DATA REPORT

PCS/MVA syndrome caused by an Alu insertion in the BUB1B gene

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We report a case of premature chromatid separation/mosaic variegated aneuploidy syndrome identified by microcephaly on fetal ultrasound and confirmed by cytogenetic analysis of amniotic fluid. Initial mutational analysis of the entire coding region of the BUB1B gene failed to identify any causative mutations. However, further analysis revealed a known compound heterozygous mutation in the upstream region of this gene and a novel Alu insertion mutation in the intron.

Human Genome Variation (2017) 4, 17021; doi:10.1038/hgv.2017.21; published online 8 June 2017

Premature chromatid separation (PCS) is a phenomenon observed as separate and splayed chromatids with discernible centromeres and involves most chromosomes during metaphase. PCS is a genetic trait that is dominantly inherited and can be observed in metaphase chromosomes in a standard cytogenetic examination. Since PCS is innocuous and is observed in the normal healthy population, it is occasionally identified as an incidental finding during screenings for other purposes.

The homozygosity of PCS manifests another clinical entity, PCS/MVA syndrome (OMIM #176430, #257300), an autosomal recessive disorder that is characterized by an increased frequency of PCS in >50% metaphase cells and mosaic aneuploidies.1,2 PCS/MVA also manifests a variety of mosaic aneuploidies, especially trisomies, double trisomies and monosomies. Affected patients have clinical characteristics such as growth retardation, microcephaly, cataracts, Dandy–Walker complex, uncontrollable clonic seizures, polycystic kidneys, and a high risk of Wilms tumor and rhabdomyosarcoma.1

PCS is caused by mutations in the BUB1B (budding uninhibited by benzimidazoles 1 homolog beta) gene (encoding BubR1) that is the core component of the mitotic spindle checkpoint for mitotic fidelity and genome stability.2 PCS/MVA syndrome is mostly caused by compound heterozygous mutations in the BUB1B gene. Both parents are often the healthy carrier of a heterozygous PCS trait as they harbor the monallelic BUB1B mutation.

In this study, we report a case of PCS/MVA syndrome in which the initial mutational analysis of the entire coding region of the BUB1B gene failed to identify any causative mutations. However, further analysis revealed a compound heterozygous known mutation in the upstream region and a novel Alu insertion mutation in the intron of BUB1B.

A 24-year-old G1P1 pregnant female in a nonconsanguineous Japanese couple was referred to our facility because of fetal abnormalities. In the 24th week of gestation, her fetus was found to have a growth restriction with extreme microcephaly (~5.0 s.d.). Screening for an intrauterine infection was negative and no family history was recorded. We performed standard cytogenetic test of the amniotic fluid and analyzed 50 metaphases. The results were 46, XY, inv(9)(p11q13)[11]/47, XY, inv(9)(p11q13), +7[12]/48, XY, inv(9)(p11q13), +3, +17[6]/47, XY, inv(9)(p11q13), +5[4]/48, XY, inv(9)(p11q13), +2, +7[4]/49, XY, inv(9)(p11q13), +3, +17, +18[4]/47, XY, inv(9)(p11q13), +16[2]/others [7]. Besides the well-known but innocuous inv(9)(p11q13) polymorphism, a mosaicism of aneuploidy was observed. A baby boy was born via caesarian section at 38 weeks of gestation. The Apgar score was 8 points for 1 min and 9 points for 5 min after birth. The body weight was 1,934 g (~3.2 s.d.) and the head circumference was 26.7 cm (~4.5 s.d.). He also had congenital cataracts, a prominent nasal bridge, a low-set ear, micrognathia and ambiguous genitalia.

Since PCS/MVA syndrome was suspected, we evaluated the PCS frequencies in blood samples from the infant and his parents. Cord blood or peripheral blood lymphocytes were cultured in RPMI1640 in the presence of PHA-M, arrested with 0.1 μg/ml colcemid for 2 h and treated with 0.075 M KCl at 32 °C for 20 min. Chromosomes were spread onto glass slides and stained with Giemsa solution. Hundreds of metaphases were used to evaluate the frequency of cells with PCS. Whereas the metaphases from the baby showed a 27.2% frequency of PCS, those from his father and mother showed 11.9% and 7.3%, respectively.

After receiving approval from the Ethics Review Board for Human Genome Studies at Fujita Health University and written informed consent from the patients, we obtained cord blood or peripheral blood lymphocyte samples for genomic analyses. We screened all 23 coding exons of the BUB1B gene baby and parents by means of PCR direct sequencing. However, we failed to detect any mutations within the coding region. Recently, a G>A mutation located 44 kb upstream of BUB1B (rs576524605) was identified among Japanese PCS cases with only one mutation detected in the coding region.3 We therefore examined the upstream region of BUB1B using the Sanger method. The expected G>A mutation was identified both in the baby and the unaffected mother (Figure 1a).

When we further surveyed the coding mutation in exon 9 of BUB1B, we noticed an extra larger sized PCR product in both the

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Received 10 March 2017; revised 4 April 2017; accepted 14 April 2017
Figure 1. Genomic sequence of the BUB1B gene. (a) Sequence of the 44 kb upstream region of the BUB1B gene. A heterozygous G>A mutation (rs576524605) was detected in the current study patient and mother, but not in the father. (b) Agarose gel electrophoresis of PCR products that were amplified with primers for intron 8 and exon 9 of BUB1B. A ~400 bp normal band is indicated with a black arrow and a ~700 bp aberrant band with a red arrow. (c) Sequence analysis of BUB1B PCR products from the baby and parents. The upper panel is a schematic illustration of the region around the boundary between intron 8 and exon 9. Arrowheads indicate the PCR primers used. An Alu element was found to be inserted just before the AG consensus dinucleotide at the splicing acceptor in the proband and father. The lower panel shows the sequence around the inserted element. Target site duplications are indicated by dashed lined boxes.

Figure 2. RNA analysis of the BUB1B gene in the proband and parents. (a) Agarose gel electrophoresis of RT-PCR products of exons 8–10. Larger products (black arrows) were found to be derived from the normal allele and smaller products (red arrows) from the allele with the Alu insertion. The lower panel shows the quantification of these amplified products. Vertical bar indicates the ratio of the product from normal splicing to aberrant splicing (internal control). Red bars indicate the smaller PCR product levels, whereas black bars indicate the relative amounts of the larger amplicons. (b) Sequence analysis of the RT-PCR products. The upper panel displays a predictive structure of the mutant transcript. Arrowheads indicate the position of the PCR primers. The lower panel shows the sequence around the exon–exon boundaries of BUB1B. RT-PCR, reverse transcription PCR.
PCR using primers specific for splice site (′AG′ in the BUB1B gene, suggesting a possible influence on normal splicing. We performed reverse transcription PCR using primers specific for flanking exons 8 and 10. In addition to the product derived via normal splicing, we detected an additional shorter product in both the infant and his father (Figure 2a). Direct sequencing of these reverse transcription PCR products revealed that the smaller product did not include exon 9 of BUB1B, indicating that exon skipping had occurred due to the insertion mutation (Figure 2b). This exon skipping would result in a frameshift that might produce a truncated nonfunctional protein. Further, this analysis also allowed us to separately quantify the transcript level from normal splicing and aberrant splicing using ImageJ software (Bethesda, MD, USA). In the baby, the ratio of the product from normal splicing to aberrant splicing was reduced compared to the father. This suggested that the BUB1B transcript level from the maternal allele was decreased due to the upstream mutation (Figure 2a).

We have identified a novel Alu insertion mutation in the BUB1B gene in a patient with PCS/MVA syndrome. The insertion of a mobile element is a common pathway for the generation of new splicing, possibly leading to the production of a nonfunctional protein. The other allele was found to be a known hypomorphic allele due to a mutation in the upstream region of the BUB1B gene. In mice, complete loss of the BUB1B allele due to a mutation in the upstream region of the 3′ gene, suggesting a possible influence on normal splicing. We performed reverse transcription PCR using primers specific for flanking exons 8 and 10. In addition to the product derived via normal splicing, we detected an additional shorter product in both the infant and his father (Figure 2a). Direct sequencing of these reverse transcription PCR products revealed that the smaller product did not include exon 9 of BUB1B, indicating that exon skipping had occurred due to the insertion mutation (Figure 2b). This exon skipping would result in a frameshift that might produce a truncated nonfunctional protein. Further, this analysis also allowed us to separately quantify the transcript level from normal splicing and aberrant splicing using ImageJ software (Bethesda, MD, USA). In the baby, the ratio of the product from normal splicing to aberrant splicing was reduced compared to the father. This suggested that the BUB1B transcript level from the maternal allele was decreased due to the upstream mutation (Figure 2a).

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