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# Value of next-generation sequencing in inherited arrhythmia syndromes

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## Abstract

**Background** Genetic studies are clinically recommended in some cases of inherited arrhythmia syndromes. Next-generation sequencing (NGS) would be helpful because of its high analytical throughput and relative speed. This study aimed to assess the mutation-detection yield obtained by NGS compared with conventional Sanger sequencing method.

**Methods** Patients with aborted sudden cardiac death and their families who underwent gene sequencing tests for inherited arrhythmia syndromes were retrospectively and enrolled in this study from 2017 to 2022 at Chonnam National University Hospital. We evaluated NGS study results of 17 patients (NGS group) and Sanger study results of 19 patients (Sanger group).

**Results** 64.7% of NGS and 94.7% of Sanger group were probands. Type 1 Brugada pattern ECG was more frequent in NGS group (64.7% vs. 21.1%; $p=0.007$). BrS was the most common disorder in NGS group (76.5%), and idiopathic ventricular fibrillation was the most common one in Sanger group (63.2%). Mutations with uncertain significance were the most common ones in NGS group (89.5%), and pathogenic or likely pathogenic mutations were the most common ones in Sanger group (45.7%). When positive yield was defined as the ratio of pathogenic or likely pathogenic mutations that were detected by sequencing, the yields were 10.5% and 45.7% in NGS and Sanger groups, respectively. The NGS arrhythmia panel did not cover two inherited arrhythmia-related mutations (RYR1, APOA5) that were detected by the Sanger method. The extended NGS arrhythmia panel was able to detect 84.8% of inherited arrhythmia-related mutations that were detected in Sanger group.

**Conclusions** NGS study has some limitations in obtaining the full genetic data of probands. Well-designed NGS panels are needed to increase the efficiency of the NGS study. With the well-designed panels, large-scale gene sequencing can efficiently and rapidly be applied in real clinical practices, especially in inherited fatal arrhythmia syndromes that have a high detection yield in genetic analyses.

**Keywords** Next-generation sequencing, Inherited arrhythmia syndrome

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## Introduction

Genetic studies are clinically recommended in some selected inherited arrhythmia syndromes such as long QT syndrome (LQTS) and catecholaminergic polymorphic ventricular tachycardia (CPVT). Genetic tests can be performed in J wave syndromes such as Brugada syndrome (BrS) or early repolarization syndrome, but the test results only partially affect diagnosis, treatment, or prognosis prediction. Nevertheless, genetic counseling is needed in some cases [1]. But, genetic tests could be under-used due to their high cost or limited availability. Next-generation sequencing (NGS) would be helpful in such situations [2, 3]. Its high analytical throughput and relative speed make NGS very attractive for early clinical implementation. Therefore, an in-depth understanding of the strengths and limitations of each platform in clinical diagnostics is required [4].

This study aimed to assess the mutation-detection yield obtained by NGS compared with conventional Sanger sequencing method in patients with aborted sudden cardiac death and their families.

## Materials and methods

### Patient enrollment

Patients with aborted sudden cardiac death due to clinically suspected inherited arrhythmia syndrome and their families who agreed gene sequencing test were retrospectively enrolled in Chonnam National University Hospital from 2017 to 2022.

The study was approved by the Ethics Committee of Chonnam National University Hospital, Gwangju, South Korea (IRB No., CNUH-2022–327). The requirement for informed consent for this study was waived, because the study constituted a retrospective analysis, and informed consents for the gene sequencing had already been obtained from the patients.

### Next-generation sequencing

Blood samples from patients with aborted sudden cardiac death and their families were obtained in our institute. Genetic analyses of 30 genes were performed using NGS on the Miseq Platform (Illumina Inc., San Diego, CA, USA). The list of targeted genes, enrichment method, and coverage information are shown in Table 1. Pathogenicity was determined in accordance with the American College of Medical Genetics and Genomics (ACMG)/Association for Molecular Pathology (AMP) guideline [5].

### Sanger sequencing

The PCR amplicons were sequenced in both directions using the Big Dye Terminator v 3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA, USA) on an ABI PRISM 3100 gene analyzer (Applied Biosystems, Foster City, CA, USA).

The chromatograms were analyzed in Sequencer software version 5.0 (Gene Codes, Ann Arbor, MI, USA). To classify the variants, standards and guidelines of the ACMG for the interpretation of sequence variants were used [5], and the variants were classified into

**Table 1** The profile of NGS test panel. The list of targeted genes, enrichment method, and coverage of the next-generation sequencing panel that is used in this study

| | | | | | |
|---|---|---|---|---|---|
| Test panel | | | Arrhythmia panel | | |
| Target enrichment method | | | Hybridization with oligonucleotide probes (GRM v1) | | |
| Massively parallel sequencing | | | MiSeq Dx (150 bp × 2; paired-end) | | |
| Reference genome | | | hg19 | | |
| Bioinformatic pipeline | | | BI_GRM v1.1 (Alignment: BWA, Variant calling: GATK) | | |
| *Coverage* | | | | | |
| Mean coverage of depth (X): 240.93 X | | | % of target bases ≥ 10X: 100% | | |
| Basic target regions (30 genes; 117,771 bp) | | | Extended target regions (30 genes) | | |
| ABCC9 | HCN4 | PKP2 | ACTN2 | JUP | PRKAG2 |
| AKAP9 | KCND3 | RANGRF | ANKRD1 | KCNA5 | RBM20 |
| ANK2 | KCNE1 | RYR2 | BAG3 | LDB3 | SALL4 |
| CACNA1C | KCNE2 | SCN1B | DES | LMNA | SCN2B |
| CACNA2D1 | KCNE3 | SCN3B | DSC2 | MYH6 | TBX5 |
| CACNB2 | KCNH2 | SCN4B | DSG2 | MYH7 | TGFB3 |
| CALM1 | KCHJ2 | SCN5A | DSP | NKX2-5 | TMEM43 |
| CASQ2 | KCNJ5 | SNTA1 | EMD | NPPA | TNNI3 |
| CAV3 | KCNJ8 | TRN | GJA5 | PDLIM3 | TNNT2 |
| GPD1L | KCNQ1 | TRPM4 | HADHA | PLN | TTN |

five pathogenicity groups: benign, likely benign, uncertain significance, likely pathogenic, and pathogenic. To define new variants, we searched locus-specific databases (LOVDs) as well as public databases (HGMD, ExAC, 1000genomes, dbSNP) including the Korean genome database containing 1244 alleles (Korean Reference Genome Database [KRGDB]; http://152.99.75.168/KRGDB/). All mutations were described using the Human Genome Variation Society nomenclature.

### Statistical analysis

All analyses were performed using SPSS® Statistics 27.0 for Windows (IBM Corp., NY, USA). The continuous variables were presented as the average values ± standard error, and categorical baseline characteristics as counts and percentages. The continuous variables were compared and evaluated using Student's t-test, and the categorical baseline characteristics by Pearson's chi-square test. Differences with p values less than 0.05 were considered as statistically significant.

**Table 2** Basic demographics. The number refers to the count of patients

| | NGS ($n=17$) | Sanger ($n=19$) | Total ($n=36$) |
|---|---|---|---|
| Gene sequencing method | | | |
| Age | 42.6 ± 3.6 | 47.2 ± 2.1 | 45.1 ± 2.1 |
| Sex (male) | 16 (94.1%) | 18 (94.7%) | 34 (94.4%) |
| Gene sequencing indication | | | |
| Proband | 11 (64.7%) | 18 (94.7%) | 29 (80.6%) |
| Family member | 6 (35.3%) | 1 (5.2%) | 7 (19.4%) |
| Past history | | | |
| HTN | 2 (11.8%) | 2 (10.5%) | 4 (11.1%) |
| DM | 0 (0.0%) | 1 (5.2%) | 1 (2.8%) |
| CVA | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) |
| MI | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) |
| Smoking | 3 (17.6%) | 7 (36.8%) | 10 (27.8%) |
| FHx of SCD | 3 (17.6%) | 3 (15.8%) | 6 (16.7%) |

*NGS* next-generation sequencing; *HTN* hypertension; *DM*, diabetes mellitus; *CVA*, cerebrovascular accident; *MI*, myocardial infarction; *FHx*, family history; *SCD*, sudden cardiac death

## Results

We evaluated NGS data of 17 patients (42.6 ± 3.6 years old; 16 males) and Sanger sequencing data of 19 patients (47.2 ± 2.1 years old; 18 males). There was not any overlapping patient who received both tests. 64.7% of NGS and 94.7% of Sanger group were probands. Basic demographics are shown in Table 2.

There were no differences in electrocardiography (ECG) rhythm at initial recovery. ECG parameters showed some differences (Table 3); Type 1 Brugada pattern ECG was more frequent in NGS group (64.7% vs. 21.1%; $p=0.007$).

In terms of clinical diagnosis (Table 3), BrS was the most common disorder in NGS group (76.5%), and idiopathic ventricular fibrillation (IVF) was the most common one in Sanger group (63.2%). In terms of documented ECG, ventricular fibrillation and tachycardia were the most common ones in both NGS and Sanger groups.

Overall, nineteen mutations by NGS and 70 mutations by Sanger method were detected. When analyzed based on the number of patients, among the 17 NGS patients, two (11.8%) had pathogenic or likely pathogenic mutations, thirteen (76.5%) had uncertain significance, and four (23.5%) had no mutation detected. Among the 19 Sanger patients, seventeen (89.5%) had pathogenic or likely pathogenic mutations, and 14 (73.7%) had uncertain significance. The details of the mutations, including mutation name, DNA change, amino acid change, Online Mendelian Inheritance in Man (OMIM) disease class, and ACMG/AMP class, can be seen in Additional file 1: Tables 1 and 2.

In terms of ACMG/AMP class, uncertain significance was the most common one in NGS group (89.5%); pathogenic or likely pathogenic were the most common ones in Sanger group (45.7%). RYR2 and CASQ2 mutations were the most common ones in NGS and Sanger groups, respectively (Table 4). The NGS arrhythmia panel did not cover 15 mutations that were detected by the Sanger method. Among these 15 mutations, RYR1 and APOA5 are associated with arrhythmogenic right ventricular cardiomyopathy and familial atrial fibrillation, respectively. But the remaining thirteen mutations in ALMS1, APOB, CETP, FBN1, GCKR, LAMA2, LAMA4, MAP2K2, MYBPC3, NEXN, PRDM16, SDHA, and TMPO are not related to inherited arrhythmia. When the number of mutations was counted allowing repeats, the extended arrhythmia NGS panel was able to detect 84.8% of inherited arrhythmia-related mutations that were detected in Sanger group (Table 4).

There were 2 LQTS patients in the Sanger group. One of them showed a mutation in SCN5A (pathogenic) and the other one in ANK2 (uncertain significance). There were 13 BrS patients in NGS group and four in Sanger group. Among the 13 BrS patients in NGS group, four showed mutations in KCND3 (uncertain significance), KCNE3 (uncertain significance), GPD1L (uncertain significance), and SCN5A (likely pathogenic). Thus, the yield was about 7.7%. Among the four BrS patients in Sanger group, two showed mutations in CACNB2 (likely benign) and CACANA1C (likely pathogenic). Thus, the yield was about 25%.

**Table 3** Clinical and electrocardiographic characteristics

| Gene sequencing method | Total ($n=36$) | NGS ($n=17$) | Sanger ($n=19$) | *P* value |
|---|---|---|---|---|
| *Clinical diagnosis* | | | | |
| Brugada syndrome | 17 (47.2%) | 13 (76.5%) | 4 (21.1%) | < 0.001 |
| Long QT syndrome | 2 (5.6%) | 0 (0.00%) | 2 (10.5%) | 0.163 |
| Catecholaminergic polymorphic VT | 0 (0.00%) | 0 (0.00%) | 0 (0.00%) | – |
| Arrhythmogenic RV cardiomyopathy | 0 (0.00%) | 0 (0.00%) | 0 (0.00%) | – |
| Idiopathic ventricular fibrillation | 12 (33.3%) | 0 (0.00%) | 12 (63.2%) | < 0.001 |
| etc | 2 (5.6%) | 1 (5.9%) | 1 (5.2%) | 0.938 |
| Non-diagnosed family | 3 (8.3%) | 3 (17.6%) | 0 (0.00%) | 0.083 |
| *ECG rhythm at initial recovery* | | | | |
| Heart rate | 83.8 ± 4.0 | 76.2 ± 4.2 | 90.5 ± 6.2 | 0.073 |
| PR interval | 175.2 ± 4.9 | 188.0 ± 6.1 | 161.6 ± 6.1 | 0.001 |
| QRS width | 106.8 ± 3.2 | 104.6 ± 3.5 | 108.7 ± 5.3 | 0.534 |
| QT interval | 385.4 ± 7.3 | 383.9 ± 8.1 | 386.8 ± 12.0 | 0.839 |
| QTc interval | 443.2 ± 7.6 | 424.8 ± 7.0 | 459.6 ± 11.9 | 0.017 |
| Normal sinus rhythm | 18 (50.0%) | 10 (58.8%) | 8 (42.1%) | 0.331 |
| Atrial fibrillation | 7 (19.4%) | 1 (5.9%) | 6 (31.6%) | 0.048 |
| AV block (2nd or 3rd degree) | 4 (11.1%) | 4 (23.5%) | 0 (0.00%) | 0.041 |
| Premature ventricular complex | 7 (19.4%) | 2 (11.8%) | 5 (26.3%) | 0.276 |
| Brugada pattern ECG | 15 (41.7%) | 11 (64.7%) | 4 (21.1%) | 0.007 |
| *ECG diagnosis at SCD* | | | | |
| Ventricular fibrillation | 21 (58.3%) | 5 (29.4%) | 16 (84.2%) | < 0.001 |
| Ventricular tachycardia | 3 (8.3%) | 1 (5.9%) | 2 (10.5%) | 0.627 |
| Sinus pause | 0 (0.00%) | 0 (0.00%) | 0 (0.00%) | – |
| Not documented | 12 (33.3%) | 11 (64.7%) | 1 (5.2%) | < 0.001 |

*NGS* next-generation sequencing; *VT* ventricular tachycardia; *RV* right ventricular; *ECG* electrocardiogram; *AV* atrioventricular; *SCD* sudden cardiac death

## Discussion

To identify genetic variants and to assess the clinical value of using NGS in cases of inherited arrhythmia syndromes, we analyzed the NGS data of patients who had suffered a sudden cardiac death or showed ECG abnormality. NGS is now favored for sequencing thousands of genomic variants simultaneously, sequencing the entire genome/exome to find novel variants, and detection of rare mutations via cell-free DNA sequencing. On the other hand, Sanger sequencing is still a method of choice for sequencing single genes or gene regions of up to 500 base pairs, and short tandem repeat analysis. In terms of the cost and time, those would be depending on situation of each institutes and nations. Nowadays in Korea, the cost of NGS (30 genes) is about 370 USD (when covered by national health insurance), it takes about 3–4 weeks to get reports (when requested to a gene-lab institute). The cost of Sanger (1 gene) is about 500 USD (when covered by national health insurance), it takes about 1–2 weeks (when requested to a gene-lab institute).

In this study, NGS and Sanger groups both consisted largely of males (94.1% and 94.7%, respectively). We think that the high proportion of males may be due to the high number of BrS patients enrolled in the study, especially in NGS group, as BrS exhibits high prevalence in men. Indeed, 13 of 17 participants in the NGS group were BrS patients. But, in Sanger group, only 4 of 19 patients had BrS. IVF was predominant in Sanger group (12 of 19). Among the 12 IVF patients, some might have been BrS patients, because IVF is diagnosed exclusively, and lots of IVF patients turn out to have other disorders, including BrS. Additionally, type 1 Brugada pattern ECGs were more frequently observed in NGS group than in the Sanger group (Table 3). This observation, also, could be explained with the high number of BrS patients in NGS group.

When positive yield was defined as the ratio of pathogenic or likely pathogenic mutations detected, the yields were 10.5% and 45.7% in NGS and Sanger groups, respectively. Considering that the yield of genetic studies of BrS is about 20% [6] and that BrS patients constitute 76.5% (13 of 17 patients) of the NGS group, the yield of 10.5% would be a reasonable result. When analyzed based on the number of patients, the rate of pathogenic or likely pathogenic mutations was relatively low. Indeed, among the 17 NGS patients, the yield was 11.8% in NGS.

**Table 4** The list and frequencies of mutations. The number refers to the count of genetic mutations

| **ACMG/AMP class (2015)** | | **NGS (*n* = 19)** | | | | **Sanger (*n* = 70)** |
|---|---|---|---|---|---|---|
| Pathogenic | | 0 (0.0%) | | | | 29 (41.4%) |
| Likely pathogenic | | 2 (10.5%) | | | | 3 (4.3%) |
| Likely benign | | 0 (0.0%) | | | | 12 (17.1%) |
| Benign | | 0 (0.0%) | | | | 0 (0.0%) |
| Uncertain significance | | 17 (89.5%) | | | | 26 (37.1%) |
| **Inherited arrhythmia-related mutation** | | ***n* = 18** | | | ***n* = 33** | |
| | RYR2 | 4 | CPVT | CASQ2 | 6 | CPVT, NCCM, HCM |
| | SCN5A | 2 | BrS, LQTS 3 | LDB3 | 4 | ARVC, HCM, DCM |
| | SCN4B | 2 | LQTS10 | APOA5 | 3 | FAF |
| | DSP | 2 | ARVC, DCM | SCN5A | 3 | BrS, LQTS |
| | GPD1L | 2 | BrS 2 | TRDN | 3 | CPVT, LQTS |
| | AKAP9 | 1 | LQTS 11 | CACNA1C | 2 | BrS, LQTS |
| | DSC2 | 1 | ARVC 11 | CACNB2 | 2 | BrS |
| | KCND3 | 1 | BrS 9 | DSC2 | 2 | ARVC, FAF, DCM |
| | KCNE3 | 1 | BrS 6 | DSP | 2 | ARVC, DCM |
| | PKP2 | 1 | ARVC 9 | RYR1 | 2 | ARVC |
| | SNTA1 | 1 | LQTS 12 | ANK2 | 1 | LQTS |
| | | | | JUP | 1 | ARVC, DCM |
| | | | | TMEM43 | 1 | ARVC |
| | | | | TRPM4 | 1 | BrS |
| Basic NGS ability | Reference | 18/18 (100%) | | | 15/33 (45.4%) | |
| Extended NGS ability | Reference | 18/18 (100%) | | | 28/33 (84.8%) | |
| **General mutation** | | ***n* = 1** | | | ***n* = 37** | |
| | LMNA | 1 | DCM 1A | APOB | 7 | FHC |
| | | | | LAMA2 | 4 | DCM |
| | | | | RBM20 | 4 | DCM |
| | | | | GCKR | 3 | FHC |
| | | | | MYH6 | 3 | FAF, HCM, DCM |
| | | | | SDHA | 3 | DCM |
| | | | | LAMA4 | 2 | DCM |
| | | | | MYBPC3 | 2 | NCCM, HCM, DCM |
| | | | | ALMS1 | 1 | DCM |
| | | | | BAG3 | 1 | DCM |
| | | | | CETP | 1 | FHC |
| | | | | FBN1 | 1 | AVD, FAA, Marfan Syndrome |
| | | | | MAP2K2 | 1 | Noonan Syndrome, HCM |
| | | | | MYH7 | 1 | RCM, NCCM, HCM, DCM |
| | | | | NEXN | 1 | HCM, DCM |
| | | | | PRDM16 | 1 | NCCM, DCM |
| | | | | TMPO | 1 | DCM |
| Basic NGS ability | | 0/1 (0.0%) | | | 0/37 (0.0%) | |
| Extended NGS ability | | 1/1 (100%) | | | 11/37 (29.7%) | |

*ACMG/AMP* The American College of Medical Genetics and Genomics/the Association for Molecular Pathology; *NGS* next generation sequencing; *CPVT* catecholaminergic polymorphic ventricular tachycardia; *NCCM* non-compaction cardiomyopathy; *HCM* hypertrophic cardiomyopathy; *BrS* Brugada syndrome; *LQTS* long QT syndrome; *ARVC* arrhythmogenic right ventricular cardiomyopathy; *DCM* dilated cardiomyopathy; *FAF* familial atrial fibrillation; *FHC* familial hypercholesterolemia; *AVD* aortic valve disease; *FAA* familial aortic aneurysm; *RCM* restrictive cardiomyopathy

However, yield of NGS seems generally low in unexplained SCD or suspicious inherited arrhythmia syndromes. In a study of Proost et al. [1] which analyzed 114 patients with primary electrical disease or SCD using 51 genes for NGS, 107 variants were identified in 36 different genes. Eighteen (16.8%) were classified as pathogenic or likely pathogenic. In a study of Herzt et al. [7] which analyzed 47 sudden unexpected deaths in infancy using 100 genes for NGS, Eight (17%) of the SUDI cases had variants in genes affecting ion channel functions.

In the Sanger group, the pathogenic and likely pathogenic mutations constituted 45.7% of all detected mutations (Table 4). Considering that the yields in cases of BrS and LQTS were 20% and 75% [8], respectively, and there were four BrS, two LQTS, and 12 IVF patients in Sanger group, we could assume that IVF-associated variations constitute a significant amount of mutations. In other words, the diagnosed cases of IVF may not have been true IVF cases.

In inherited channelopathies, including LQTS, CPVT, and BrS, the structure and function of the ion channels are affected in the cardiac cells leading to perturbed ion channel function, disrupted action potential propagation, and development of arrhythmias. Given that functional changes in ion channels in heart are not visible microscopically or observable at the macro scale with standard techniques, the cause of death in cases involving perturbed ion channel function is not detectible by conventional medical investigations, and genetic analyses will be needed to detect the cause of death [7].

Genetic testing using NGS for BrS revealed several de novo genetic variants but their association with the disease remain uncertain. Accumulation of NGS data and functional studies will increase the yield of genetic tests in BrS in the future. Fundamentally, Sanger method is superior to NGS in its accuracy and coverage. But as mentioned in the introduction section, its high analytical throughput and relative speed make NGS very attractive for early clinical implementation. However, it still seems unreasonable to apply NGS to all genetic diseases for diagnosis or screening. We could assume that for a disease like BrS, for which genetics studies show low yields of detection, NGS can be practically disadvantageous compared to Sanger method. On the contrary, it is expected to be highly effective in diseases where genetic studies exhibit high rates of successful variant detection.

#### Long QT syndrome

The prevalence of LQTS is estimated to be 1/2000 in the general population [9] and about the same among different ethnicities [10]. At least 13 LQTS-related genes have been reported so far [10], following the discovery of the first LQTS-related mutation at 11p15.5 [11], the core cardiac potassium channel genes KCNQ1 [12] and KCNH2 [13], and sodium channel gene SCN5A [14] as causative genes for LQTS1-3. The overall mutation detection yield in LQTS is about 70%, and most of the identified mutations are found in three major LQTS genes: 42–52% of the mutations are in KCNQ1 (LQTS1), 32–45% in KCNH2 (LQTS2), and 8–13% in SCN5A (LQTS3) [10]. All kinds of mutations are found in LQTS; about 70% are missense mutations, 15% are frame-shift mutations, and in-frame deletions, nonsense, and splice site variants account for 3–6% of the total mutations.

In the present study, several LQTS-related mutations were detected. Mutations in SCN5A, SCN4B, AKAP9, and SNTA1 were detected by NGS, while those in SCN5A, TRDN, CACANA1C, and ANK2 were detected by Sanger sequencing. There were two LQTS patients analyzed by Sanger sequencing. One of them exhibited SCN5A mutation (pathogenic) and the other one ANK2 mutation (uncertain significance) [15]. Though the number of enrolled patients was small, the yield of Sanger method was 50% in LQTS.

Considering that the overall yield of gene studies in LQTS is known to be about 70%, the yield of Sanger method was relatively low in the present study. But the number of enrolled patients was only two; thus, the results may not be conclusive.

#### Brugada syndrome

BrS is highly associated with sudden cardiac death, especially in young males. Several genetic variations such as those in SCN5A are known to be related to BrS [1, 6]. In BrS, the identification yield for pathogenic gene variants is low.

In the present study, several BrS-related mutations were detected. SCN5A, GPD1L, KCND3, and KCNE3 mutations were detected in NGS group. SCN5A, CACANA1C, CACNB2, and TRPM4 mutations were detected in Sanger group. There were 13 BrS patients in NGS group and four in Sanger group. Among the 13 BrS patients in NGS group, four had mutations in KCND3 (uncertain significance), KCNE3 (uncertain significance), GPD1L (uncertain significance), and SCN5A (likely pathogenic). Thus, the yield of NGS was about 7.7% in the case of BrS. Among the four BrS patients in the Sanger group, two had mutations in CACNB2 (likely benign) and CACANA1C (likely pathogenic). Thus, the yield of Sanger method was about 25% in the case of BrS. Considering that the overall yield of BrS gene studies is about 20%, the yield of NGS was relatively low [6]. In the present study, the NGS arrhythmia panel did not cover two inherited arrhythmia-related mutations (RYR1 and APOA5) that were detected by Sanger method. The target gene coverage

in NGS could be variable or designed to sequence a particular set of genes. Thus, it might be suggested that the yield of NGS analysis is variable, *i.e.*, it depends on the genes included in the NGS panel. But, as seen in Table 4, the extended arrhythmia NGS panel was able to detect 84.8% of inherited arrhythmia-related mutations that were detected in Sanger group. Therefore, a well-designed NGS panel is expected to have a high yield.

### Limitations

The sample size in this study is small to represent a real clinical situation. Indeed, this study enrolled entirely 36 subjects. In a study of Herzt et al. [7] which analyzed small sample size of 47 subjects, the yield was not so different from our result. So, we think our result is not so far from the real clinical situation. However, a large sample size would show a more accurate clinical situation.

The present study was not performed in all patients with aborted sudden cardiac death and inherited arrhythmia syndromes; it was performed on selected patients. The decision to perform the genetic study was made by the patients and their families, and was not influenced by medical teams. Thus, it would be difficult to describe the incidence or prevalence of genetic abnormality in patients with aborted sudden cardiac death and inherited arrhythmia syndromes. However, we believe it is valuable to show the differences in coverage between NGS and Sanger sequencing in a certain disease, inherited arrhythmia syndromes, in this study.

NGS was conducted on DNA samples. Exonic deletion/duplication, regulatory or deep intronic regions, repeat expansion, and imprinting defects cannot be detected. Also, the target region might have not been captured. In cases of homologous region, mutation detection accuracy would be reduced. The variant pathogenicity was classified in accordance with the 2015 ACMG/AMP guideline [5]. To date, there is a lack of a Korean genetics library, especially for arrhythmia. Thus, it is challenging to determine the pathogenicity of the variants detected.

Sanger sequencing and NGS were not performed in the same group of subjects simultaneously. So, an exact comparison between the two methods was not possible, which is the most critical limitation of this study. Since the insurance coverage was limited in patients and the cost was high, simultaneous sequencing was not available. However, we believe that this study shows a practical example of genetic studies in a real clinical situation. In such a practical situation, we compared the usefulness and coverage differences of two methods of genetic analysis.

## Conclusions

NGS study has some limitations in obtaining the full genetic data of probands. In the present study setting, the yield was definitely lower in NGS. As the result showed, the more extended NGS panel detects pathogenic mutations with a better yield. Thus, well-designed NGS panels are needed to increase the efficiency of the NGS study. When such a condition is met, large-scale gene sequencing can efficiently and rapidly be applied in real clinical practices, especially in inherited fatal arrhythmia syndromes that have a high detection yield in genetic analyses.

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s42444-023-00097-z.

**Additional file 1: Table 1**. Mutations verified with Sanger sequencing; **Table 2**. Mutations verified with next-generation gene sequencing.

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#### Author contributions

MJK was a major contributor to the writing of the manuscript. YRK contributed to the manuscript review, approval of the final version of the manuscript, and agreement of all aspects of the work. KHL contributed to the manuscript review, approval of the final version of the manuscript, and agreement of all aspects of the work. NY contributed to the design, literature search, data acquisition, data analysis, manuscript preparation, manuscript editing, manuscript review, approval of the final version of the manuscript, and agreement of all aspects of the work. HWP contributed to the conception of the work, manuscript review, approval of the final version of the manuscript, and agreement of all aspects of the work. All authors read and approved the final manuscript.

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#### Availability of data and materials

Data are available from the authors upon reasonable request with permission of Institutional Review Board of Chonnam National University Hospital.

## Declarations

#### Ethics approval and consent to participate

All patients that fulfilled the acceptance criteria were eligible to be included in the study and were asked to participate. All patients who agreed and provided written informed consent (approved by the institutional review board, IRB No., CNUH-2022-327) were included.

#### Consent for publication

All authors have permitted the publication.

#### Competing interests

The authors declare that there are no conflicts of interest regarding the publication of this article.

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