CLINICAL REPORT

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A Unique *TBX5* Microdeletion with Microinsertion Detected in Patient with Holt–Oram Syndrome

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Holt–Oram syndrome (HOS) is an autosomal dominant condition characterized by upper limb and congenital heart defects and caused by numerous germline mutations of *TBX5* producing preterminal stop codons. Here, we report on a novel and unusual heterozygous *TBX5* microdeletion with microinsertion (microindel) mutation (c.627delinsGTGACTCAGGAAACGCTTTCCTGA), which is predicted to synthesize a truncated *TBX5* protein, detected in a sporadic patient with clinical features of HOS prenatally diagnosed by ultrasonography. This uncommon and relatively large inserted sequence contains sequences derived from nearby but not adjacent templates on both sense and antisense strands, suggesting two possible models, which require no repeat sequences, causing this complex microindel through the bypass of large DNA adducts via an error-prone DNA polymerase-mediated translesion synthesis.

Key words: microindel; *TBX5*; Holt–Oram syndrome; error-prone DNA polymerase

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INTRODUCTION

Holt–Oram syndrome (HOS; MIM# 142900) is a rare autosomal dominant disorder characterized by bilateral upper limb defects involving mainly the pre-axial radial ray and variable congenital heart defects (CHD), most commonly ostium secundum atrial septal defect (ASD) and ventricular septal defect (VSD) [Holt and Oram, 1960; Basson et al., 1994; Huang, 2002]. Although HOS is a highly penetrant disorder, inter- and intra-familial variability is frequently described [Basson et al., 1994; Newbury-Ecob et al., 1996; Brassington et al., 2003]. Mutations in the *TBX5* gene (MIM# *601620) encoding a member of the T-box family of transcription factors cause HOS [Basson et al., 1997; Li et al., 1997]. More than 70% of individuals who meet strict diagnostic criteria for HOS had *TBX5* mutations spread throughout coding exons [Heinritz et al., 2005; McDermott et al., 2005]. Most *TBX5* mutations cause premature truncation of the primary transcript, leading to haploinsufficiency.

Among the various types of germline and somatic mutations causing human genetic diseases, deletions with insertions (indels) are a special and uncommon mutation class, defined as a colocalized insertion and deletion of nucleotides resulting in a net change in the total number of nucleotides, where the two changes are near each other on the DNA [Scaringe et al., 2008]. A microindel (microdeletion with microinsertion) is defined as an indel that results in a net change of one to 50 nucleotides [Gonzalez et al., 2007]. Although a deletion followed by an insertion, or vice-versa, has been suggested to occur by simple combinations of the same mechanisms that cause pure microinsertions and pure microdeletions in microindelogenesis [Chuzhanova et al., 2003],

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Scaringe et al. [2008] proposed that microindels are not caused predominantly by combinations of the mutational mechanisms of insertion and deletion but occur during error-prone repair of DNA adducts by translesional polymerase in a one-step manner.

Here, we describe a prenatally diagnosed girl with sporadic HOS in whom we found a novel and unusual microindel of the *TBX5* gene having a 1-nucleotide deletion and a 24-nucleotide insertion with a net gain of 23 nucleotides, resulting in the production of a truncated protein. The inserted sequence with its nearby sequence and a deletion observed in the present case supports the hypothesis that microindels are caused via the activity of a highly error-prone translesional polymerase to bypass a bulky DNA adduct formed by various causes, e.g., genotoxic chemicals and oxidative stress, in the germline, and seems to be consistent with two models, the "Tarzan model" [Scaringe et al., 2008] and the "template switching model" [Sale, 2012].

CLINICAL REPORT

A 29-year-old Japanese woman, gravida 2, para 1, with an uneventful spontaneous vaginal delivery two years previously was referred to our tertiary medical centre at 30 + 3 weeks of gestation for further evaluation of fetal bilateral upper limb abnormalities and CHD. She had no relevant past medical history and there was no history of consanguinity, structural anomalies, or genetic disorders on either side of the family. Prenatal three-dimensional (3-D) ultrasonography (Voluson E8 Expert; GE Healthcare, Waukesha, WI) showed absence of thumb in the right hand and absence of thumb and index finger in the left hand with bilateral radial deviation of the wrist (Fig. 1A and B). Echocardiography revealed multiple muscular and perimembranous ASD and VSD and a persistent left superior vena cava with sinus bradycardia (Fig. 1C and D). No sign of cardiac failure or hydropic findings were present. Biparietal diameter, abdominal circumference and length of the femur, tibiae, fibulae, and humerus were normal for the gestational age, and all the other organs appeared normal. Normal fetal growth, amniotic fluid volume and blood flow velocity waveforms in the umbilical and middle cerebral arteries were recorded. Because the parents opted for fetal karyotyping after counselling, an amniocentesis was performed at 33 + 2 weeks of gestation and the karyotype analysis revealed a normal female karyotype of 46,XX in all examined metaphases. According to the clinical findings, a prenatal diagnosis of HOS was suspected.

At 41 + 1 weeks of gestation, a 2,922 g female with Apgar scores of eight and nine at 1 and 5 min, respectively, was delivered vaginally. On examination, the right upper limb had a radially bowed forearm, radial deviation of the wrist associated with flexion deformity at the elbow and wrist joint caused by absence of the radius and absence of thumb (Fig. 1E). The left upper limb had a shortened middle segment, stiff fingers, and absence of thumb and index finger (Fig. 1F). Radiological examination showed bilateral radial dysplasia with complete absence of the right radius and thumb, the absence of the left radius, thumb and index finger, and hypoplasia of the left humerus (Fig. 1G). Echocardiography confirmed muscular and perimembranous VSD, large secundum ASD, and persistent left superior vena cava draining into a dilated coronary sinus with sinus bradycardia. In addition, there was a right folded ear and abdominal ultrasonography revealed a large bladder. Brain ultrasonography and radiography of the spine were normal. On the basis of the established criteria for clinical diagnosis, the patient was diagnosed with HOS [McDermott et al., 2005].

MATERIALS AND METHODS

This study was approved by the ethical committees of Shikoku Medical Center for Children and Adults and Tokushima University. Molecular diagnosis was performed using genomic DNA extracted from the patient's whole blood after informed consent was obtained from her parents. Each coding exon of *TBX5* including exon–intron boundaries was amplified by polymerase chain reaction (PCR) using PrimeSTAR® GXL DNA Polymerase (TAKARA Bio, Shiga, Japan) and specific primer sets [Basson et al., 1997; Gruenauer-Kloevekorn and Froster, 2003], and bidirectional sequencing of PCR products was performed using the BigDye® Terminator v. 3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA) in an ABI 3130 Genetic Analyzer Sequencer (Applied Biosystems).

RESULTS

Genetic Analysis

Molecular analysis of the proband identified a novel heterozygous microindel mutation in exon 6 of the *TBX5* gene, NM_000192.3(TBX5_v001):c.627delinsGTGACTCAGGAAACGCTTTCCTGA (Fig. 2AA), which introduces a stop codon at position 210 (NM_000192.3(TBX5_i001):p. (Ala210*)) and creates a truncated *TBX5* protein of 209 amino acids lacking part of the T-box domain sequences involved in DNA binding [Bruneau et al., 2001]. Given that most previously reported *TBX5* mutations cause premature truncation of the primary *TBX5* transcript, resulting in haploinsufficiency, this microindel is a likely pathogenic mutation for HOS in this patient. Indeed, the mutation is not present in the NHLBI GO Exome Sequencing Project (ESP6500) Variant Server (http://evs.gs.washington.edu/EVS/), the 1,000 Genomes Project database (http://www.1000genomes.org/) or the Human Genetic Variation Database (HGVD, http://www.genome.med.kyoto-u.ac.jp/SnpDB/), although it has never been reported in the Human Gene Mutation Database professional 2015.2 (HGMD, http://www.hgmd.org/) or ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/). Because parental DNA was not available, the mutation was not confirmed as de novo.

DISCUSSION

Both germline and somatic indels are uncommon and tend to shorten the nucleotide sequence, with the majority resulting in a net deletion that also shifts the reading frame [Chuzhanova et al., 2003; Scaringe et al., 2008; Stenson et al., 2014]. In mutation databases including HGMD and ClinVar, only one case of a *TBX5* microindel, which had a one-nucleotide deletion and a two-nucleotide insertion, was reported [Basson et al., 1999]. In addition, only one case with an insertion of more than three bases, which contained a 6-base duplication, has been reported in *TBX5* [Debeer et al., 2007]. Thus, the one-base deletion and 24-base insertion with a net 23-base gain detected in our HOS case is the first microindel with an unusually long insertion in the *TBX5* gene.

The 24-base inserted sequence contains 8-base sense and antisense (reverse complementary) sequences, which are likely to derive from the same nearby but not adjacent template, with three nucleotides of separation and an additional five nucleotides (Fig. 2A). This finding is mostly consistent with the characteristics of microindels reported by Scaringe et al. [2008] from their analysis of somatic microindels. In microindels, the inserted sequences derive from nearby but not adjacent template sequences on the sense or antisense strand, in contrast to the slippage that characterises the great majority of pure microinsertions. It was also shown that the mechanisms of microindels, at least those with larger insertions, are highly error-prone overall, with an estimated error rate of 13% per base pair, consistent with the error rates of certain Y-family translesion polymerases [Scaringe et al., 2008]. Thus, the mutation detected in our case seems to be a microindel with a relatively large and complex insertion that arises from the error-

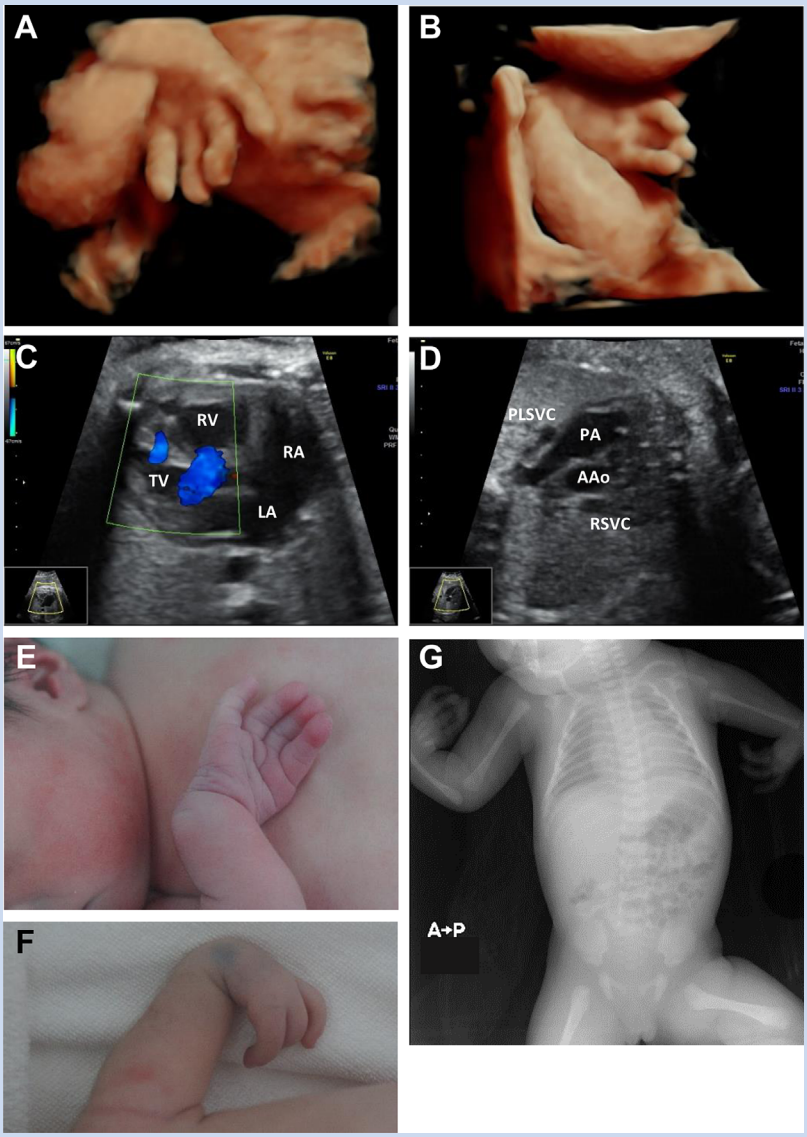

FIG. 1. Clinical photographs of an HOS patient. (A and B) 3-D ultrasonographic images of the fetal upper limbs. The right hand (A) showed absence of thumb, with radial deviation of the wrist, whereas the left hand (B) showed stiff fingers, and absence of thumb and index finger with radial deviation of the wrist. (C and D) Echocardiographic images of the fetus. A four-chamber view (C) showed large perimembranous and muscular VSD, and a three-vessel view (D) showed supernumerary fourth vessels (persistent left superior vena cava, PLSVC) to the left of the pulmonary artery (PA), LV, left ventricle; LA, left atrium; RV, right ventricle; RA, right atrium; AA, ascending aorta; RSVC, right superior vena cava. (E and F) Postnatal appearance of bilateral upper limbs. The right upper limb (E) had a radially bowed forearm, radial deviation of the wrist associated with flexion deformity at the elbow and wrist joint and absence of thumb, whereas the left upper limb (F) had a shortened middle segment, stiff fingers and absence of thumb, and index finger. (G) X-rays of the infant showing the absence of the radius and thumb in the right upper limb and absence of radius, thumb and index finger with hypoplastic humerus in the left upper limb. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/ajmga].

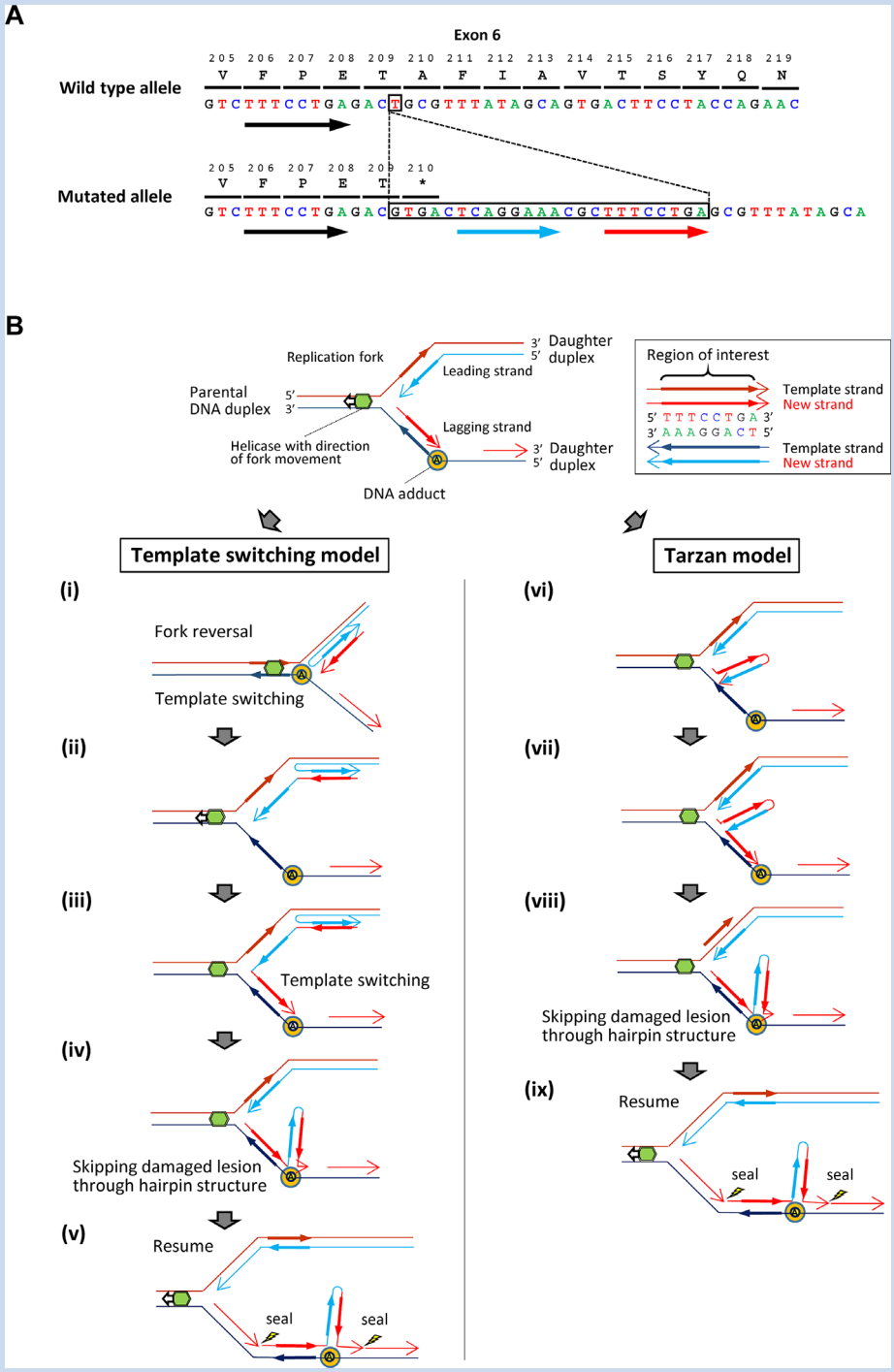

FIG. 2. Characterization of the *TBX5* microindel detected in an HOS patient. A: Schematic representation of wild-type (top) and mutated (bottom) sequences observed in *TBX5* exon 6 in the HOS patient. Boxes indicate a 1-nucleotide deletion (top) and a 24-nucleotide insertion (bottom). Red and blue arrows indicate a match and reverse complement to the putative template (black arrows), respectively, observed in the inserted sequence. B: Schematic representation of two alternative models causing the *TBX5* microindel in this case. Replication by normal DNA polymerase is blocked by a DNA adduct (orange circle) located around the deletion on the template DNA (upper). For lagging strand synthesis to progress in the presence of a DNA adduct, a sequential nucleotide synthesis from the template strand (ii and iii) occurs after the association of the two nascent DNA strands (i; "template switching model," lower left) or the nucleotide synthesis from nascent and template strands (vi and vii) after the dissociation of the nascent strand from the template (vi; "Tarzan model" [Scaringe et al., 2008], lower right) with some errors using an error-prone translesion polymerase. In both models, the translesion polymerase is able to swing across a DNA adduct (iv and viii), resulting in skipping of one nucleotide on the template and the normal polymerase can proceed with replication. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/ajmga].

prone translesion polymerase-mediated post-replication repair to bypass a DNA adduct, blocking the replication of lagging-strand synthesis (Fig. 2B, upper). On the basis of the inserted sequence containing both sense and antisense sequences derived from the nearby template observed in our case, two alternative models for indelogenesis may be presented. To save a cell with a DNA adduct that blocks replication by normal polymerase, an error-prone translesion polymerase complex may be recruited for the progression of lagging-strand synthesis [Scaringe et al., 2008; Sale, 2012]. In the "template switching model" (Fig. 2B; lower left, i–v), the association of the two nascent DNA strands is followed by additional synthesis on the nascent strand while constituting the inserted sequence from two templates sequentially when switching templates [Sale, 2012]. In the "Tarzan model" previously proposed by Scaringe et al. [2008] (Fig. 2B; lower right, iv–ix), the helicase unwinds the nearby nucleotides of the nascent strand from the template so that the translesion polymerase can loop back on itself and back up on the template strand. In both models, the association of sense and antisense sequences synthesized on the nascent strand from the nearby template form a hairpin structure with some additional length acting like a vine that the translesion polymerase uses to swing across the adduct [Scaringe et al., 2008]. In addition, existence of sequences whose template is undetermined in the insertion, other than sense, and antisense sequences derived from the nearby template, supports the involvement of error-prone translesion polymerase. Because of the lack of repeat sequences around the microindel in our case, serial replication of slippage models, a possible alternative mechanism for microindels with large insertion sizes [Chen et al., 2005], may not be involved in the indelogenesis of the microindel in the present case. Although nonhomologous end joining after DNA double-strand breaks is unable to be completely excluded as another possible mechanism for microindel generation, the inserted sequence containing both sense and antisense sequences derived from the nearby template is difficult to be explained by this mechanism.

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