTC	MCCC1				SLC52A3
PAH	MCCC2				SLC5A6
PCBD1	MMAA				SPR
PTS	MMAB				
QDPR	MMACHC				
SLC25A13	MMADHC				

variants of the 63 metabolic disease-related genes. The overall mean depth of each coding region was described in Supplementary Table 2.

Arrhythmia-related gene variants

Genetic arrhythmia. Most cases of genetic arrhythmia have autosomal dominant inheritance, and a familial history is not always present. Sporadic cases are always caused by *de novo* mutation and sometimes by germline mosaicism, but in some cases, either one of the parents may be a subclinical patient.

Among our 71 cases, 56 cases had at least one heterozygous amino acid change in an arrhythmia-related gene. Out of these 56 cases, 6 cases had at least one heterozygous amino acid change in an arrhythmia-related gene that had previously been reported to be an arrhythmia-related variant (Table 3). Novel or rare variants with uncertain significance were also detected in the arrhythmia-related genes (Supplementary Table 3).

Long QT syndrome-related variant. Case 10 had a R148W-*KCNH2* variant. The *KCNH2* gene encodes potassium voltage-gated channel subfamily H member 2. Mutation of this gene results in long QT syndrome, which causes ventricular arrhythmia and increases the risk of sudden death.²¹ It has been contested whether the R148W-*KCNH2* variant is indeed a mutation^{10,22,23} or just a polymorphism.^{24,25} Recently, Mechakra *et al.*²⁶ revealed that the human ether-à-go-go-related gene (hERG) current was decreased when the hERG/R148W variant was co-expressed with the wild-type gene, indicating that the heterozygous R148W-*KCNH2* variant causes long QT syndrome and is associated with *Torsade de pointes*, and that heterozygous carriers of the R148W-*KCNH2* variant may be at risk of cardiac sudden death.

Case 68 had a A283V-*KCNQ1* variant. The *KCNQ1* gene encodes potassium voltage-gated channel subfamily Q member 1, and mutation of this gene causes long QT syndrome. This variant has not been reported before, but the A283G-*KCNQ1* and A283T-*KCNQ1* variants have been previously reported in long QT syndrome patients.^{27,28}

Case 25 had D85N-*KCNE1* variant. The D85N-*KCNE1* variant is a rare variant, but it has been reported to be disease-causing.²⁹ In *Xenopus* oocytes, *KCNQ1*-encoded currents were reduced by about 50% by the D85N-*KCNE1* variant.³⁰ Nishio *et al.*²⁹ also demonstrated

that the D85N-*KCNE1* variant significantly reduced wild-type *KCNH2/KCNE1*-encoded currents by about 30%.

Arrhythmogenic right ventricular dysplasia-related variant. Case 60 had a V158A-*DSG2* variant. The *DSG2* gene encodes a member of the desmoglein family and cadherin cell adhesion molecule superfamily of proteins, and mutation of this gene causes arrhythmogenic right ventricular dysplasia, familial, 10. This variant has not been reported before, but the V158G-*DSG2* variant has been reported as non-pathogenic,³¹ although it has been detected in a family with arrhythmogenic right ventricular cardiomyopathy/dysplasia.³²

Brugada syndrome-related variant. Case 16 had a F386C-*SCN10A* variant. The *SCN10A* gene encodes sodium voltage-gated channel alpha subunit 10, and mutation of this gene is related to Brugada syndrome.^{33,34} The F386C-*SCN10A* variant has been reported as putative pathogenic mutation.³⁵

Case 22 had a V110I-*SCN3B* variant. The *SCN3B* gene encodes sodium voltage-gated channel beta subunit 3 and is a modifier protein of the cardiac sodium channel complex. We reported that the V110I-*SCN3B* variant is a relatively common cause of *SCN5A*-negative Brugada syndrome in Japan, which results in a reduced sodium current because of the loss of cell surface expression of a pore-forming subunit of the cardiac sodium channel complex.³⁶

Digenic variants. Case 43 had T4A-*KCNE3* and A283V-*KCNQ1* variants. The T4A-*KCNE3* variant has previously been reported in a patient with Brugada syndrome, and its functional consequence was a gain of function of the transient outward potassium current, which may underlie the pathogenesis of Brugada-pattern electrocardiogram (ECG).³⁷ In this case, the *KCNE3* and *KCNQ1* variants may have affected each other, leading to fatal arrhythmia.

Nonsense variants. There were three cases that had a stop-gain deletion with immature protein. Case 30 and 45 had deletions in the *TRPM4* gene, and case 55 had a deletion in the *KCNA5* gene. The *TRPM4* gene encodes transient receptor potential cation channel subfamily M member 4 and is related to progressive familial heart block type I and progressive cardiac conduction disturbances,^{38–40} long QT syndrome and Brugada syndrome.⁴¹ The *KCNA5* gene, which encodes potassium voltage-gated channel subfamily A member 5, is related to arterial fibrillation.⁴² However, according to the ExAC database, there was no significant difference between the expected number and the observed number of truncation variants, which means that both genes might have little effect on the disease.

Metabolic disease-related gene variants

Metabolic disease. Most inherited metabolic diseases have autosomal recessive inheritance. Homozygous amino acid changes or at least two heterozygous amino acid changes are necessary to cause metabolic disease, although a single heterozygous amino acid change with a large deletion or an intronic mutation may also cause disease.

Homozygous variants. During the filtering steps, 1 case out of the 71 cases had homozygous amino acid changes in the *NAGS* genes (Table 4).

Case 17 had a homozygous V241A-*NAGS* variant. The *NAGS* gene encodes *N*-acetylglutamate synthase, and mutation of this gene causes *N*-acetylglutamate synthase deficiency, a form of hyperammonemia.^{43,44} As the variant was not detected in controls and the *in silico* algorithms predicted that it was deleterious and

Table 3 Results of known arrhythmia-related variants

Case	Gene	Position	Substitution	Amino acids	Sift score	PolyPhen-2 score	dbSNP ID	ExAC frequency	ToMMo frequency	References
10	<i>KCNH2</i>	150656690	C>C/T	R148W	Deleterious (0.01)	Possibly damaging (0.478)	rs139544114	0/8640	N.R.	10,22,23,26
16	<i>SCN10A</i>	38798298	T>T/G	F386C	Deleterious (0)	Probably damaging (1)	rs78555408	61/8654	N.R.	35
22	<i>SCN3B</i>	123513271	G>G/A	V110I	Tolerated (0.13)	Probably damaging (0.993)	rs147205617	20/8654	N.R.	36
25	<i>KCNE1</i>	35821680	G>G/A	D85N	Deleterious (0.04)	Benign (0.277)	rs1805128	48/8636	N.R.	29
43	<i>KCNQ1</i>	2594143	C>C/T	A283V	Tolerated (0.4)	Probably damaging (1)	—	N.R.	N.R.	27,28
43	<i>KCNE3</i>	74168599	A>A/G	T4A	Tolerated (0.76)	Benign (0)	rs200856070	9/8646	N.R.	37
68	<i>KCNQ1</i>	2594143	C>C/T	A283V	Tolerated (0.4)	Probably damaging (1)	—	N.R.	N.R.	27,28

Abbreviation: N.R.: not reported.

possibly damaging, it remains possible that this variant is related to the disease.

Heterozygous variants. Among the 71 cases, 49 cases had at least one heterozygous non-synonymous variant in the metabolic diseases-related genes; three of these cases had two variants in the same gene (in the *ACADVL* gene, *HMGCL* gene or *CPT2* gene, respectively; Table 4).

Case 9 had G546R-*ACADVL* and C630S-*ACADVL* variants. Mutation of the *ACADVL* gene causes very long-chain acyl-CoA dehydrogenase (VLCAD) deficiency. Although these two variants have not been reported to be common polymorphisms,⁴⁵ this case may have had VLCAD deficiency.

Case 24 had T205S-*HMGCL* and M217V-*HMGCL* variants. Mutation of the *HMGCL* gene causes 3-hydroxy-3-methylglutaric aciduria.⁴⁶ These two variants have not been reported before, but the T205 amino acid is conserved among species and the D204N-*HMGCL* and G203E-*HMGCL* variants have respectively been reported in two patients.^{47–49} It is possible that the case had 3-hydroxy-3-methylglutaric aciduria.

Case 33 and 63 had a heterozygous F383Y-*CPT2* variant and splice-site variant (c.233+2T>A). The F383Y-*CPT2* variant causes a decrease in CPT II activity and has been reported in CPT II deficiency patients.^{14,50,51} Splice-site variants cause exon skipping and reduces CPT II activity.^{52,53} These two cases may have had CPT II deficiency.

Three cases (cases 9, 17 and 63) also had arrhythmia-related variant, but all of them were variants with uncertain significance.

DISCUSSION

Newborn screening

The first successful newborn screening was reported in the 1960s as the Guthrie qualitative bacterial inhibition test, which formed the basis for phenylketonuria programs.⁵⁴ Millington *et al.* applied fast-atom bombardment tandem mass spectrometry to newborn screening of metabolic diseases in 1990,^{11,55} and newborn screening has been continually improving since then.^{55–62} In Japan, newborn screening with tandem mass spectrometry had only been performed in restricted regions as a pilot study,¹² but it is now available nationwide.

Neonatal ECG screening has been started in some regions.^{13,63} Yoshinaga *et al.*¹³ reported that among 4285 Japanese infants who were screened at the age of 1 month, four infants showed prolonged QT intervals. These patients were also genetically diagnosed. Two of them showed progressive prolongation of QT intervals, and medical intervention could be started. However, there have been many reports of fatal arrhythmia in SUD cases. Screening had not been performed in these fatal cases, and so the disease could not be diagnosed at an asymptomatic phase.

Genetic screening

The most common types of screening are tandem mass screening and neonatal ECG screening; they are performed biochemically and physiologically. However, no genetic screening is currently being widely performed in neonates. Although biochemical or physiological screening can identify many potential patients, there still remain some cases in which the diseases are missed. Edmondson *et al.*⁶⁴ reported a 4-year-old CPT II deficiency patient whose disease was initially missed by newborn screening, demonstrating that not all cases of CPT II deficiency are being detected by the currently used newborn screening methods. Some potential long QT patients do not show extended QT intervals during the neonatal period because QT intervals change with aging; in contrast, a genetic variant remains the same in an individual throughout their lifetime, indicating the usefulness of genetic screening even in neonates despite the associated ethical issues and cost matters. More recently, next-generation sequencing has been developed and applied for the detection of genetic abnormalities in arrhythmia. This technique makes it possible to examine a much larger number of genes and exons in less time and at a lower cost, and it may enable genetic screening to become more feasible than it has been in the past.

Benefits and disadvantages

There are many benefits of genetic screening. First, in combination with biochemical and physiological screening, many potential patients may be detected before they reach the symptomatic phase. Second, genetic screening is useful for detecting diseases in which a genotype–phenotype correlation has been established. In such cases, appropriate therapeutic approaches could be applied on the basis of the identified genetic variants. Furthermore, the obtained genetic information could be useful for asymptomatic siblings and future pregnancies.

There are also some disadvantages to such approaches. Next-generation sequencing, such as whole-exome sequencing, might identify variants of unknown significance and coincidental pathogenic variants. Familial genetic counseling would also be necessary. Furthermore some undetected variants due to low-coverage regions or non-sequenced regions might be misdiagnosed. In this present study, some coding regions were not sequenced (Supplementary Table 2) and further analysis such as Sanger’s method is necessary for the future, but it is time consuming. In addition, the cost of next-generation sequencing is still rather expensive: it costed several hundred dollars per sample in this study.

Molecular autopsy

Postmortem genetic testing or molecular autopsy, including metabolic autopsy, has been commonly performed, but they targeted only one or a few genes. There are many genes that cause arrhythmia and metabolic disease, and thus, in many cases, the mutations were not detected. Recently, next-generation sequencing has also been used for

Table 4 Results of metabolic disease-related variants

Case	Gene	Position	Substitution	Amino acids	Zygoty	Sift score	PolyPhen-2 score	dbSNP ID	ExAC frequency	ToMMo frequency
17	<i>NAGS</i>	42083412	T>C/C	V241A	Homozygote	Deleterious (0)	Possibly damaging (0.76)	rs201142696	0/2319 ^a	N.R.
9	<i>ACADVL</i>	7127674	G>G/A	G546R	Heterozygote	Deleterious (0.04)	Benign (0.045)	rs139425622	3/8626	N.R.
9	<i>ACADVL</i>	7128196	G>G/C	C630S	Heterozygote	Deleterious (0)	Possibly damaging (0.651)	rs200117742	6/8638	N.R.
24	<i>HMGCL</i>	24134761	C>C/G	T205S	Heterozygote	Deleterious (0)	Probably damaging (0.966)	—	N.R.	N.R.
24	<i>HMGCL</i>	24134726	A>A/G	M217V	Heterozygote	Tolerated (0.22)	Probably damaging (0.91)	—	0/8652	N.R.
33	<i>CPT2</i>	53666473	T>T/A	c.233+2T>A	Heterozygote	—	—	—	N.R.	N.R.
33	<i>CPT2</i>	53675837	T>T/A	F383Y	Heterozygote	Tolerated (0.41)	Benign (0.192)	rs74315295	3/8644	N.R.
63	<i>CPT2</i>	53666473	T>T/A	c.233+2T>A	Heterozygote	—	—	—	N.R.	N.R.
63	<i>CPT2</i>	53675837	T>T/A	F383Y	Heterozygote	Tolerated (0.41)	Benign (0.192)	rs74315295	3/8644	N.R.

Abbreviation: N.R.: not reported.
^aThe frequency of homozygotes.

Table 5 Summary of previously reported cases with arrhythmia-related variants

Gene	Amino acids	Age	Sex	Diagnosis	ECG	References
<i>KCNH2</i>	R148W	19 y.o.	Female	Long QT type 2	Torsade de pointes	22
		4 m.o.	Male	Sudden death	N.R.	10
		—	—	SCD survivor	VF	23
		13 m.o.	Male	Sudden death	N.R.	26
<i>SCN10A</i>	F386C	Over 15 y.o.	—	BrS	BrS	35
		Over 15 y.o.	—	SUNDS	N.R.	35
<i>SCN3B</i>	V110I	19 y.o.	Female	Asymptomatic Br-like	Coved ST-elevation	36
		33 y.o.	Male	Asymptomatic BrS	Saddleback ST-elevation	36
		42 y.o.	Male	Symptomatic BrS	Coved ST-elevation	36
		51 y.o.	Male	BrS with syncope	Coved ST-elevation	36
<i>KCNQ1</i>	A283T	16th gestational week	Male	Intrauterine fetal death	N.R.	28

Abbreviations: ARVC, arrhythmogenic right ventricular cardiomyopathy; BrS, Brugada syndrome; m.o., months old; N.R., not recorded; SCD, sudden cardiac death; SUNDS, sudden unexplained nocturnal death syndrome; VF, ventricular fibrillation; y.o., years old.

postmortem genetic testing.^{65–68} We also reported the first metabolic autopsy using next-generation sequencing.¹⁵

In this study, we analyzed 71 cases of SUD, most of which had not been undergone screening at the neonatal period. The number of targeted genes was 129, which covered most of the arrhythmia- and metabolic disease-related genes. The diseases related to these genes are preventable and worth screening. Among our cases, six cases (cases 10, 16, 22, 25, 43 and 68) had at least one heterozygous amino acid change in an arrhythmia-related gene that had previously been reported as an arrhythmia-related variant, 1 case (case 17) had homozygous amino acid changes in the *NAGS* gene, two cases (cases 9 and 24) had two heterozygous amino acid changes in the *ACADVL* and *HMGCL* genes, and two cases (cases 33 and 63) had a heterozygous amino acid change and splice-site variant in the *CPT2* gene.

Arrhythmia diseases

There are two types of variants: crucial mutations and modifier substitutions. The variants of the *KCNH2* and *KCNQ1* genes are crucial mutations for long QT syndrome, whereas the variants of the *SCN5A* and *SCN3B* genes might be modifier substitutions for Brugada syndrome. Indeed, Probst *et al.*⁶⁹ reported that *SCN5A* variants are not directly causal to the occurrence of Brugada phenotype in familial analyses. Therefore, those who have a crucial mutation should be

investigated and provided intensive intervention, whereas familial analyses should be recommended to those with modifier substitutions.

Variants are also classified according to the disease onset period. Table 5 summarizes the data of previously reported patients with the variants detected in this study. The R148W-*KCNH2* and A283T-*KCNQ1* variants were detected during childhood, whereas the others were detected during adolescence. If large cohorts of patients are studied and an accurate genotype–phenotype correlation is established, the patients with a severe genotype could be provided intervention from an early period, and those with a mild genotype could be followed regularly.

Metabolic diseases

In this study, no definite diagnosis was made, although there were some cases in which metabolic diseases were suspected (cases 9, 17, 24, 33 and 63). Owing to the postmortem nature of the samples, enzymatic and biochemical analyses were not performed and definite diagnoses were not made. If genetic screening and tandem mass biochemical screening were combined, these patients could be filtered for potential diseases. Fatty acid oxidation disorders may be responsible for as much as 5% of SUD cases in infancy, and as we reported in our previous metabolic autopsy study, *CPT II* deficiency was the cause of SUD in two cases.^{14,15} These deaths could have been prevented by newborn screening, demonstrating the necessity of early detection.

Limitations

There are some limitations in this study. First, diagnosis was only performed genetically, but actual diagnoses should be made in consideration of the clinical symptoms and examinations. In addition, not all patients with a genetic variant develop symptoms, and not all patients who are diagnosed with the diseases have a genetic abnormality. Second, this is a retrospective study, and not a prospective cohort study. This study focused on SUD in infancy and included only SUD cases in certain areas of Japan. It is also important to keep in mind that not all patients with these diseases die, and there are far more living patients suffering from the diseases than those who died. Third, segregation in families was not performed. It is valuable to clarify the significance of each variant. Familial analysis is needed for the future. Fourth, we filtered out many genes other than the 129 arrhythmia- and metabolic disease-related genes studied. There are undoubtedly many other candidate genes that can cause SUD. For example, variants in some structural genes such as sarcomere genes and desmosome genes may induce arrhythmias without cardiac alteration.

CONCLUSION

In this study, we diagnosed several supposed cases of arrhythmia (6/71 cases; 8.5%) and metabolic disease (5/71 cases; 7%) among the 71 SUD cases analyzed. In addition to the two previously reported cases of CPT II deficiency, we found that the diseases in a certain number of infants could have been diagnosed and treated had genetic screening been performed. Genetic newborn screening may enable the prevention of SUD in patients who would otherwise die.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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