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Case Report

CHARGE syndrome in a child with a *CHD7* variant and a novel pathogenic *SOX2* variant: A case report

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Highlights

- We present a male child diagnosed with CHARGE syndrome with variants in *CHD7* and *SOX2*.
- Interactions between these variants may have contributed to his symptoms.

Abstract. CHARGE syndrome is a clinically heterogeneous condition that typically presents with a loss-of-function mutation in *CHD7*. SOX2 anophthalmia syndrome is a rare condition associated with hypogonadism and hearing loss. Herein, we describe the case of a Japanese boy presenting with a micropenis, bilateral cryptorchidism, cupped ear, right facial nerve palsy, and bilateral hearing loss, clinically meeting the diagnostic criteria for CHARGE syndrome, but with optic nerve hypoplasia, which is atypical for the syndrome. Therefore, a genetic analysis (next-generation sequencing) was performed. In addition to the missense variant p.[Arg1940Cys] in *CHD7*, a novel nonsense variant, p. [Tyr110*] in *SOX2* was identified. Although most features, including genital abnormalities and hearing loss, were clinically compatible with CHARGE syndrome caused by a *CHD7* variant, optic nerve hypoplasia may have been caused by a pathogenic *SOX2* variant. Prior research has shown that SOX2 is related to the development of male genitalia and the inner ear. Therefore, the genital abnormalities and hearing loss in this patient may be attributed to both the *CHD7* and *SOX2* variants. Furthermore, the interactions between SOX2 and CHD7 may have affected symptoms independently or reciprocally.

Key words: CHARGE syndrome, optic nerve hypoplasia, *CHD7*, *SOX2*

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Introduction

CHARGE syndrome is a clinically heterogeneous condition characterized by the following specific features: coloboma of the eye, heart defects, atresia of the choanae, retardation of growth and/or development, genital and/or urinary abnormalities, and ear abnormalities. The following clinical features have also been reported in patients with this syndrome: cranial nerve dysfunction (VII, VIII, and others), semicircular canal anomaly, cleft lip and/or palate, feeding difficulties, facial palsy, anosmia, and tracheoesophageal anomaly. The criteria set by Blake and Verloes have been used to diagnose CHARGE syndrome clinically (1, 2). Loss-of-function mutations in *CHD7* have also been found in most patients with this syndrome. *CHD7* analysis in patients who fulfilled the clinical criteria for CHARGE syndrome detected a pathogenic variant in more than 90% of cases, while 81% of CHARGE patients with *CHD7* variants developed eye coloboma (3). Haploinsufficiency of CHD7 causes failure of proper closure of the optic fissure, leading to coloboma (4).

Pathogenic mutations in *SOX2* are common causes of anophthalmia and microphthalmia (5). SOX2 anophthalmia syndrome is sometimes associated with hypopituitarism, forebrain defects, developmental delays, esophageal atresia, sensorineural hearing loss, and hypogonadotropic hypogonadism (HH). Coloboma and optic nerve hypoplasia are also observed in *SOX2* variant-positive patients (5). Therefore, the phenotypes of SOX2 anophthalmia syndrome may overlap with those of CHARGE syndrome.

Herein, we describe the case of a Japanese boy with optic nerve hypoplasia who was clinically diagnosed with CHARGE syndrome and found to harbor variants in *CHD7* and *SOX2*.

Case Report

The patient was born to nonconsanguineous parents at 40 wk 2 d of gestation. His birth height was 48.0 cm (−0.9 SD), and birth weight was 3,350 g (+0.6 SD). During the neonatal period, he presented with a cupped right ear, right facial nerve palsy, and bilateral hearing loss (50–70 dB). At the age of 5 mo, a micropenis was pointed out and he was referred to our hospital. At this initial visit, he was 67 cm (+0.3 SD) tall and weighed 8,770 g (+1.3 SD). The stretched penile length was 1.1 cm. The right testicular volume in the scrotum was 1 mL; however, the left testis was not palpable. The patient had a square-shaped face and no congenital heart defects. Laboratory results are presented in **Table 1**. His thyroid and adrenal functions were normal, and SmC was within the normal range (normal range at 0 yr: 11–149). A GnRH stimulation test was performed at 6 mo. The peak LH was 27.73 and the peak FSH level was 29.27. Peak FSH was delayed (90 min after GnRH injection) and prolonged LH and FSH responses were detected (**Table 2**). After human chorionic gonadotropin (hCG) stimulation, the T and dihydrotestosterone (DHT) levels were 715 ng/dL and 0.76 ng/mL, respectively (T/DHT ratio, 9.41). Chromosomal analysis revealed a 46,XY karyotype. Ophthalmological examination revealed no coloboma; however, bilateral optic nerve hypoplasia was detected. Computed tomography (CT) of the temporal bone revealed dilatation of the right lateral semicircular canal and vestibule, and absence of the right anterior semicircular canal and right stapes. Magnetic resonance imaging of the brain revealed a normal pituitary, with no olfactory nerve anomalies. From 8 to 10 mo of age, the patient received three separate intramuscular injections of testosterone enanthate (25 mg) to treat the micropenis. The patient was diagnosed with bilateral cryptorchidism and underwent orchidopexy at 19 mo of age. He started to walk at 20 mo of age, and had a developmental quotient of 61 and stretched penile length of 3.0 cm at 5 yr of age. At the time of his last visit to our hospital (age, 9 yr and 6 mo), his height was 135.8 cm (+0.38 SD), and weight was 28.9 kg (−0.13 SD). He had attended a support class in elementary school. His LH and T levels were below the measured sensitivity values, and there were no signs of puberty.

The clinical findings of the patient fulfilled two of the major criteria (characteristic ear anomaly and cranial nerve dysfunction) and three of the minor criteria (genital hypoplasia, developmental delay, and distinctive facial features) defined by the Blake criteria

Table 1. Laboratory test results at 5 mo

T	< 3 ng/dL
E_2	< 10 pg/mL
FSH	5.09 mIU/mL
LH	0.62 mIU/mL
DHEA-S	290 ng/mL
ACTH	32.5 pg/mL
Cortisol	12.4 μg/dL
Aldosterone	43.2 ng/dL
PRA	5.2 ng/mL/h
17-OHP	0.7 ng/mL
SmC	28 ng/mL
FT_4	1.37 ng/dL
FT_3	3.55 pg/mL
TSH	2.39 μIU/mL

Table 2. Results of the GnRH stimulation test at 6 mo

	0 min	30 min	60 min	90 min	120 min
FSH (mIU/mL)	5.28	22.07	26.33	29.27	25.36
LH (mIU/mL)	1.72	27.73	26.95	20.88	15.24

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for CHARGE syndrome (1). He was therefore diagnosed with probable CHARGE syndrome, and genetic analyses were performed to clarify the genetic etiology of this condition.

After obtaining informed consent from the mother, genomic DNA was collected from white blood cells, and next-generation sequencing was performed for the following 29 genes related to HH: *CHD7*, *FGF8*, *FGFR1*, *FSHB*, *GNRH1*, *GNRHR*, *HESX1*, *HS6ST1*, *KAL1*, *KISS1*, *KISS1R*, *LEP*, *LEPR*, *LHB*, *LHX3*, *LHX4*, *NELF*, *NR0B1*, *OTX2*, *POU1F1*, *PROK2*, *PROKR2*, *PROP1*, *SEMA3A*, *SOX2*, *SOX3*, *TAC3*, *TACR3*, and *WDR11*. This study was approved by the Ethics Committees of the National Research Institute for Child Health and Development and the Tohoku University Graduate School of Medicine.

The patient harbored heterozygous *CHD7* (NM_017780.3:c.5818C > T; NP_0602250.2: p).[Arg1940Cys]) and *SOX2* (NM_003106.3:c.330C > G, NP_003097.1:p. [Tyr110*]) variants (**Fig. 1**). The *CHD7* variant was predicted to be probably damaging, with a PolyPhen-2 score of 0.999 and a CADD Phred score of 32. The frequency of the *CHD7* variant was 1/152148 alleles, whereas the nonsense *SOX2* variant was not registered in the gnomAD database. The c.5818C>T in the *CHD7* was scored as "Uncertain Significance" (PM6, PP3 and PP4) according to the American College of Medical Genetics and Genomics standards and guidelines for the interpretation of sequence variants (6). His parents did not agree to undergo genetic analysis themselves.

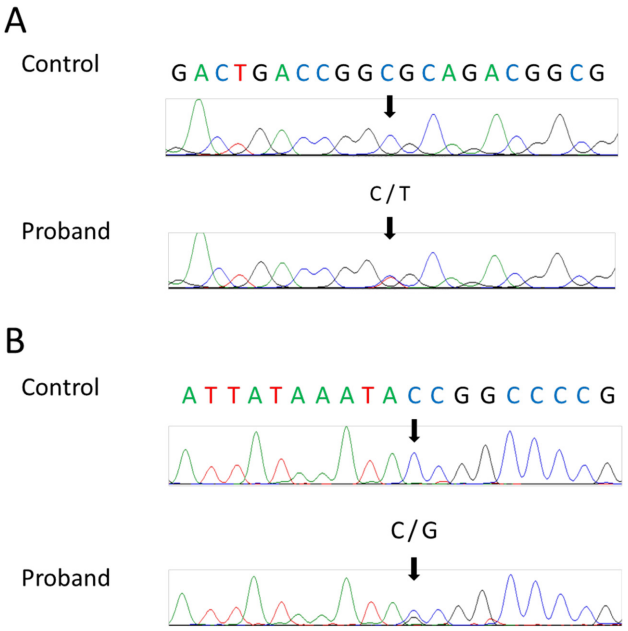

Fig. 1. Results of gene analysis of the patient. A: *CHD7* analysis in the proband. A missense variant, c.5818C > T, p.[Arg1940Cys], was identified. B: *SOX2* analysis in the proband. A nonsense variant, c.330C > G, p.[Tyr110*], was identified.

Discussion

The patient in this report was clinically diagnosed with CHARGE syndrome, but presented with a rare phenotype. Coloboma, a typical finding in patients with CHARGE syndrome, was not observed; however, bilateral optic nerve hypoplasia, a rare occurrence in patients with this syndrome, was detected. As shown in **Fig. 1**, the patient had heterozygous variants in *CHD7* (c.5818C > T: p.[Arg1940Cys]) and *SOX2* (c.330C > G: p.[Tyr110*]).

SOX2 anophthalmia syndrome is characterized by a spectrum of ocular malformations (anophthalmia, microphthalmia, coloboma, and optic nerve hypoplasia) associated with anomalies of the brain, pituitary, genitourinary, and gastrointestinal system (5). SOX2 is a transcription factor involved in early embryonic development, and plays a critical role in the development of the eye, forebrain, and hypothalamus-pituitary. Haploinsufficiency of SOX2 results in SOX2 anophthalmia syndrome (7). *SOX2* variants are associated with a broad spectrum of phenotypes; patients with nonsense/frameshift variants in *SOX2* tend to have more severe ocular phenotypes than those with missense variants (5). However, a few cases of mild ocular phenotypes, including optic nerve hypoplasia and normal ocular manifestations with *SOX2* nonsense/frameshift mutations, have also been reported (8–10).

Apart from the ocular manifestations, it was difficult to identify the variants responsible for the other signs and symptoms in the patients in this study, because of the overlapping phenotypes of CHARGE and SOX2 anophthalmia syndromes. Genital anomalies caused by *CHD7* and *SOX2* variants are believed to be caused by HH (11, 12). Some patients harboring *CHD7* or *SOX2* variants respond to GnRH stimulation. CHD7 plays a critical role in the development and maintenance of GnRH neurons (13). Moreover, HH cases caused by *CHD7* variants generally have a hypothalamic origin. Haploinsufficiency of SOX2 leads to impaired generation of GnRH neurons and complete absence of projections in the median eminence (14). Therefore, HH caused by a pathogenic *SOX2* variant is presumed to be of hypothalamic origin.

The GnRH stimulation test was performed at six months of age. Reference values for LH and FSH levels in GnRH stimulation tests in infancy have not been reported. In boys, LH and FSH elevations in mini-puberty end by 6–9 mo of age, and the basal LH level in this case was within the reference value. However, the basal FSH level was high (15). Moreover, the GnRH stimulation test showed exaggerated LH and FSH responses (normal range of prepubertal boy; basal LH 0.0–0.4, peak LH 0.4–6.0, basal FSH 0.6–3.0 and peak FSH 6.3–15.6) (16), indicating that his gonadotropin secretion was preserved and that his hypogonadism was of primary origin. However, hCG stimulation test results indicated that his testosterone secretion was normal. In the present case, the LH and FSH peaks were delayed and the responses were prolonged, suggesting that his

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hypogonadism was caused by hypothalamic dysfunction. Based on the above evidence and clinical findings, we believe that genital hypoplasia may be caused by the *CHD7* or *SOX2* variants. Accurate prediction of gonadal function after puberty may be difficult using the GnRH stimulation test in infancy, and re-evaluation is therefore necessary.

Ear anomalies, hearing loss, and facial nerve palsy are common in patients with CHARGE syndrome. External ear and semicircular canal anomalies have been observed in more than 90% of patients with *CHD7*-mutation-positive CHARGE syndrome (3). A mild cupped ear has been reported in only a few cases of anophthalmia and microphthalmia with *SOX2* mutation (5), and there have been no reports of facial nerve dysfunction in patients with SOX2 anophthalmia syndrome. The *CHD7* variant in the present case may have caused the cupped ear, semicircular canal anomaly, and facial nerve palsy. Variability in the type of hearing loss (conductive, sensorineural, or mixed) may be observed in patients with CHARGE syndrome. Anomalies of the cochlea, cochlear nerves, and middle ear structures are common in patients with CHARGE syndrome. The right stapes was removed from the patient in this report. One study involving Chd7 conditional deletion mice showed that Chd7 haploinsufficiency causes defects in the middle ear ossicles and neurogenesis in the inner ear (17). Therefore, a pathogenic *CHD7* variant may cause conductive and sensorineural hearing loss. Conversely, sensorineural hearing loss has been reported in some cases of SOX2 anophthalmia syndrome (12). In the current study, CT indicated the absence of the right stapes, but the type of hearing loss was not determined because of inadequate growth of the middle ear ossicles. Therefore, whether *CHD7* or *SOX2* variants are responsible for hearing loss currently remains unclear.

Most features in this patient were compatible with CHARGE syndrome caused by the *CHD7* variant. The *SOX2* variant may not be associated with semicircular canal and ossicle anomalies or facial nerve palsy, but can cause optic nerve hypoplasia. Moreover, the genital abnormalities and hearing loss observed in this patient may have been caused by *CHD7* or *SOX2* variants. The pathogenicity of the *CHD7* variant was scored as "Uncertain Significance," however, oligogenicity may synergize to produce a more severe phenotype (18), and the combination of the *SOX2* variant may intensify the pathogenicity of the *CHD7* variant. Additionally, CHD7 and SOX2 are thought to interact with each other. Chd7 and Sox2 are similarly expressed during development; CHD7 is an important SOX2 cofactor that cooperates with the latter to activate common target genes (19). Therefore, variants in both genes may modify and contribute to the phenotype of patients.

The CHARGE and SOX2 anophthalmia syndromes are autosomal dominant malformations. Unfortunately, informed consent for genetic analysis was not obtained from the parents in this study, and no obvious clinical manifestations were observed in the parents. Despite identical mutations, there is significant intrafamilial variability in the phenotypic spectra of CHARGE and SOX2 anophthalmia syndromes. Parental mosaicism of *CHD7* and *SOX2* variants has been previously reported (5, 20). Thus, either of the patients' parents in the current study may have had a *CHD7* or *SOX2* variant.

Conclusion

The patient presented in this study was diagnosed with CHARGE syndrome driven by *CHD7* and *SOX2* variants. Variants in both of these genes may have contributed to the patient's phenotype.

Conflict of interests: The authors have no potential conflicts of interest to disclose.

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