Molecular Syndromology

Novel Insights from Clinical Practice

Mol Syndromol 2022;13:517–521
DOI: 10.1159/000524375

Received: November 1, 2021
Accepted: March 24, 2022
Published online: May 5, 2022

Mosaicism of a Truncating Variant of *CASK* Causes Congenital Heart Disease and Neurodevelopmental Disorder

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Established Facts

- Mutations in calcium/calmodulin-dependent serine protein kinase (*CASK*) cause microcephaly with pontine and cerebellar hypoplasia (MICPCH) and X-linked intellectual disability with various severity.
- Congenital heart disease (CHD) is a rare complication reported only in male patients with full loss-of-function mutations of *CASK*.

Novel Insights

- We identified a male patient with mosaicism of a truncating variant of *CASK* with CHD. Truncating variants in *CASK*, even in a mosaic state, may be associated with CHD.
- The remaining normal allele may contribute to the longer survival of the male patient with mosaic mutation of *CASK*.

Keywords
CASK · Mosaicism · Truncating variant · Congenital heart disease · Neurodevelopmental disorders

Abstract
Introduction: Calcium/calmodulin-dependent serine protein kinase (*CASK*) gene mutations cause microcephaly with pontine and cerebellar hypoplasia (MICPCH) and X-linked intellectual disability. Congenital heart disease (CHD) is a rare complication reported in only 4 male patients with full loss-of-function mutations. Here, we report the first male patient with mosaicism of a truncating variant of *CASK* complicated by CHD. ***Case Presentation:*** The patient is a 6-year-old male with MICPCH, ventricular septal defect, and developmental delay. He achieved rolling over but can not speak meaningful words. We identified a somatic mosaic variant of *CASK*: c.[725=/G>A], p.(W242*) and high mosaic ratios of 90% and 84% for mutant alleles in peripheral blood lymphocytes and skin fibroblasts, respectively. His developmental delay was severe but milder than that of previously reported CHD patients. ***Discussion:*** Truncating *CASK* variants may be associated with CHD, even in a mosaic state, and even a low normal allele ratio could lengthen survivorship.

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Introduction

Pathogenic variants of the calcium/calmodulin-dependent serine protein kinase (*CASK*) gene cause microcephaly with pontine and cerebellar hypoplasia (MICPCH), X-linked intellectual disability, and ophthalmic abnormalities including nystagmus [Najm et al., 2008; Burglen et al., 2012; Moog et al., 2015]. The clinical severity depends on the sex and variant type [Moog et al., 2015]. Loss-of-function variants result in a more severe phenotype than hypomorphic missense variants. Male patients, who generally present with more severe symptoms than females, can be divided into the following 3 groups according to their clinical features and variant types [Moog et al., 2015]: Group I – MICPCH with epileptic encephalopathy due to a loss-of-function variant, group II – MICPCH due to somatic mosaicism of a loss-of-function variant, and group III – X-linked intellectual disability with or without nystagmus due to a hypomorphic variant. Congenital heart disease (CHD) is a rare complication that has been previously reported in only 4 male patients in group I [Nakamura et al., 2014; Moog et al., 2015]. Here, we report the case of a male patient with CHD who had a truncating variant in mosaic state belonging to group II.

Case Report

The proband is a 6-year-old male patient. He was born to non-consanguineous parents at 39 weeks of gestation as their first child. His birth weight, height, and head circumference were 2,334 g (−1.4 SD), 45 cm (−1.5 SD), and 30.0 cm (−2.2 SD), respectively. After birth, he was noted to have retrognathia, nystagmus, and heart murmur due to a perimembranous type ventricular septal defect (VSD) (2.5 mm × 3.3 mm). Echocardiography at the age of 5 months revealed mild right coronary cusp prolapse and mitral valve regurgitation. The VSD spontaneously closed at the age of 6

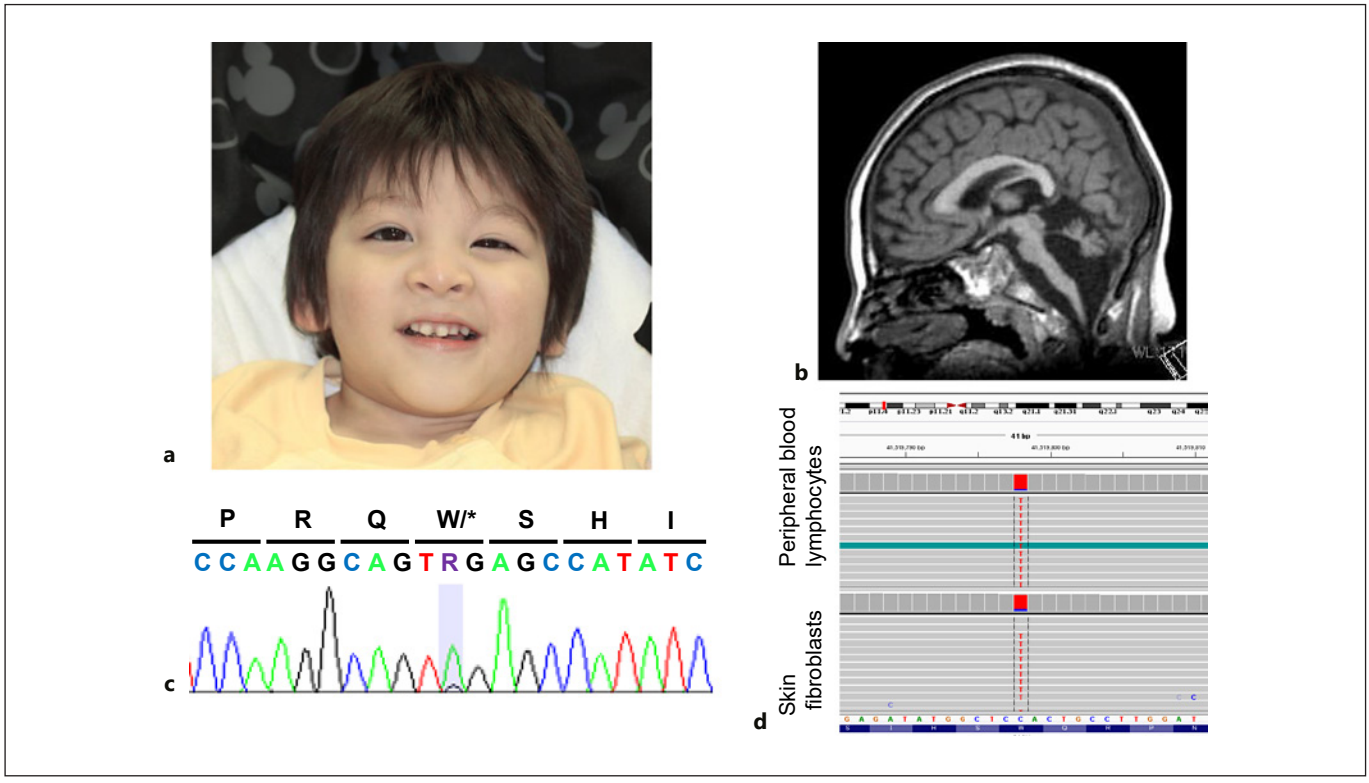

Fig. 1. Clinical features and the *CASK* mutation in the patient. **a** Photograph of the patient at 4 years of age. Facial features included microcephaly, arched eyebrow, wide-spaced teeth, thin upper lip, and retrognathia. **b** A sagittal section of brain MRI. Severe pontocerebellar hypoplasia was noted. **c** Sequencing of the variant of *CASK* for peripheral blood lymphocytes. Electropherogram showed mosaic state with low ratio of normal allele in peripheral blood lymphocytes, consistent with the results of targeted sequencing using TruSight One Sequencing Panel. **d** Deep sequencing of the variant of *CASK* in peripheral blood lymphocytes (upper panel) and cultured skin fibroblasts (lower panel). Visualization on the Integrative Genomics Viewer determined a high proportion of mosaicism in peripheral blood lymphocytes (mutant allele 90%, read depths 3,288) and skin fibroblasts (mutant allele 84%, read depths 3,408).

years. His physical and psychomotor development was severely delayed. By the age of 2 years, he was able to control his head and roll over. At the age of 3 years and 6 months, his weight, length, and head circumference were 9,075 g (−3.2 SD), 82.0 cm (−4.0 SD), and 39.0 cm (−7.2 SD), respectively, suggesting short stature and progressive microcephaly (Fig. 1a). At 4 years of age, he moved by rolling over but could not sit without support and spoke no meaningful words. Magnetic resonance imaging (MRI) of his brain showed severe pontocerebellar hypoplasia (Fig. 1b). He developed epileptic seizures at the age of 6 years and was successfully treated with valproate. He could bring his hand to his mouth and suck on it but could not grasp a rattle. Instead, he could follow moving things with his eyes to the midline, smile responsively, and laugh aloud. Chromosomal studies revealed a 46,XY karyotype. Cytogenetic microarray analysis revealed no significant copy number variants.

Molecular Studies

Targeted Sequencing

Genomic DNA was obtained from peripheral blood lymphocytes using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) and from cultured skin fibroblasts using the QIAamp DNA Micro Kit (Qiagen) according to the manufacturer's instructions. Targeted sequencing with peripheral blood lymphocytes was first performed using the TruSight One Sequencing Panel (Illumina, Inc., San Diego, CA, USA), which enables the analysis of 4,813 genes causing a mendelian disease, using the MiSeq platform (Illumina) with 150-bp paired-end reads. Sequencing data were analyzed using the Burrows-Wheeler alignment tool (http://bio-bwa.sourceforge.net/) and the Genome Analysis Toolkit (https://gatk.broadinstitute.org/hc/en-us), and visualized using the Integrative Genomics Viewer (IGV). Variants identified by targeted sequencing were analyzed and confirmed by Sanger sequencing as previously described [Enomoto et al., 2018; Murakami et al., 2020].

We found a somatic mosaic variant of *CASK* (NM_003688.3): c.[725=/G>A], p.(W242*), with a mosaic ratio of 93% for the variant allele in peripheral blood lymphocytes. Sanger sequencing confirmed that this variant was de novo, and that the normal allele was retained with a low proportion of mosaicism (Fig. 1c). This variant was absent in gnomAD (https://gnomad.broadinstitute.org/), Jmorp (https://jmorp.megabank.tohoku.ac.jp/), and the Human Genetic Variation Database (http://www.hgvd.genome.med.kyoto-u.ac.jp/). This variant was registered in the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/) as a likely pathogenic variant (VCV000976007.1); however, detailed clinical information was unavailable.

Evaluation of Mosaic Ratio

To evaluate the mosaic ratios of the identified variant in peripheral blood lymphocytes and skin fibroblasts, we performed sequence analysis of polymerase chain reaction amplicons encompassing the variants by preparing the library using a Nextera XT kit (Illumina) with the MiSeq platform.

Deep sequencing by Nextera XT revealed that the mosaic ratio in lymphocytes and fibroblasts was 90% and 84%, respectively (Fig. 1d).

Discussion

Here, we report a male patient with a high proportion of mosaicism of the *CASK* truncating variant who exhibited CHD and MICPCH. He developed epilepsy but was

Table 1. *CASK* variants and clinical features of the male patients with congenital heart disease

	Present patient	Moog et al., 2015			Jinnou et al., 2012 Nakamura et al., 2014
		patient 1	patient 3	patient 4	
Mutation	c.725G>A, p.(W242*)	c.704_708del, p.(K236Efs*10)	c.1A>G	c.79C>T, p.(R27*)	c.227_228del, p.(E76Vfs*6)
Age at last follow-up	6 years	7 months (died)	5 years	1 year 3 months	8 months
Somatic mosaicism	+	–	–	–	–
Development	Rolling over, no meaningful words	No development, tube feeding, mechanical respiratory support	No development, tube feeding	Apnea, tube feeding	No head or trunk control, tube feeding, tracheostomy and mechanical ventilation
Epilepsy	+	+	+	+	+
Brain MRI findings	Hypoplasia of cerebellum and pons	Hypoplasia of cerebellum, pons, and medulla	Hypoplasia of cerebellum and pons	Hypoplasia of cerebellum and pons, progressive cortical atrophy	Hypoplasia of cerebellum and pons
Head circumference, SD	−7.2	−5.9	−5	−9.0	−5.4
Congenital heart disease	Ventricular septal defect	Atrial septal defect	Atrioventricular septal defect	Ventricular septal defect	Tetralogy of Fallot
Group classification of Moog et al., 2015	II	I	I	I	I

successfully treated and did not require tube feeding or respiratory support. He showed severe developmental delay but was able to roll over by the age of 2 years. To date, 4 male patients have been reported to have a *CASK* variant and CHD with profound developmental delay requiring tube feeding. To the best of our knowledge, no female patients with CHD have been previously reported. All 4 patients with CHD belonged to group I according to the classification of Moog et al. [2015] (Table 1). Our patient showed a less severe phenotype of neurodevelopment than the 4 patients, but a more severe phenotype than the previously reported patients with a lower rate of mosaicism in group II (online suppl. Table 1; see www.karger.com/doi/10.1159/000524375). Thus, the results indicate that the variant may cause severe neurodevelopmental disorders and CHD, but mosaicism may slightly reduce the severity of neurodevelopmental delay [Jinnou et al., 2012; Nakamura et al., 2014; Moog et al., 2015]. In the human heart, CASK interacts with Ca/calmodulin-dependent kinase II (CaMKII) and is expressed in the cardiovascular system during both fetal and adult stages. Functionally, CASK is associated with the regulation of sodium and potassium channels in cardiomyocytes and contractile functions in adults [Leonoudakis et al., 2004; Beuriot et al., 2020; Mustroph et al., 2021]. The detailed role of CASK in fetal heart development remains unknown. However, considering the expression of CASK in the fetal heart and the role of CASK in cardiac function in adults, loss-of-function variants of *CASK* might affect the development of cardiac malformations. Nevertheless, we acknowledge that more data are required to offer a more meaningful insight.

The proportion of mosaicism of loss-of-function mutations in *CASK* varies widely among male patients of type II [Burglen et al., 2012; Moog et al., 2015]. In our patient, mutant alleles were detected in 90% of lymphocytes in the peripheral blood and in 84% of fibroblasts of the skin, respectively (Fig. 1d). The normal allele with a low ratio remained, which may have allowed the patient to survive longer. These results suggest that male patients with severe phenotypes due to loss-of-function mutations may harbor a low proportion of normal alleles. Thus, in male patients of group I, but with a less severe phenotype, it may be necessary to review the sequence of exome or panel analysis data using Integrative Genomics Viewer or to reanalyze the potential variants by deep sequencing to detect the normal alleles with low allele frequency.

In conclusion, we identified a truncating variant with a mosaic state of *CASK* in a male patient with MICPCH and CHD. CHD is a rare complication but is observed among male patients with truncating variants even in a mosaic state. These results suggest that truncating variants in *CASK* may be associated with congenital heart defect even in mosaicism state and corroborate the importance of medical management in patients with mosaic mutation of *CASK*.

Acknowledgement

We would like to thank the patient and his family for their cooperation.

Statement of Ethics

This study was approved by the Institutional Review Board of Kanagawa Children's Medical Center (approval number 2018-1). Written informed consent was obtained from the parents of the patient for publication of the details of their medical case and any accompanying images.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Funding Sources

This research was supported in part by a Health Labour Sciences Research Grant from the Ministry of Health, Labour and Welfare, Japan, the Initiative on Rare and Undiagnosed Diseases (19ek0109301h0002) from the Japan Agency for Medical Research and Development, and JSPS KAKENHI 20K08270 (K.K.).

Author Contributions

C.A.-H. and K.K. contributed to the writing and editing of the manuscript. T.Y. and K.I. provided critical feedback and helped shape the manuscript. All authors approved the final manuscript.

Data Availability Statement

Data sharing is not applicable to this article, as no new data were created or analyzed in this study.

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