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Original Research

Rapid diagnostic testing of a neonate in a family with hypertrophic cardiomyopathy

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Summary

Familial hypertrophic cardiomyopathy (HCM) is a common but severe genetic disease. A pregnant woman with familial HCM was referred to our hospital as both the couple and their families were concerned that the baby would later develop HCM. Therefore, we determined the risk of HCM in the neonate. Using whole-exome sequencing, mutational analysis was performed on the patient, her family members (including her father, mother, sister, and husband), and the neonate. Sanger sequencing was also performed. We found that HCM in this family was caused by a mutation in the cardiac heavy chain β-myosin (*MYH7*) gene. Encouragingly, the neonate did not carry this *MYH7* mutation as the father was also negative. We were able to determine that the neonate had no risk of familial HCM. Obstetricians should consider genetic screening if a pregnant woman has a severe risk of such familial complications. *Content*: We demonstrated absence of familial HCM in a neonate and suggest appropriate genetic screening in pregnant women with familial complications.

Key words: Early diagnosis; Hypertrophic cardiomyopathy; Mutation; *MYH7*; Whole-exome sequencing.

Introduction

Familial hypertrophic cardiomyopathy (HCM) is a primary disorder of cardiac muscle associated with a thickened ventricular wall and septum, increased myocardial fibrosis, disorganized myofibers, and always accompanied by an arrhythmic heart beat [1]. HCM has a prevalence of 0.2% in the general population [2] and is the most common cause of sudden cardiac death in individuals younger than 35 years [3]. Additionally, HCM is the most common autosomal dominant cardiovascular disease [1]. Mutations in at least 30 different genes have been identified in patients with HCM, but the genes encoding cardiac myosin-binding protein C3 (*MYBPC3*) and cardiac heavy chain β-myosin (*MYH7*) account for approximately 50% of the mutations [4, 5]. Because of the large size of these genes, Sanger sequencing of single amplicons is labor-intensive and expensive, therefore next-generation sequencing (NGS) technologies can facilitate genetic screening in such cases [6, 7]. Most reported NGS procedures are based on polymerase chain reaction (PCR) amplification of coding exons using primers that match flanking introns, followed by pooling and digestion of PCR products to achieve a readable size, then by ligation of specific oligonucleotides to each fragment [8-10]. Putative mutations within a pool can be assigned to specific individuals by Sanger sequencing of the corresponding exon.

Familial HCM is a common but severe disease. Accordingly, pregnant women with familial HCM sometimes present at hospital with both parents and their families concerned to know whether the baby will develop this disease. Here, we were able to identify a gene mutation causing familial HCM, and thereby succeeded in early diagnosis of the neonate, who fortunately was negative for the causative mutation.

Materials and Methods

Subjects and DNA samples

A 29-year-old primigravid woman with a spontaneous pregnancy was referred to Asahikawa Medical University Hospital (Asahikawa, Japan) following a positive pregnancy test. Her personal and family histories were as follows. In 2000, the patient was diagnosed with HCM at 16 years of age. Since then she had been on drug therapy, and was put on a cardiac pacemaker in 2013 at 28 years of age. Her father and sister were also diagnosed with familial HCM, and have had pacemakers installed since 2006 and 2011, respectively (Figure 1a). All of the study participants provided informed consent, and the study was approved by the institutional review board of Asahikawa Medical University. The expected delivery date was calculated from the fetal crown-rump length at 10 weeks of gestation, which was obtained by transvaginal ultrasonography. Because of the mother's HCM, elective caesarean section was performed at 39 weeks and 4 days of gestation. The neonate was female and weighed 2,670 g with Apgar scores of 8 and 8 after 1 and 5 min, respectively.

Genomic DNA samples for individuals II-2 (patient's father), III-1 (patient's sister), III-2 (patient), and III-3 (patient's husband) were extracted from peripheral blood,

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Table 1. — *Three programs (SIFT, PolyPhen-2, and Mutation Taster) predicted that the c.2770G>A mutation in MYH7 would influence protein function. This mutation was first reported in 1992 [11].*

Gene	Mutation	SIFT	PolyPhen-2	Mutation Taster
MYH7 (NM_000257)	c.2770G > A p.E924K	0 (deleterious)	0.995 (probablydamaging)	Disease-causing

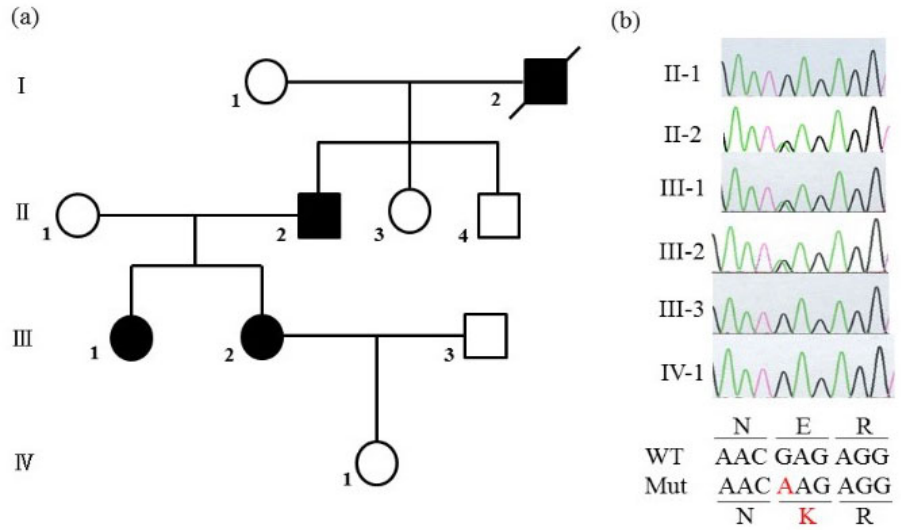

Figure 1. — Genetic analysis of the *MYH7* mutation in the family. (a) The family pedigree. (b) Electropherograms of the *MYH7* mutation. MT, mutant allele; WT, wild type allele.

while genomic DNA for individual IV-1 (the neonate) was extracted from umbilical cord blood, using NucleoSpin Blood QuickPure kits (TaKaRa Bio Inc., Shiga, Japan; Figure 1a).

Whole-exome sequencing

Whole-exome sequencing was performed on five individuals in the family (Figure 1a). One microgram of high-quality genomic DNA was captured using SureSelect Human All Exon v. 5 kits (Agilent Technologies, Santa Clara, CA, USA), according to the manufacturer's instructions. Captured DNA was sequenced using HiSeq2000 (Illumina, San Diego, CA, USA) with 100-bp paired-end reads. Image analysis and base calling were performed using Sequence Control Software with Real-Time Analysis (Illumina) and CASAVA software (v. 1.8.2 or 1.8.4) (Illumina). Reads were mapped to the human reference genome (UCSC hg19, NCBI build 37.1) using Novoalign (v. 3.00.02). After removal of PCR duplications using Picard (v. 1.55), single-nucleotide variants and short insertions and deletions were called using the Genome Analysis Toolkit (GATK) (v1.6-5) and annotated using ANNOVAR (June 2013).

Prioritization of variants

Of all the variants within exons and ± 30 bp of intronic regions of exon-intron boundaries, those registered in either dbSNP137 (non-flagged variants), the National Heart Lung and Blood Institute Exome Sequencing Project Exome Variant Server (NHLBI-ESP 6500), Human Genome Variation Database, and our in-house database (exome data from 575 individuals), as well as synonymous variants, were removed. Therefore, our analysis focused on heterozygous variants. All called variants used the Human Genome Mutation Database as a reference for disease-causing mutations. Variants were confirmed as true positives by Sanger sequencing (below).

Sanger sequencing

MYH7 exon 23 was amplified by nested PCR from the patient, her father, sister, husband, and the neonate. The primers used were: MYH7F1, 5′-GGGATATCCTAGAGTTACCC-3′; MYH7F2, 5′-TTTGAGTGATGTGCCTCTCC-3′; MYH7R1, 5′-CTGGGTGAAGGTCAGTATGG-3′; MYH7R2, 5′-TGAGAGTCCTGATGAGACCC-3′. Amplified nested PCR products were sequenced using an ABI 3500xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The Human Genetic Variation Database (http://www.hgvd.genome.med.kyoto-u.ac.jp) was used to determine whether the variants had been reported previously.

Results

The patient, her sister, and father were diagnosed with HCM and all used a cardiac pacemaker; additionally, her grandfather had died because of HCM (Figure 1a). Consequently, the family wanted to know if the baby was likely

to develop HCM after birth. We offered them genetic counseling for familial HCM, explaining that many causative genes had already been identified. Thus, if whole-exome sequencing was successful, the causative gene within this family was likely to be identified. We could then determine if the fetus had HCM by umbilical blood aspiration and subsequent genetic analysis. The patient and her husband had hoped for a prenatal diagnosis, but the patient's father and sister were not able to reach the hospital easily, so we could not obtain blood from them before delivery. However, on the day of surgery, the patient's father, mother, and sister came to the hospital and we collected blood from all of them.

The mean read depth of RefSeq coding sequence regions (CDS) was 53.19-92.18 reads, with 86.0%-91.2% of CDS bases covered by 20 or more reads. We identified 37 potential pathogenic variants using the Human Genome Mutation Database as a reference. Of these 37 variants, a missense mutation (c.2770G>A (p.E924K)) was identified in *MYH7*. This mutation had been reported as causative for HCM (Table 1) [11]. This heterozygous missense mutation was confirmed in individuals II-2, III-1, and III-2 by Sanger sequencing, but was not found in II-1, III-3, or IV-1 (Figure 1b). The mutation was predicted to be damaging by SIFT, Polyphen-2, and MutationTaster. The patient's husband had a normal *MYH7* sequence that was also detected in the neonate (Figure 1b), indicating there was no risk of transmission of HCM. We provided this result to the parents and their families.

Discussion

HCM is the most common cause of sudden cardiac death at a young age, and a major cause of morbidity and mortality in elderly people. HCM is frequently familial and inherited as a Mendelian dominant disease caused by mutations in sarcomeric genes [12]. According to most studies, approximately 50% of cases of HCM have mutations in *MYBPC3* or *MYH7*, with the other genes mutated in fewer than 10% of patients [13-18]. Mutations in *MYH7* are more frequent in severe forms of hypertrophy and patients with a family history of HCM or of sudden cardiac death [19].

Here, we identified three patients with HCM within a family with a heterozygous *MYH7* missense mutation (c.2770G>A (p.E924K)). *MYH7* encodes the β-cardiac myosin heavy chain protein that is expressed predominantly in the heart. To date, many *MYH7* mutations have been identified that cause HCM [20]. The p.E924K mutation was previously reported in a pedigree by Watkins *et al*. [11]. This missense mutation is located at subfragment 2 (S-2) domain [20]. The S-2 functional element plays an important role in regulating smooth muscle activity and binding of myosin-binding protein C, a modulator of sarcomere contractility in striated muscle [21].

Many pregnant women with various complications attend our university hospital. Initially, it is important to carefully obtain a detailed anamnesis and family history. If a hereditary disease is in doubt, then genetic counseling is required. In this case, the patient, her husband, and their parents were concerned that the baby might develop familial HCM. We were able to show that a *MYH7* gene mutation caused HCM in this family, and that the neonate had a normal *MYH7* sequence. We were able to reassure them that the baby girl will not develop familial HCM.

Many genes causing human diseases have been identified because of NGS technologies, which have also facilitated genetic screening. Obstetricians should consider this as routine when a pregnant woman has such severe familial complications.

Ethics Approval and Consent to Participate

All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of Asahikawa Medical University (approval number: 15112).

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Conflict of Interest

The authors declare no conflict of interest.

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