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Short communication

A submicroscopic deletion involving part of the *CREBBP* gene detected by array-CGH in a patient with Rubinstein–Taybi syndrome

Angeline H.M. Lai [a], Maggie S. Brett [b], Wai-Hoe Chin [b], Eileen C.P. Lim [b], Jasmine S.H. Ng [b], Ene-Choo Tan [b,c,*]

[a] Genetics Service, KK Women's and Children's Hospital, Republic of Singapore
[b] KK Research Centre, KK Women's and Children's Hospital, Republic of Singapore
[c] Department of Psychological Medicine, Yong Loo Lin School of Medicine, National University of Singapore, Republic of Singapore

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ABSTRACT

We report a girl with Rubinstein–Taybi syndrome (RSTS) who was found to have copy number loss on 16p13.3 by array-CGH. She has developmental delay and other features of RSTS including downslanting palpebral fissures, a prominent nose with the nasal septum extending below the alae nasi, broad thumbs and big toes, postaxial polydactyly of the right foot and constipation from birth. We report the junction sequence across the breakpoint region for a microdeletion in RSTS. The sequencing results also showed that the deletion was 81.4 kb involving three genes *DNASE 1*, *TRAP 1*, and *CREBBP*.

1. Introduction

Rubinstein–Taybi syndrome (RSTS, OMIM 180849) is a rare multiple congenital anomaly/intellectual disability disorder characterized by delays in growth and psychomotor development, typical facial dysmorphology, broad and duplicated distal phalanges of thumbs and great toes and an increased risk of tumors (Hennekam, 2006; Petrij et al., 1995).

Mutations in two genes—*CREBBP* and *EP300*—have been identified in individuals with RSTS. Approximately 50% of RSTS patients have mutations in the CREB binding protein (*CREBBP* or *CBP*) gene. About 10% of RSTS patients have large deletions including *CREBBP*, detectable by FISH (Stef et al., 2007). The other abnormalities involving *CREBBP* include smaller deletions and duplications. Mutations in *EP300*, a gene that codes for a product homologous to *CREBBP*, have been reported in a small proportion (3%) of cases (Roelfsema et al., 2005). The etiology in the remaining cases is unknown, suggesting that there are other causative genes that have not been identified.

We describe a patient with typical clinical features of RSTS in whom a deletion at 16p13.3 that involved the *CREBBP* gene was detected by microarray-based comparative genomic hybridization (array-CGH). The breakpoint was further mapped by using long-range PCR amplification and the sequence in the breakpoint region was also determined.

1.1. Clinical description

The patient is a 7-year-old girl who was first assessed at 2 years of age. She is the second of three children of healthy unrelated parents. She has an 8-year-old sister and a 1-year-old brother, both of whom are well. Her mother has a female cousin who has absent speech at 15 years of age, and two male cousins with preaxial polydactyly. Antenatally, her mother had impaired glucose tolerance in the third trimester. She was born at full term by forceps delivery, with a birth-weight of 3.3 kg. Her length and head circumference at birth were not available. Her parents reported that she fed slowly in the first 5 months of life. She also had a urinary tract infection in infancy and constipation since infancy. Global developmental delay was noted in infancy. She sat independently at 9 months, crawled at 21 months and walked independently at 3 1/2 years of age. Her first word was “mum” at 2 1/2 years of age.

At the initial assessment at 2 years of age, her height was 86 cm (50–75th centiles), weight was 13.7 kg (90–97th centiles) and head circumference was 45.6 cm (10th centile). She was noted to have downslanting palpebral fissures, a prominent nose with the nasal septum extending below the alae nasi, broad thumbs and big toes

Abbreviations: RSTS, Rubinstein–Taybi syndrome; OMIM, Online Mendelian inheritance in man; CREBBP, CREB binding protein; EP300, E1A binding protein p300; CGH, comparative genomic hybridization; PCR, polymerase chain reaction; Cy3-dUTP, deoxyuridine 5′-triphosphate coupled to Cy3 fluorescent dye; Cy5-dUTP, deoxyuridine 5′-triphosphate coupled to Cy5 fluorescent dye; qRT-PCR, quantitative real time-polymerase chain reaction; GRCH36/hg18, Genome Reference Consortium Human Reference Build 36; DNASE 1, deoxyribonuclease I; TRAP1, Tumor Necrosis Factor receptor-associated protein 1; AluSg, Alu repeat subfamily Sg; AluSz, Alu repeat subfamily Sz; HAT, histone acetyltransferase; NAHR, non-allelic homologous recombination; NHEJ, non-homologous end joining.

* Corresponding author at: KK Research Centre, Level 5 Children's Tower, KK Women's and Children's Hospital, 100 Bukit Timah Road, Republic of Singapore. Tel.: +65 63943792; Fax: +65 63941618.
E-mail address: tanec@bigfoot.com (E.-C. Tan).

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and postaxial polydactyly on the right foot. Initial investigations done included a renal ultrasound scan, which revealed bilateral hydronephrosis, and a peripheral blood karyotype analysis, which was normal at a G-banding resolution of 500 (46,XX). A clinical diagnosis of Rubinstein–Taybi syndrome was made based on her physical features.

The patient has had physiotherapy, and speech and language therapy since diagnosis. Her height and weight are on the 25th and 97th centiles for age, respectively. She is in good general health, apart from persistent constipation, which is managed with oral medication. Currently at 7 years of age, she walks well and can feed herself with a spoon. She does not speak but understands instructions and communicates with gestures. Formal psychological testing has not been done.

2. Materials and methods

Written informed consent for array-CGH testing and follow-up investigations was obtained from the parents. Genomic DNA was extracted from whole blood using the Puregene DNA extraction kit (Gentra Systems Inc., Minneapolis, USA). DNA was checked for quantity and purity using the NanoDrop Spectrophotometer (NanoDrop Technologies, Wilmington, USA).

Human CGH array consisted of 400 K 60-mer oligonucletode probes from Agilent (Agilent Technologies Inc., Santa Clara, CA, USA) and reference used was male human genomic DNA from Promega (Promega Corp., Madison, WI, USA). Test DNA was labeled with Cy3-dUTP and reference DNA with Cy5-dUTP (Sigma-Aldrich, St. Louis, MO, USA) according to Agilent's protocol for enzymatic labeling (Version 6.3). The efficiency of the labeling was measured using a Nanodrop Spectrophotometer. Hybridization and washing were done according to the manufacturer's instructions and the array was scanned with Agilent G2505C microarray scanner at 5 μm resolution. Data were extracted from the scanned image using Agilent Feature Extraction (Version 10.7.31) and analyzed for copy number change using Agilent Genomic Workbench Lite (Edition 6.0.130.24) and Partek Genomics Suite (Version 6.5) (Partek Inc., St. Louis, MO, USA).

Quantitative real time-polymerase chain reaction (qRT-PCR) was performed on the Step-One Plus™ Real-Time PCR Systems (Applied Biosystems, Foster City, CA). Each 20 μl reaction contains 2× *Power* SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) according to manufacturer's recommendations.

Nested PCR primers were designed from the proximal and distal breakpoint segments as determined by the array results. Long-range PCR was performed using the TaKaRa LA Kit (TaKaRa Bio Inc., Shiga, Japan) using different combinations of primers. Each 10 μl reaction contain 10 ng of DNA, 100 μM of dNTPs, 0.2 μM of each primer and 0.5 U TaKaRa LA Taq polymerase in 1× LA PCR buffer II. The smallest PCR product was purified with QIAquick Gel Extraction Kit (QIAGEN GmbH, Hilden, Germany). Resulting DNA was sequenced in both directions with the ABI BigDye Terminator kit and electropheresed through an Applied Biosystems 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

3. Results

Agilent 400 K Human CGH array showed a loss in copy number of the short arm of chromosome 16. The last distal probe that was normal for copy number mapped at 3,641,282–3,642,383 while the first distal probe with copy number loss was at 3,646,249–3,646,401. The last proximal probe with copy number loss was at 3,720,510–3,720,569 while the first proximal probe with normal copy number was at 3,728,522–3,728,581 (GRCH36/hg18). From the position of the probes, the estimated minimum size of the deletion interval was about 74.3 kb while the maximum size was 87.4 kb. The deletion included the 5′ end of the *DNASE1* gene, the whole of the *TRAP1* gene and part of the *CREBBP* gene (Fig. 1). This copy number loss was validated by quantitative real-time polymerase chain reaction with primers targeting the *CREBBP* gene (Fig. 2).

Using nested primers, amplification of the region across the breakpoint resulted in PCR products of various sizes. Sequencing of the smallest amplicon (approximately 700 bp) revealed that the distal breakpoint mapped at 3,643,600 bp (within the 5′ upstream region of the *DNASE1* gene) and the proximal breakpoint at 3,724,995 bp (within the intron between exon 28 and 29 of the *CREBBP* gene). The deletion interval of 81,414 bp would include all coding regions of the *DNASE1* gene, the entire *TRAP1* gene and exons 29–31 of the *CREBBP* gene.

Analysis of the sequence at the breakpoint junction showed no homologous regions. The breakpoint occurred within a 9 bp (CTGAGGTCA) segment of DNA of which the last 6 bp (AGGTCA) showed sequence identity to the *DNASE1* gene (Fig. 3). The distal breakpoint occurred within a 310 bp *AluSg* repetitive element. There was no repetitive element at the proximal breakpoint but an *AluSz* element occurs within 1 kb of the proximal breakpoint.

4. Discussion

The diagnosis of RSTS is still essentially based on clinical presentation. Typical facial features, broad thumbs and great toes, and growth and developmental delays are the main diagnostic features. Our patient had the typical RSTS phenotype, including characteristic facial features, broad thumbs and big toes, initial feeding difficulties, persistent constipation and global developmental delay, particularly affecting her speech. In addition, she had postaxial polydactyly in her right foot, which has been reported in RSTS but is rare. Preaxial polydactyly or partial duplication of the first ray in either the hands or feet is more common.

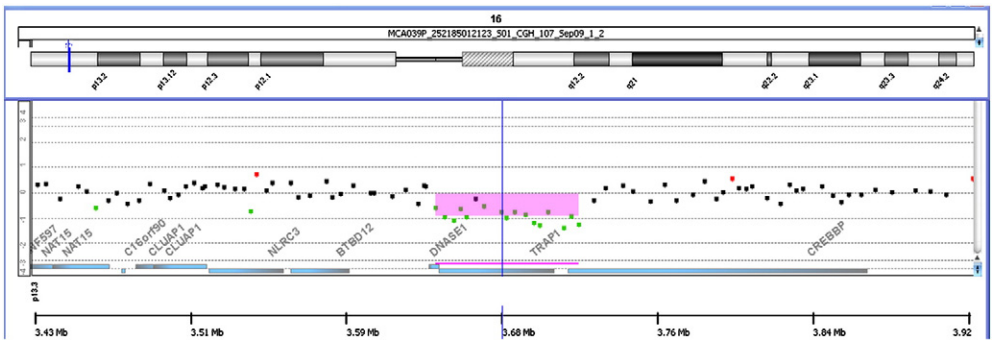

Fig. 1. Array-CGH result showing the deletion at 16p13.3. The screenshot from Genomic Workbench Lite displayed the region with the copy number loss as the area shaded in pink.