

## DATA REPORT

A novel frameshift mutation of *CHD7* in a Japanese patient with CHARGE syndromeTomohiro Kohmoto<sup>1,2,4</sup>, Miki Shono<sup>3,4</sup>, Takuya Naruto<sup>1</sup>, Miki Watanabe<sup>1,2</sup>, Ken-ichi Suga<sup>3</sup>, Ryuji Nakagawa<sup>3</sup>, Shoji Kagami<sup>3</sup>, Kiyoshi Masuda<sup>1</sup> and Issei Imoto<sup>1</sup>

CHARGE syndrome is a rare autosomal dominant developmental disorder involving multiple organs. *CHD7* is a major causative gene of CHARGE syndrome. We performed targeted-exome sequencing using a next-generation sequencer for molecular diagnosis of a 4-month-old male patient who was clinically suspected to have CHARGE syndrome, and report a novel monoallelic mutation in *CHD7*, NM\_017780.3(CHD7\_v001):c.2966del causing a reading frameshift [p.(Cys989Serfs\*3)].

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CHARGE syndrome (OMIM 214800) is an autosomal dominant congenital multi-system disorder occurring in an estimated 1:10,000 births worldwide and displaying broad clinical variability.<sup>1,2</sup> Hall<sup>3</sup> and Hittner *et al.*<sup>4</sup> were the first to independently describe a non-random cluster of malformations that was later referred to as CHARGE association, named by the acronym summarizing the six major clinical features: ocular coloboma, heart malformations, atresia of the choanae, retardation of growth, genital hypoplasia and ear abnormalities.<sup>5</sup> The clinical diagnosis of CHARGE syndrome with major and minor criteria was then proposed by Blake *et al.*<sup>6</sup> and updated by Verloes.<sup>7</sup> As CHARGE syndrome can closely resemble several other genetic and teratogenic conditions, genetic testing should be emphasized as a useful tool in patients with clinical suspicion.<sup>8</sup> Monoallelic mutations or deletions of *CHD7* (OMIM 608892), encoding the chromodomain helicase DNA-binding protein 7 at chromosome 8q12, are present in 65–70% of patients clinically diagnosed with CHARGE syndrome.<sup>9</sup> *CHD7* mutations in typical CHARGE syndrome patients occur *de novo* in the vast majority of the cases.<sup>8</sup> There is also evidence that CHARGE syndrome can be caused by mutations in *SEMA3E* (OMIM 608166) at chromosome 7q21, although only one case has been reported.<sup>10</sup>

The difficulty in clinical diagnosis of CHARGE syndrome lies in differentiating it from various other diseases sharing its clinical features, such as 22q deletion syndrome, Kabuki syndrome, velo-cardio-facial syndrome, cat eye syndrome, retinoic acid embryopathy, VACTERL association and *PAX2* abnormalities.<sup>8</sup> Molecular diagnosis is the most accurate and definitive method for differential diagnosis.<sup>11,12</sup> Conventional Sanger sequencing has routinely been used to identify disease-causing mutations for genetic diseases, but is laborious, expensive and time-consuming for large genes with numerous exons, such as *CHD7* (37 exons covering 8,994 bp of the coding sequence), as well as for multiple candidate genes. Recently, targeted-exome sequencing for candidate or known disease-associated genes using next-generation sequencing (NGS) has been used to diagnose patients with various types of disorders including CHARGE syndrome.<sup>13</sup>

Here, we report a novel monoallelic frameshift mutation of *CDH7*, NM\_017780.3(CHD7\_v001):c.2966delG, detected in a targeted-exome panel for the coding regions of 4,813 clinical phenotype-associated genes including CHARGE syndrome-associated genes in a Japanese patient with CHARGE syndrome.

A 4-month-old male infant was the second child born to non-consanguineous Japanese healthy parents with an unremarkable family history, after uncomplicated pregnancy and delivery at a local hospital. He was born at 38 weeks and 2 days of gestation with a birth weight of 3,086 g (−0.1 s.d.), a length of 47 cm (−1.1 s.d.) and an occipitofrontal circumference of 33.4 cm (+0.1 s.d.). Apgar scores were 9 and 9 at 1 and 5 min, respectively. At birth, he showed cleft lip and palate resulting in poor feeding, and required tube feeding as well as oxygen; he was transferred to our hospital at the age of 11 days. Results of repeatedly performed auditory brainstem response test showed bilateral profound deafness with auditory brainstem response thresholds > 95 dB. Computed tomography revealed bilateral hypoplastic semicircular canals and filled tympanic cavities. Two-dimensional color-Doppler echocardiography showed incomplete atrioventricular septal defect, patent ductus arteriosus, aortic valve stenosis and bicuspid aortic valve. As the child had ventilation dysfunction due to glossotropis and upper airway obstruction detected by multi-detector row computed tomography were observed, he required artificial ventilation with endotracheal intubation at 14 days, and was subjected to a tracheotomy owing to difficulties of extubation at 81 days. He still required nasogastric tube feeding owing to swallowing difficulty. Symptoms of heart failure due to the progression of aortic valve stenosis developed at 90 days. He showed dysmorphic features, including choanal stenosis, bilateral low-set ears, micropenis and cryptorchidism. His motor development, such as head control and rolling over, was almost normal. Karyotype analysis revealed a normal male karyotype of 46,XY. Biochemical and gas chromatography-mass spectrometry metabolic disorder tests were negative, with no evidence of metabolic disease. No clinical characteristics of CHARGE syndrome were detected in the patient's parents. Although coloboma was

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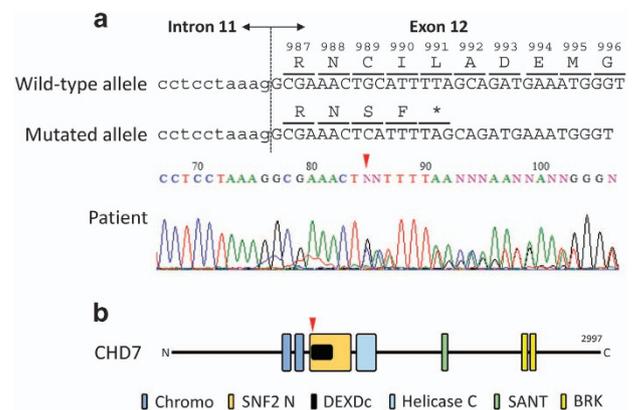
**Table 1.** Symptoms of the patient compared with major and minor diagnostic criteria in CHARGE patients<sup>8</sup>

Diagnostic criteria	Symptoms of the patient	Frequency in CHARGE patients <sup>8</sup>
<i>Major</i>		
Ocular coloboma	–	80–90%
Choanal atresia or stenosis	+	50–60%
Cranial nerve dysfunction	Unclear (VIII, IX/X)	Frequent (VIII) or 70–90% (IX/X)
Characteristic CHARGE syndrome ear	+ (Characteristic outer and inner ears and deafness)	80–100%
<i>Minor</i>		
Genital hypoplasia due to congenital hypogonadotropic hypogonadism	+ (Micropenis, cryptorchidism)	50–60% (male; micropenis, cryptorchidism)
Developmental delay	–	≤ 100%
Cardiovascular malformations	+	75–85%
Growth deficiency	–	70–80%
Orofacial cleft	+	15–20%
Tracheoesophageal fistula	–	15–20%
Distinctive facial feature	–	70–80%

not observed, the patient was suspected to have CHARGE syndrome because two of four major characteristics (choanal atresia and characteristic outer and inner ears, and deafness) and three minor characteristics (cardiovascular malformation, genital hypoplasia and cleft lip and palate) of CHARGE syndrome were observed (Table 1).<sup>8</sup>

Molecular diagnosis was performed using genomic DNA extracted from a blood sample, after informed consent was obtained from the parents. The study was approved by the ethics committees of Tokushima University. To screen known disease-associated genes including *CHD7*, we first used a MiSeq bench-top sequencer (Illumina, San Diego, CA, USA) to perform NGS with a TruSight One Sequencing Panel (Illumina), and our pipeline for NGS data analysis as described elsewhere.<sup>14,15</sup> To identify presumably pathogenic single-nucleotide variants (SNVs), we excluded sequence variants with minor allele frequency > 0.05 from the 1000 Genomes Project database (<http://www.1000genomes.org/>), NHLBI Grand Opportunity Exome Sequencing Project (ESP6500, <http://evs.gs.washington.edu/EVS/>) and the Human Genetic Variation Database (HGVD, <http://www.genome.med.kyoto-u.ac.jp/SnpDB/>). Copy number aberration (CNA) analysis was also performed as described elsewhere.<sup>16</sup> These analyses identified a monoallelic, one-base (guanine) deletion of *CHD7*, NM\_017780.3(CHD7\_v001):c.2966del (NM\_017780.3:n.3458del), which was confirmed by Sanger sequencing (Figure 1a). Primer sequences are available upon request. This mutation causes a reading frameshift starting from residue Cys989, with the new reading frame ending with a premature stop codon two codons downstream, NM\_017780.3(CHD7\_i001):p.(Cys989Serfs\*3). As shown in Figure 1, the mutation is located in exon 12, encoding the SNF2 family N-terminal (SNF2 N) and DEAD-like helicase superfamily including an ATP-binding (DEXDc) domains.<sup>9,17</sup> This mutation is not present in dbSNP138, 1000 Genomes Project, ESP6500 and HGVD. The mutation is also not present in the Human Gene Mutation Database professional 2015.2 (HGMD, <http://www.hgmd.org/>) or ClinVar (<http://www.ncbi.nlm.nih.gov/clinvar/>), suggesting it to be novel. No other potentially pathogenic SNVs and CNAs were detected in genes possibly responsible for CHARGE syndrome or other related diseases.<sup>17</sup> The mutation was not confirmed as *de novo* because of the unavailability of parental DNA.

In HGMD professional 2015.2, 615 *CHD7* mutations were reported in CHARGE syndrome. The majority of these mutations are nonsense, frameshift or splice site mutations, which are predicted to be loss-of-function mutations, likely leading to an aberrant messenger RNA targeted for degradation via nonsense-mediated decay (NMD).<sup>18</sup> Therefore, haploinsufficiency for *CHD7* is



**Figure 1.** (a) Electropherogram of *CHD7* (NM\_017780.3) exon 12 and flanking intron 11 sequences showing the monoallelic germline mutation NM\_017780.3:c.2966del (arrowhead) in the patient's DNA. The DNA and corresponding amino acid sequences of the wild-type and mutant *CHD7* alleles are also shown. (b) A schematic representation of the *CHD7* protein. Domains are depicted approximately to the scale adapted from human reference sequence (NM\_017780.3(CHD7\_i001)) and reference 17. The arrowhead shows the position corresponding to the mutation. BRK, Brahma and Kismet domain; Chromo, chromodomain; DEXDc, DEAD-like helicase superfamily including an ATP-binding domain; Helicase C, helicase superfamily C-terminal domain; SANT, switching-defective protein 3, adaptor 2, nuclear receptor corepressor, transcription factor IIIB domain; SNF2 N, SNF2 family N-terminal domain.

the most likely pathogenic mechanism underlying CHARGE syndrome.<sup>18</sup> Even if the mutated transcript escapes NMD, the truncated *CHD7* protein predicted in this patient contains only the N-terminal tandem chromodomains, which are incapable of performing the protein's functions owing to the loss of chromatin remodeling activity (Figure 1b).

NGS emergence for the use in diagnostic laboratories will facilitate the characterization of new sequence variants in patients with genetic disorders. In a diagnostic setting for CHARGE syndrome, *CHD7* mutation analysis using Sanger sequencing may miss genetic alterations causing CHARGE syndrome or phenocopies of CHARGE syndrome, such as gross deletions or deep intronic mutations of *CHD7*, alterations in *SEMA3E*, unique chromosomal imbalances and 22q11.2 deletion syndrome.<sup>17</sup> Given that targeted-exome or whole-exome sequencing can detect both exonic SNVs and CNAs in disease-causing genes,<sup>15,16</sup> those platforms can facilitate differential diagnosis, appropriate therapy and genetic counseling for patients with this

syndrome and related diseases in a cost-effective manner. Coverage of all genetic alterations causing CHARGE and CHARGE-like diseases will be extended with whole-genome sequencing when the cost of sequencing is lowered.

## HGV DATABASE

The relevant data from this Data Report are hosted at the Human Genome Variation Database at <http://dx.doi.org/10.6084/m9.figshare.hgv.777>.

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## COMPETING INTERESTS

The authors declare no conflict of interest.

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