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CASE REPORT

Contiguous gene deletion neighboring *TWIST1* identified in a patient with Saethre-Chotzen syndrome associated with neurodevelopmental delay: Possible contribution of *HDAC9*

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ABSTRACT **Saethre-Chotzen syndrome (SCS) is an autosomal dominant craniosynostotic disorder characterized by coronal synostosis, facial asymmetry, ptosis, and limb abnormalities.**

Haploinsufficiency of *TWIST1*, a basic helix–loop–helix transcription factor is responsible for SCS. Here, we report a 15-month-old male patient with typical clinical features of SCS in addition to developmental delay, which is a rare complication in SCS. He showed a *de novo* 0.9-Mb microdeletion in 7p21, in which *TWIST1*, *NPMIP13*, *FERD3L*, *TWISTNB*, and *HDAC9* were included. In comparison with previously reported patients, *HDAC9* was suggested to contribute to developmental delay in SCS patients with 7p21 mirodeletions.

Key Words: 7p21 deletion, Craniosynostosis, *HDAC9*, Saethre–Chotzen syndrome, *TWIST1*

INTRODUCTION

Craniosynostosis, which involves the premature fusion of multiple cranial sutures, occurs in 1 out of every 2000–2500 births. At least 20% of cases are caused by genetic mutations, comprising 86% single gene mutations and 14% chromosome abnormalities (Wilkie et al. 2010). Saethre-Chotzen syndrome (SCS), also known as acrocephalosyndactyly III (ACS III) (MIM 101400), is an autosomal dominant craniosynostotic disorder characterized by uni- or bilateral coronal synostosis, facial asymmetry, ptosis, hypertelorism, small and dysmorphic ears, limb abnormalities, brachydactyly, and partial syndactyly. The prevalence of SCS is 1 in 25 000–50 000 people. The mutation rate of the twist homolog 1 (*TWIST1*: MIM 601622) was 3.6% for craniosynostosis cases in a recent review (Twigg and Wilkie. 2015). Generally, SCS patients do not show developmental delay. However, most of the previously reported SCS patients due to contiguous gene deletions including *TWIST1* showed developmental delay (Howard et al. 1997; Johnson et al. 1998; Busche et al. 2011). This suggests that some of the genes neighboring *TWIST1* would be related to neurodevelopment. Here, we report an additional case of a SCS patient with developmental delay due to a microdeletion in 7p21 and discuss which genes are responsible.

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CLINICAL REPORT

The patient was a 15-month-old male who was born at 38 weeks of gestation with a birth weight of 3220 g (+0.6 SD) and an occipitofrontal circumference (OFC) of 33.5 cm (+0.2 SD). His parents and older brothers had no notable medical conditions. At one month of age, cranial computed tomography (CT) scans revealed unilateral craniosynostosis (Fig. 1). The patient was able to lift his head at the age of 4 months, sit up independently at 8 months, and walk with support at 9 months. At 15 months, he could not yet walk independently or speak any meaningful words, indicating mild developmental delay. He had a unilateral coronal suture, plagiocephaly, and a wide anterior fontanelle. Characteristically, he exhibited brachycephaly, facial asymmetry, low-set frontal hairline, ptosis, hypertelorism, posteriorly rotated ears, mild syndactyly, and cleft palate. Surgeries to repair the cleft palate and ptosis were performed at 1 year of age. The methods for the genetic analysis are described in the Supporting Information text and Supporting Information Figure S1. Sanger sequencing of *TWIST1* revealed no nucleotide alterations, small deletions, or insertions. The quantitative real-time PCR (qPCR) analysis of exon 1 of *TWIST1* identified a heterozygous deletion. Further analysis indicated that six genes were deleted, including *NPM1P13*, *TWIST1*, *FERD3L*, *TWISTNB*, and *TMEM196* and the C-terminus of histone deacetylase 9 (*HDAC9*) (MIM 606543). *TMEM196* is a non-protein coding gene. (Supporting Information Fig. S2). *TWIST1* and *HDAC9* are OMIM disease genes. To precisely determine the breakpoint, we obtained a 1.3-kb PCR product from the patient's DNA but not the control or parental DNA (Fig. 3A). We sequenced the PCR product with primers flanking the deletion interval. The 5′ end of the breakpoint was identified in intron 21 of *HDAC9* (NM_178425.3), and the 3′ end was identified in non-coding region between *TMEM196* and *RPL21P75*-using primers flanking the deleted region (Fig. 2). The deletion spanned approximately 0.9 Mb (chromosome 7, NC_000007.13: 18 925 715–19 815 446) based on the assembly of the UCSC Genome Browser GRCh37/hg19 (Fig. 2). The deletion in the chromosomal region was confirmed by fluorescence *in situ* hybridization (FISH) analysis (Fig. 3B). There was no deletion in both parents, indicating a *de novo* deletion in the patient.

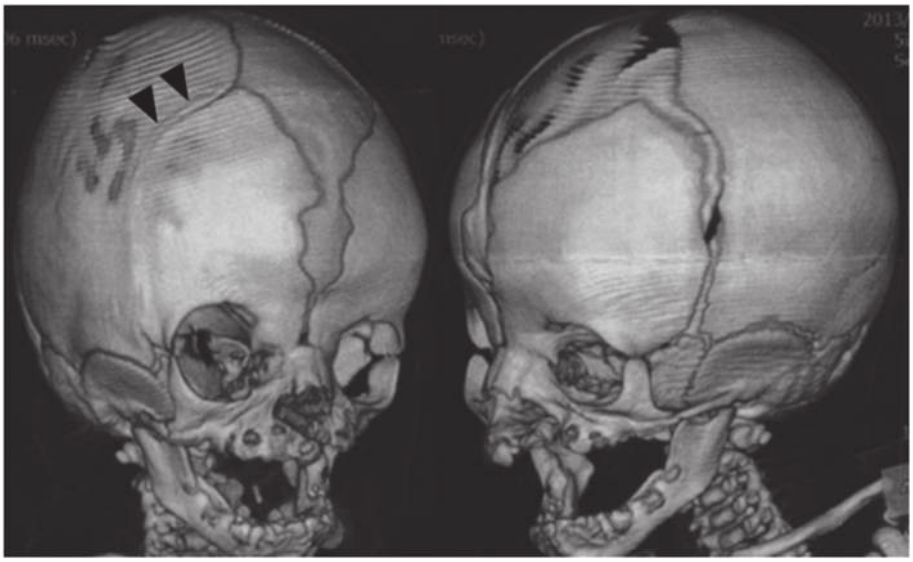

Fig. 1 Three dimensional computed tomography (CT) image of patient at one month of age. Craniosynostosis of the right coronal suture is shown (arrowheads).

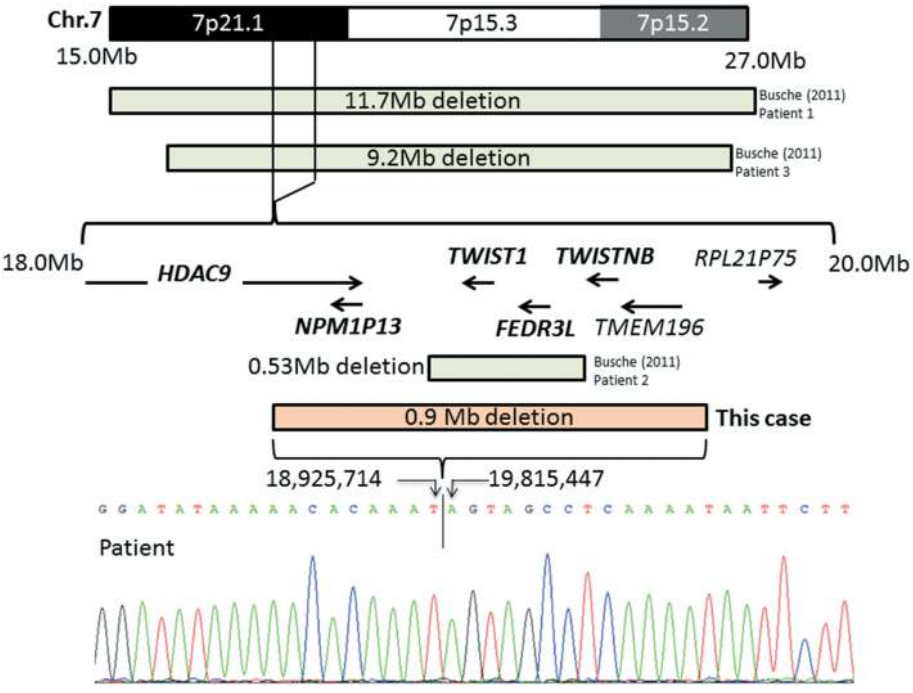

Fig. 2 A schematic representation of the 7p21 microdeletions in the patients in this study and the previous studies. The deleted region in the present patient is 0.9 Mb in size and includes six genes; *TWIST1*, *NPM1P13, FERD3L, TWISTNB, HDAC9* and *TMEM196*. *TMEM196* is a non-protein coding gene. The sequence chromatogram including the genomic breakpoints in the present patient is shown.

DISCUSSION

TWIST1 haploinsufficiency usually leads to craniofacial abnormalities but no neurodevelopmental delay (Paznekas et al. 1998; Kress et al. 2006). We describe a 15-month-old male patient presenting with SCS features, including unicoronal synostosis, facial asymmetry, ptosis, mild syndactyly, associated with developmental delay. According to the genetic screening flowchart for SCS (Supporting Information Fig. S1), we identified a heterozygous deletion of *TWIST1*. To confirm the size of the deletion, we precisely determined the breakpoint positions using primers flanking the deleted region (Fig. 2). We found no low-copy repeats in the vicinity of either breakpoint, excluding the possibility of complex chromosomal rearrangements. The patient's clinical symptoms were attributed to a *de novo* 0.9-Mb deletion of 7p21, which encompassed a complete deletion of the protein-coding region of genes *TWIST1*, *NPM1P13, FERD3L, TWISTNB,* and the C-terminal region of *HDAC9*. Patients with large 7p21 deletions that include *TWIST1* or 7p21-related chromosomal rearrangements exhibit severe intellectual disabilities (Busche et al. 2011; Shimada et al. 2013). Most SCS patients with intragenic mutations of *TWIST1* do not show severe developmental delay (Paznekas et al. 1998; Kress et al. 2006). In comparison with SCS patients due to *TWIST1* nucleotide changes, patients with contiguous gene deletions in 7p21 often show severe intellectual disability. Busche et al. reported three cases with 526-kb, 9.2-Mb, and 11.7-Mb deletions of 7p21 that included *TWIST1*. Two patients with large deletions showed severe intellectual disabilities, whereas a patient with a smaller deletion encompassing

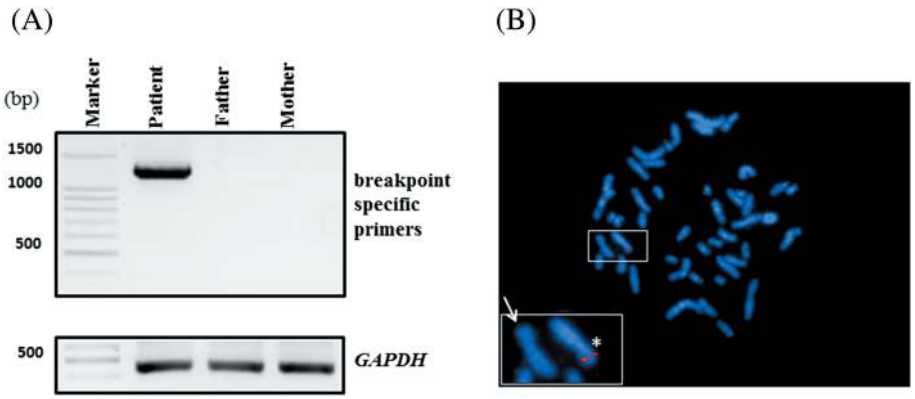

Fig. 3 The results of analyses. (A) The deletion-specific polymerase chain reaction (PCR) amplification. A 1248-bp PCR product is successfully amplified only in the present patient, but not in either parent. *GAPDH* is used as a reference gene. (B) FISH analysis for the patient's metaphase specimen using a RP11-753O17 probe for the *TWIST1* region (red). The asterisk (*) indicates a normal signal and an arrow indicates the deletion of this region.

only *TWIST1* and *FERD3L* showed no developmental delay (Busche et al. 2011). Clinical features of these patients are summarized and compared in Supporting Information Table S1. The common deleted genes are *TWIST1* and *FERD3L*. *FERD3L* is a conserved basic helix–loop–helix transcription factor expressed in the developing central nervous system of the mouse and fly (Verzi et al. 2002); however, the influence of *FERD3L* in the human brain is unclear. *HDAC9* is expressed in the brain to regulate neocortical neuronal development (Sugo et al. 2010) and deletion or single-nucleotide variations of *HDAC9* were identified in autism spectrum disorder, developmental delay, and schizophrenia (Pinto et al. 2014). Among the genes commonly deleted in SCS patients with developmental delay, *HDAC9* is related to neuronal development and there is a report which suggested the relation between *HDAC9* alteration and neurological impairments. Therefore, *HDAC9* haploinsufficiency is likely to contribute to neurodevelopmental delay in SCS patients due to microdeletions.

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DISCLOSURE

None.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

Figure S1 A used genetic screening flowchart in this study.
Figure S2 The estimated results of relative copy number of the genes neighboring *TWIST1*.
Table S1 Comparison of clinical features and deletion sizes between our case and a previous report (Bushe, 2011).
Table S2 PCR primers and PCR conditions.