Gene 655 (2018) 65–70

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Research paper

Clinical and genetic characterization of a patient with *SOX5* haploinsufficiency caused by a *de novo* balanced reciprocal translocation

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ARTICLE INFO

Keywords:
SOX5
Lamb-Shaffer syndrome
De novo balanced reciprocal translocation
Autism spectrum disorder

ABSTRACT

Lamb-Shaffer syndrome (OMIM: 616803) is a neurodevelopmental disorder characterized by developmental delay, mild to moderate intellectual disability, speech delay, and mild characteristic facial appearance caused by *SOX5* haploinsufficiency on chromosome 12p12.1. There are clinical variabilities among the patients with genomic alterations, such as intragenic deletions, a point mutation, and a chromosomal translocation of t(11;12)(p13;p12.1), in *SOX5*. We report herein a 5-year-old Japanese male with a *de novo* balanced reciprocal translocation t(12;20)(p12.1;p12.3) presenting a mild intellectual disability, speech delay, characteristic facial appearance, and autistic features. We determined the translocation breakpoints of the patient to be in intron 4 of *SOX5* and the intergenic region in 20p12.3 *via* FISH and nucleotide sequence analyses. Thus, the present patient has *SOX5* haploinsufficiency affecting 2 long forms of SOX5 and is the second reported case of Lamb-Shaffer syndrome caused by a *de novo* balanced reciprocal translocation. This report confirmed that haploinsufficiency of the 2 long forms of *SOX5* presents common clinical features, including mild intellectual disability and autistic features, which could be useful for the clinical diagnosis of Lamb-Shaffer syndrome.

1. Introduction

SRY-related high-mobility-group box 5 (*SOX5*; OMIM 604975) is a transcription factor belonging to the SRY-box (Sox)-containing gene family and is located on chromosome 12p12.1 with a span of approximately 1 Mb. SOX5 has a high-mobility-group (HMG) domain that functions as a DNA-binding domain (Ikeda et al., 2002). This domain has also been shown to interact with several transcription factors (Wissmüller et al., 2006). The *SOX5* transcripts result in 3 major mRNA isoforms (7.5 kb, 7.1 kb, and 5.9 kb), which encode 2 long form proteins (82 kDa, NM_152989.4; and 84 kDa, NM_006940.5) and 1 short form protein (48 kDa, NM_178010.3) (Ikeda et al., 2002; Kiselak et al., 2010). We defined NM_152989.4 as *L1-SOX5*, NM_006940.5 as *L2-SOX5*, and NM_178010.3 as *S-SOX5* in the present study. In the cohort study of copy number variations associated with 1461 clinical samples from patients with autism spectrum disorders or autistic features, *SOX5* was identified as a novel candidate gene (Rosenfeld et al., 2010). *SOX5* haploinsufficiency in patients results from a variety of mutations, including large multigenic 12p12 deletions with *SOX5*, intragenic *SOX5* deletions, a point mutation of *SOX5*, and chromosomal translocation within *SOX5* (Lamb et al., 2012; Lee et al., 2013; Schanze et al., 2013; Arroyo-Carrera et al., 2015; Nesbitt et al., 2015; Quintela et al., 2015). These patients displayed common clinical features, including developmental delay, a variety of intellectual disabilities, a prominent speech delay, and characteristic facial appearances, all of which were defining features of Lamb-Shaffer syndrome (LAMSHF; OMIM 616803) (Lamb et al., 2012).

We present here the first Japanese patient with the *de novo* balanced reciprocal translocation 46,XY,t(12;20)(p12.1;p12.3) exhibiting mild intellectual disability, speech delay, characteristic facial appearance, and autistic features. We determined the translocation breakpoint in 12p12.1, which causes *SOX5* haploinsufficiency of L1- and L2-SOX5. We also discuss the clinical features and especially focusing on the intellectual disability of the present patient, and reported cases of *SOX5*

Abbreviations: SOX5, SRY-related high-mobility-group box 5; LAMSHF, Lamb-Shaffer syndrome; HMG, high-mobility-group; *L1-SOX5*, NM_152989.4; *L2-SOX5*, NM_006940.5; *S-SOX5*, NM_178010.3; OFC, occipitofrontal circumference; SD, standard deviation; MRI, magnetic resonance imaging; KIDS, Kinder Infant Development Scale; DQ, developmental quotient; DSM-5, Diagnostic and Statistical Manual of Mental Disorders-fifth edition; FISH, fluorescence *in situ* hybridization; BAC, bacterial artificial chromosome; DAPI, 4′,6-diamidino-2-phenylindole; der(12), derivative chromosome 12; der(20), derivative chromosome 20; CFu neurons, corticofugal neurons

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https://doi.org/10.1016/j.gene.2018.02.049
Received 15 November 2017; Received in revised form 14 February 2018; Accepted 21 February 2018

haploinsufficiency.

2. Material and methods

2.1. Cytogenetic analyses

Written informed consent was obtained from the parents of the patient. Experiments were conducted after obtaining approval from the institutional review board of the Institute for Developmental Research, Aichi Human Service Center.

Chromosomal samples were prepared from the patient's lymphoblastoid cells. Chromosomal analyses were performed using GTG-banded chromosomes at a resolution of 400–550 bands. Fluorescence *in situ* hybridization (FISH) was performed using metaphase chromosome spreads obtained from the patient by using fosmid and bacterial artificial chromosome (BAC) clones as probes. The fosmid clones WI2-2546A11 (12p12.1) and WI2-528L6 (20p12.3) were used to identify the translocation breakpoints (BACPAC Resources Center, CA, USA). The BAC clones RP11-81H3 (12q15) and RP11-101E14 (20q12) were used as chromosomal markers for chromosomes 12 and 20, respectively (Life Technologies, Carlsbad, CA, USA). The fosmid and BAC clones were labeled with digoxigenin-11-dUTP (fosmid) and biotin-16-dUTP (BAC), respectively. Finally, the chromosomes were stained with 4′,6-diamidino-2-phenylindole (DAPI) (Roche Diagnostics, Mannheim, Germany).

2.2. Characterization of the translocation breakpoints

Primer pairs used to amplify the translocation breakpoint at WI2-2546A11 and WI2-528L6 on chromosomes 12p12.1 and 20p12.3 are as follows: 12p sense (a), 5′-AGTCCTTACGCCATTTTGTTC-3′; 12p antisense (b), 5′-AGCAGTAAGTAGGTGATAGCT-3′; 20p sense (c), 5′-AAG CCCTTGGATGACTGAAAT-3′; and 20p antisense (d), 5′-TGAGCTGAGA TCACGCCATT-3′. The PCR products were purified and sequenced using the GenomeLab™ GeXP Genetic Analysis System (Beckman Coulter, Fullerton, CA, USA) and GenomeLab™ Dye Terminator Cycle Sequencing with Quick Start Kit (Beckman Coulter).

3. Results

3.1. Patient

The patient was a 5-year-old boy born to healthy non-consanguineous Japanese parents. He was born at 35 weeks and 5 days of gestation after an uneventful pregnancy. The birth weight, height, and occipitofrontal circumference (OFC) were 2554 g (−1.0 standard deviation [SD]), 46.0 cm (−1.4 SD), and 30.5 cm (−2.0 SD), respectively. Brain magnetic resonance imaging (MRI) at 8 months of age revealed no peculiar findings. He started speaking at 20 months. At 5 years, his body weight, height, and OFC were 16.3 kg (−4.0 SD), 104.8 cm (−3.0 SD), and 50.0 cm (−0.5 SD), respectively. He was noted to have broad and lateral slim eyebrows, long palpebral fissures, deeply set eyes, long eyelashes, epicanthus inversus, a depressed nasal bridge, short philtrum, and a square face (Fig. 1A, B). His third and fifth fingers as well as his third, fourth, and fifth toes showed clinodactyly (Fig. 1C, D). He did not have symptoms of constipation. He spoked three-word sentences at 5 years old. Examination of physical and mental developmental ages with the Kinder Infant Development Scale (KIDS) Type T revealed his developmental quotient (DQ) was 72 at 5 years and 7 months old. It was also observed that he continuously looked at pictures of trains and cars in a book for more than an hour. He was diagnosed with autism spectrum disorders according to the Diagnostic and Statistical Manual of Mental Disorders-fifth edition (DSM-5) system.

3.2. Identification of the balanced reciprocal translocation breakpoints

A G-banded chromosome analysis revealed that the present patient had a balanced reciprocal translocation t(12;20)(p12.1;p12.3) (Fig. 2A). To identify the translocation breakpoints of the derivative chromosome 12 (der[12]) and derivative chromosome 20 (der[20]), FISH analyses were performed using 2 fosmid clones, WI2-2546A11 at 12p12.1 (chromosomal position: 23,882,394–23,923,947 bp, genome build GRCh38) and WI2-528L6 at 20p12.3 (6,774,480–6,818,445 bp, GRCh38). The results showed that the 3 red signals of WI2-2546A11 were observed on 12p12.1 (two white arrows) and 20p12.3 (a yellow arrowhead), respectively (Fig. 2B, upper panel). Thus, the translocation breakpoint of der(12) was located within WI2-2546A11, including exon 5 of *SOX5*. Similarly, WI2-528L6 at 20p12.3 also showed that the 3 red signals were observed on 20p12.3 (two white arrows) and 12p12.1 (a yellow arrowhead), demonstrating that the translocation breakpoint of der(20) was located within WI2-528L6 (Fig. 2B, lower panel).

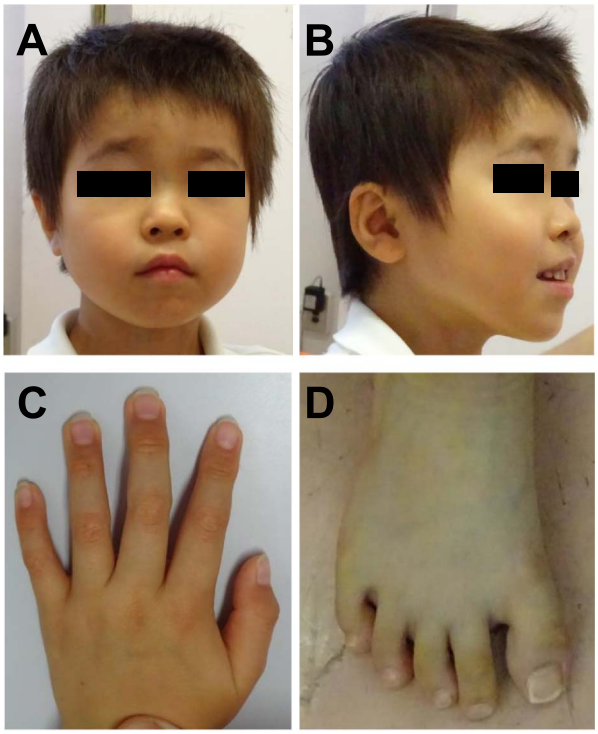

Fig. 1. Facial appearance, fingers, and toes of the patient. (A, B) Frontal and lateral view of the patient's face, respectively. (C, D) Fingers and toes of the patient, respectively.

3.3. Determining the nucleotide sequences of the translocation breakpoints

To determine the nucleotide sequences of the translocation breakpoints of der(12) and der(20), PCR was performed using the 20p sense (c) and 12p antisense (b) primers as well as the 12p sense (a) and 20p antisense (d) primers (Figs. 2, 3). Only two DNA fragments, one about 600 bp long, which contained the breakpoint of 12p12.1, and the other about 500 bp long, which contained the breakpoint of 20p12.3, were amplified in the patient with each primer pair (Fig. 3A). This result showed that the present patient had a *de novo* balanced reciprocal translocation. The nucleotide sequences of the DNA fragments revealed that the breakpoint of der(12) was within intron 4 of *SOX5* (23,901,853 bp, GRCh38), and the breakpoint of der(20) was within an intergenic region (6,802,721 bp, GRCh38) (Figs. 3B, 4A). There is a 10-bp insertion (AGGAAACCTA) on der(12) and a 4-bp deletion (GCTT)/12-bp insertion (TGGAAATCTTAT) on der(20) at these breakpoints (Fig. 3B). The nucleotide sequences of the parents around the breakpoints of chromosomes 12 and 20 were identical to the nucleotide sequences as reported on the NCBI database (data not shown). Thus, the patient had *de novo* structural abnormalities at the translocation