Non-Homologous End Joining Repair Mechanism-Mediated Deletion of *CHD7* Gene in a Patient with Typical CHARGE Syndrome

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CHARGE syndrome MIM #214800 is an autosomal dominant syndrome involving multiple congenital malformations. Clinical symptoms include coloboma, heart defects, choanal atresia, retardation of growth or development, genital hypoplasia, and ear anomalies or deafness. Mutations in the chromodomain helicase DNA binding protein 7 (*CHD7*) gene have been found in 65-70% of CHARGE syndrome patients. Here, we describe a 16-month-old boy with typical CHARGE syndrome, who was referred for *CHD7* gene analysis. Sequence analysis and multiplex ligation-dependent probe amplification were performed. A heterozygous 38,304-bp deletion encompassing exon 3 with a 4-bp insertion was identified. There were no *Alu* sequences adjacent to the breakpoints, and no sequence microhomology was observed at the junction. Therefore, this large deletion may have been mediated by non-homologous end joining. The mechanism of the deletion in the current case differs from the previously suggested mechanisms underlying large deletions or complex genomic rearrangements in the *CHD7* gene, and this is the first report of *CHD7* deletion by this mechanism worldwide.

Key Words: CHARGE syndrome, *CHD7*, Large deletion, Non-homologous end joining

INTRODUCTION

CHARGE syndrome MIM #214800 is an autosomal dominant genetic disorder with multiple congenital anomalies. The syndrome derives its name from the first letters of its main clinical manifestations: Coloboma, Heart defects, Atresia of choanae, Retardation, Genitourinary malformation, and Ear abnormalities. The clinical criteria for CHARGE syndrome were first described by Blake *et al*. [1], and then modified by Verloes [2]. According to Verloes' criteria, CHARGE syndrome can be classified as typical, partial, or atypical.

The chromodomain helicase DNA binding protein 7 (*CHD7*) gene located on chromosome 8q12.1 is 188 kb in length and consists of 38 exons. The *CHD7* protein functions as a regulator of DNA transcription [3]. *CHD7* gene mutations have been identified in 65-70% of patients with CHARGE syndrome [4-6]. As most of cases of CHARGE syndrome are caused by a *de novo* mutation [7], familial cases are rarely reported.

CHD7 gene mutations have been identified throughout the coding exons, and most of them are point mutations. Large deletions and duplications account for only 2% of the observed mutations, while translocations account for <1%. As they are rare, large *CHD7* gene deletions and duplications have not been reported previously in the Korean population. Here, we report a typical CHARGE syndrome patient with a large deletion in the *CHD7* gene and a presumptive relevant mechanism.

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CASE REPORT

A 16-month-old boy was referred for genetic workup because of typical manifestations of the CHARGE syndrome. Facial asymmetry was observed at birth, and auditory and visual dysfunctions were also noted. Heart defects, including aortic stenosis, persistent ductus arteriosus, atrial septal defect, and pulmonary stenosis, were identified on cardiological examination. Multiple abnormal findings, including incomplete cochlear turn and dysplasia of the vestibule and semicircular canal, were found on a computerized tomography scan. Two major signs (coloboma and hypoplastic semicircular canals) and four minor signs (rhombencephalic dysfunction, abnormal middle or external ear, malformation of mediastinal organs, and mental retardation) were identified according to Verloes' criteria. The patient was diagnosed as having typical CHARGE syndrome. A *CHD7* gene analysis was requested for a confirmative diagnosis.

Informed consent was obtained from his legal representative, and genomic DNA was extracted from whole blood. PCR was performed by using primers specific for the 37 coding exons of the *CHD7* gene. The sequencing reaction was performed with an ABI 3730 analyzer (Applied Biosystems, Foster City, CA, USA) by using a BigDye Terminator v3.1 Cycle sequencing kit (Applied Biosystems). Sequencher 5.0 software (Gene Codes Corporation, Ann Arbor, MI, USA) was used for the sequencing data analysis. No mutations were identified from this *CHD7* gene sequence analysis.

Gene dosage analysis was performed by using a multiplex ligation-dependent probe amplification (MLPA) kit (SALSA MLPA P201-C1 CHARGE probemix; MRC-Holland, Amsterdam, Netherlands). MLPA analysis of the *CHD7* gene revealed a heterozygous exon 3 deletion (Fig. 1). Genetic analyses of the patient's parents were not available. Further experiments were then per-

formed in order to identify the precise breakpoints of this large deletion. A total of nine *Alu* sequences were located in intron 2 and three were located in intron 3 (Fig. 2C). Long range PCR was performed by using five forward primers adjacent to *AluSx3*, *AluSg*, *AluSx1* (the more distal of the two), *AluY*, and *AluSx*, and one common reverse primer adjacent to *AluSq2*. The proximal breakpoint was localized between *AluSg* and *AluSx1* (the more distal of the two). Another long range PCR was performed by using one common forward primer adjacent to *AluSg*, and two reverse primers adjacent to *AluJr* and *AluSq2*. The distal breakpoint was localized between *AluJr* and *AluSq2*. An additional long range PCR was performed by using forward primers located 1 kb, 2 kb, and 3 kb distal to *AluSg* and reverse primers located 1 kb, 2 kb, and 3 kb proximal to *AluSq2*. The proximal breakpoint was located between 3 kb distal to *AluSg* and *AluSx1* (more distal of the two), while the distal breakpoint was located between 3 kb and 2 kb proximal to *AluSq2*. Finally, a 1kb-sized PCR fragment was obtained by using the following primers: GGTGGGCTGTGAAGTGTTCTGGC (forward primer; located in intron 2) and ACCCACAGTGCACTCCTCCCC (reverse primer; located in intron 3) (Fig. 2A). Sequence analysis revealed the exact breakpoints (Fig. 2B). The deleted region totaled 38,304 bp from c.1665+10039 in intron 2 to c.2097-3547 in intron 3, and it was accompanied by a TAAC insertion (Fig. 2D).

DISCUSSION

The majority of *CHD7* gene mutations are point mutations—nonsense mutations, 44%; frameshift deletions or insertions, 34%; splice site mutations, 11%; and missense mutations, 8% [8]. Large deletions or duplications account for only 2% of total cases. To date, 15 cases of large deletions and 5 cases of large duplications in the *CHD7* gene region have been reported (Table
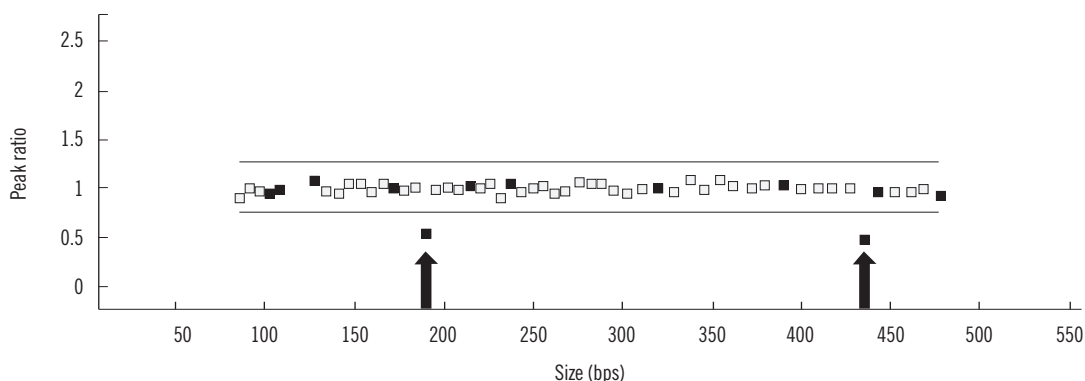
Fig. 1. Multiplex ligation-dependent probe amplification analysis of the *CHD7* gene. Arrows indicate the reduced ratio of exon 3.

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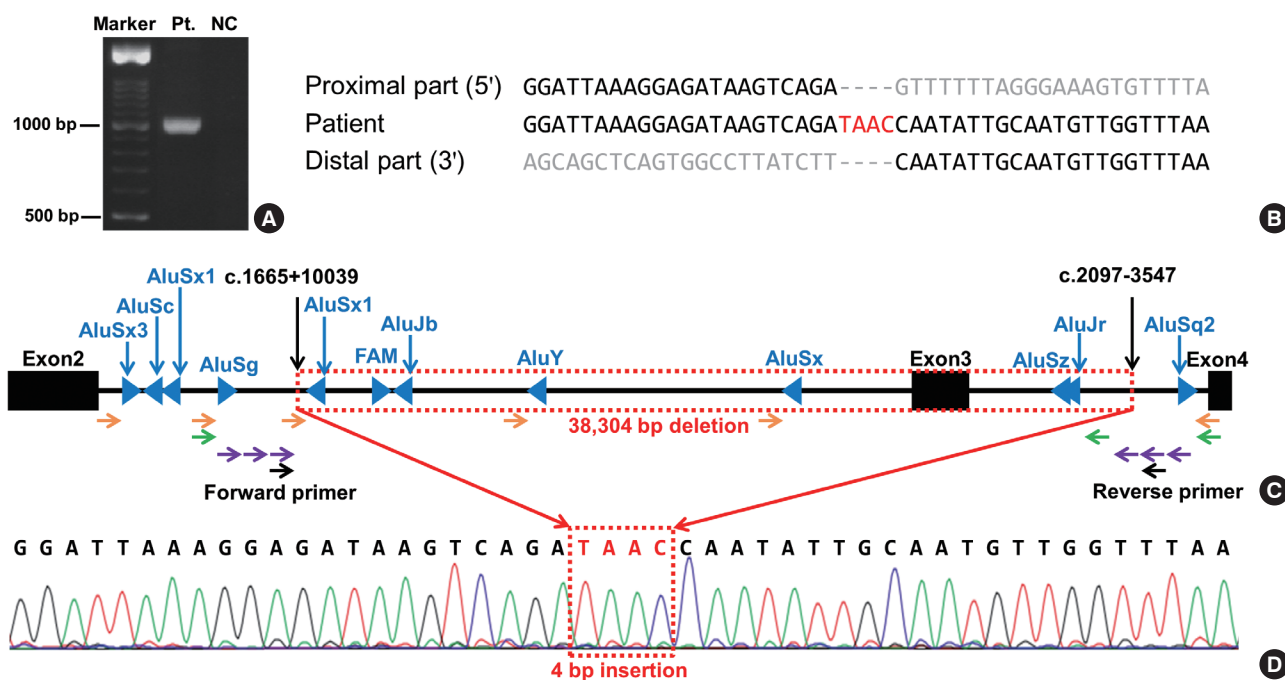
Fig. 2. Results of the sequence analysis and genomic structure encompassing exons 2-4 of the *CHD7* gene. (A) Electrophoresis of a PCR product using the forward and reverse primers designated in (C) showed an apparent band in the patient. (B) The proximal and distal parts had no homologous sequences near the breakpoints. (C) Schematic diagram showing the range of the 38,304-bp deletion from c.1665+10039 to c.2097-3547. *Alu* sequences (blue triangles) were not adjacent to the breakpoints. Orange, green, and violet arrows indicate stepwise long-range PCR primers. (D) Sequence analysis revealed a 4-bp insertion (TAAC) between the breakpoints.
Abbreviations: Pt., patient; NC, normal control.

1). Of these 20 cases, 10 cases involved whole gene deletions and duplications. To date, 16 index cases of *CHD7* gene mutations have been reported in Korea [9-12]. However, large deletions and duplications have not yet been reported in the Korean population.

Copy number variations including both additions and deletions are mediated by homologous recombination and non-homologous repair mechanisms [13]. Homologous recombination is composed of non-allelic homologous recombination and single-strand annealing. Non-homologous repair mechanisms are classified into non-replicative non-homologous repair (non-homologous end joining [NHEJ] and microhomology-mediated end joining), and replicative non-homologous repair (replication slippage or template switching, fork stalling and template switching [FoSTeS], and microhomology-mediated break-induced replication [MMBIR]).

Two different mechanisms underlying *CHD7* gene deletions have been previously reported. In the first case, a large deletion spanning exons 8-12 was detected in a Japanese girl [14]. The deletion encompassed 10,417 bases from intron 7 to intron 12. A polyadenine tract of approximately 100 bases was inserted into the junction. Therefore, this deletion was attributed to an *Alu* retrotransposition-mediated mechanism. In the second case, a complex genomic rearrangement was detected in a Caucasian girl [15]. A deletion of approximately 4,484 bases, including exon 7, was accompanied by an insertion of 347 bases from intron 4 between the breakpoints. This deletion was attributed to a FoSTeS or MMBIR mechanism.

The *CHD7* gene mutations in the current study were mediated by a distinctly different mechanism from the aforementioned cases. *Alu* sequences were not adjacent to the breakpoints of the deleted region in the current case. The nearest *Alu* sequences from the proximal and distal breakpoints were located 469 bp and 2,400 bp away, respectively. There was a 4-bp, non-template insertion at the junction site, and there was no sequence microhomology between the upstream and downstream sequences near the junction. Therefore, NHEJ is the most probable mechanism responsible for the current case. NHEJ is a part of the double-strand break repair pathway and is the predominant repair mechanism used in mammals [16]. Furthermore, it is not uncommonly related to large deletions [17]. One or more nucleotide bases can be inserted into the junctions during the repair procedure in NHEJ [18, 19]. In summary, we report the first case of a *CHD7* gene deletion mediated by NHEJ worldwide.

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Table 1. Previously reported cases of gross deletion or duplication of the *CHD7* gene

Classification	Range	Phenotype	Reference (PMID)
Deletion	exon 1	CHARGE syndrome	18445044
Deletion	exon 2	CHARGE syndrome	21158681
Deletion	exons 2-38	CHARGE syndrome	21158681
Deletion	exon 3 (38,304-bp deletion with TAAC insertion)	CHARGE syndrome	current case
Deletion	exon 4	CHARGE syndrome	18445044
Deletion	exons 8-12	CHARGE syndrome	16615981
Deletion	exons 8-12 (10,417-bp deletion with approximately 100-bp insertion)	CHARGE syndrome	[14]
Deletion	exons 13-38	CHARGE syndrome	18472328
Deletion	exon 29	CHARGE syndrome	22033296
Deletion	entire *CHD7* gene	Temporal bone malformation	19248844
Deletion	entire *CHD7* gene	CHARGE syndrome	22033296
Deletion	entire *CHD7* gene	CHARGE syndrome	22033296
Deletion	entire *CHD7* gene	CHARGE syndrome	23024289
Deletion	entire *CHD7* gene (6.04 Mb)	psychomotor retardation, hypertrichosis, facial asymmetry, synophria, failure to thrive, developmental delay, gastro-esophageal reflux	23142376
Deletion	entire *CHD7* gene (635 kb)	CHARGE syndrome	18445044
Deletion	0.1 Mb on 8q12.2 encompassing a large fraction of the *CHD7* gene	CHARGE syndrome	23747993
Duplication	exon 3	CHARGE syndrome	21158681
Duplication	entire *CHD7* gene (2.7 Mb)	Congenital heart defect, ear anomalies, and torticolis but without Duane anomaly	22902603
Duplication	entire *CHD7* gene (2.99 Mb)	Duane syndrome, mental retardation, dysmorphic face, hypotonia, deafness	18413373
Duplication	entire *CHD7* gene (3.85 Mb)	Duane syndrome, developmental delay, asymmetric and dysmorphic face, plagiocephaly, hypoplasia of the kidney	22258531
Duplication	entire *CHD7* gene (6.9 Mb)	Duane syndrome, microbrachycephaly, deafness, developmental delay, heart defect	19772954
Complex rearrangement	4,484-bp deletion including exon 7 with 347-bp insertion from intron 4	CHARGE syndrome	[15]

Abbreviations: PMID, PubMed identifier; Mb, megabase.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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