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CASE REPORT

Duplication 17p11.2 (Potocki-Lupski Syndrome) in a child with developmental delay

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Abstract

Potocki-Lupski syndrome (PTLS), also known as duplication 17p11.2 syndrome, trisomy 17p11.2 or dup(17)(p11.2p11.2) syndrome, is a developmental disorder and a rare contiguous gene syndrome affecting 1 in 20,000 live births. Among the key features of such patients are autism spectrum disorder, learning disabilities, developmental delay, attention-deficit disorder, infantile hypotonia and cardiovascular abnormalities. Previous studies using microarray identified variations in the size and extent of the duplicated region of chromosome 17p11.2. However, there are a few genes which are considered as candidates for PTLS which include *RAI1*, *SREBF1*, *DRG2*, *LLGL1*, *SHMT1* and *ZFP179*. In this report, we investigated a case of a 3-year-old girl who has developmental delay. Her chromosome analysis showed a normal karyotype (46,XX). Analysis using array CGH (4X44 K, Agilent USA) identified an ~4.2 Mb *de novo* duplication in chromosome 17p11.2. The result was confirmed by fluorescence *in situ* hybridization (FISH) using probes in the critical PTLS region. This report demonstrates the importance of microarray and FISH in the diagnosis of PTLS.

Keywords: Potocki-Lupski syndrome, duplication 17p11.2, microarray, developmental delay, fluorescence *in situ* hybridization

INTRODUCTION

Chromosome 17p11.2 duplication syndrome, also known as Potocki Lupski syndrome (PTLS) (OMIM #610883) was first described by Brown *et al*[1]. Subsequently, with the advent of high resolution methods such as microarray, more patients were reported[2,3]. Molecular analysis of the PTLS region showed that it is of the same region deleted in Smith Magenis syndrome (OMIM #182290), and consists of a unique junction fragment with an apparent fragment size. The region was also described to be preferentially paternal in origin and occured by non-allelic homologous recombination (NAHR) mechanism[4]. The majority of patients were found to have a common 3.7 Mb duplication within 17p11.2 while the remainder harbored non-recurrent duplications ranging from 1.3 to 15.2 Mb in size. The critical PTLS region was identified as a 1.3 Mb in length which included the retinoic acid inducible 1 (*RAI1*) gene and 17 other genes[3].

The common phenotypes of PTLS patients are hypotonia, poor feeding, failure to thrive, developmental delay during infancy, speech and language impairment, hyperactivity in early childhood, intellectual disability, autism and behavioral problems in older children and cardiovascular problems[5,6]. The variations in size of the duplicated region also explain the wide varieties and severities in the clinical features of the PTLS patients. In the present study, we identified microduplication 17p11.2 in a child with global developmental delay. The size of the duplication was estimated at 4.2 Mb which overlaps the common and critical region of PTLS.

CASE REPORT

Our patient was a 3-year-old girl, the second child of a non-consanguineous marriage. She presented to the Child Development Centre (CDC), UKMMC with problems of hyperactivity,

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developmental delay, central hypotonia and poor weight gain. She was born full term via caesarean section with a birth weight of 2890g. The mother was 39 years old and diagnosed with gestational diabetes mellitus during her pregnancy. During early infancy, our patient was active, but had slow sucking during feeding. At 48 hours of life, she was admitted to the neonatal intensive care unit due to respiratory depression secondary to hypoglycemia. She was referred to physiotherapy and started walking at 2 years of age. She was able to run, walk upstairs, one step at a time but unable to walk downstairs. She was able to speak 11 words with meaning and able to obey one step commands. She was unable to feed herself and still wet by day. Hearing and eye assessment were normal.

Informed consent from the parents was obtained for this case report. The research protocol and informed written consent was approved by the Universiti Kebangsaan Malaysia Research Committee Board.

Genetic findings

Blood lymphocyte culture was prepared from the patient's blood sample following standard protocol. Giemsa-banded chromosomes were analyzed from 20 metaphases which showed normal female karyotype at 550 bands per haploid (bph) resolution (Fig. 1).

Genomic DNA was extracted from the patient's blood sample using the DNeasy Blood & Tissue kit according to manufacturer's recommended procedure (Qiagen, Germany). The normal commercial female gDNA (Agilents Technologies, USA) was used as reference sample. The quality and the quantity of the DNA was assessed by electrophoresis in a 1.0% agarose gel and by using NanoDrop UV-VIS Spectrophotometer (Nanodrop Tech. Inc., USA) respectively. Array CGH was performed following the Agilent's Direct Method of Oligo Array CGH workflow (Agilent Technologies, USA) on Agilent Human Genome 4x44K oligonucleotide arrays slide. The result of microarray analysis detected an ~4.2 Mb duplication on chromosome 17p11.2 (Fig. 2). The start and stop points of the duplication were estimated at 16,782,546 and 21,050,337 kb respectively. The region contains 122 RefSeq genes which includes the genes in the critical PTLS region.

In view of the previous reports and the contents of the Database of Genomic Variants (http://dgv.tcag.ca/gb2/gbrowse/dgv2_hg19/) this duplication could be considered as a characteristic of the Potocki-Lupski syndrome duplication and contains the dosage sensitive *RAI1* gene. To confirm the microarray result, FISH using specific probes Agilent SureFISH 17p11.2 LLGL1-SHMT1 (spectrum red), Agilent SureFISH 17p11.2 RAI1 (spectrum green) and a control Agilent SureFISH 17 CEP BL (spectrum aqua) was performed. Results of the hybridization on 150 nuclei analysed showed evidence of duplication of the RAI1 and SHMT1 locus, thus confirming the microarray finding. Both parents have a normal karyotype (data not shown) and a normal FISH signal pattern ie. two red, two green and two aqua (Fig. 3).

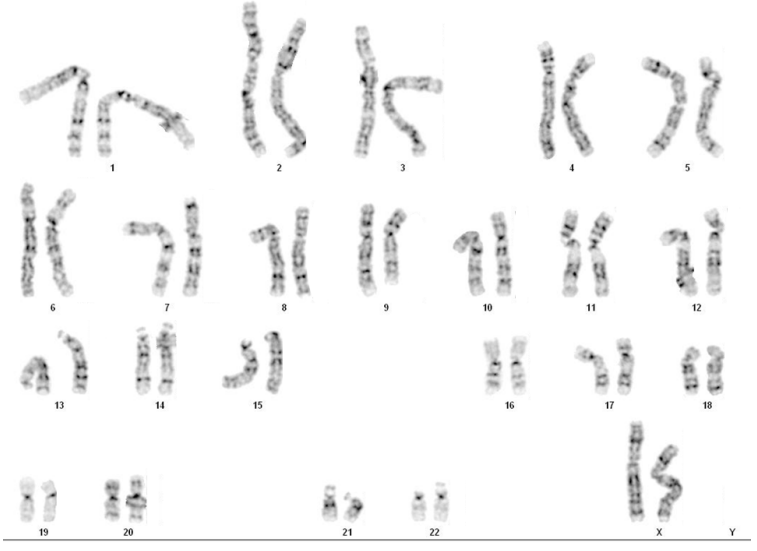

FIG. 1: G-banding karyotype of the patient showing 46,XX

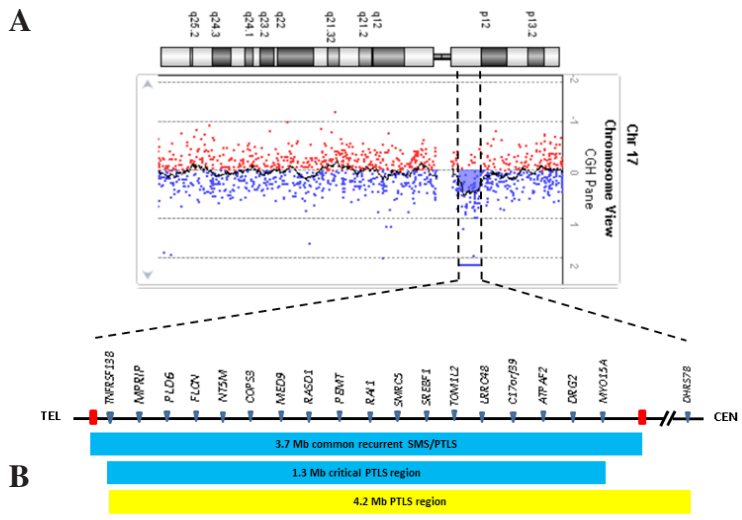

FIG. 2: (A) Microarray CGH analysis identified duplication in chromosome 17p11.2 with an estimated size of 4.2 Mb (16,782,546-21,050,337 kb). (B) A schematic diagram showing the extent of the duplicated region (yellow horizontal bar) which contained more than 122 genes starting from *TNFRSF13B* to *C17orf35B* and overlapped the 3.7 Mb common recurrent duplicated region of PTLS and the 1.3 Mb critical PTLS region

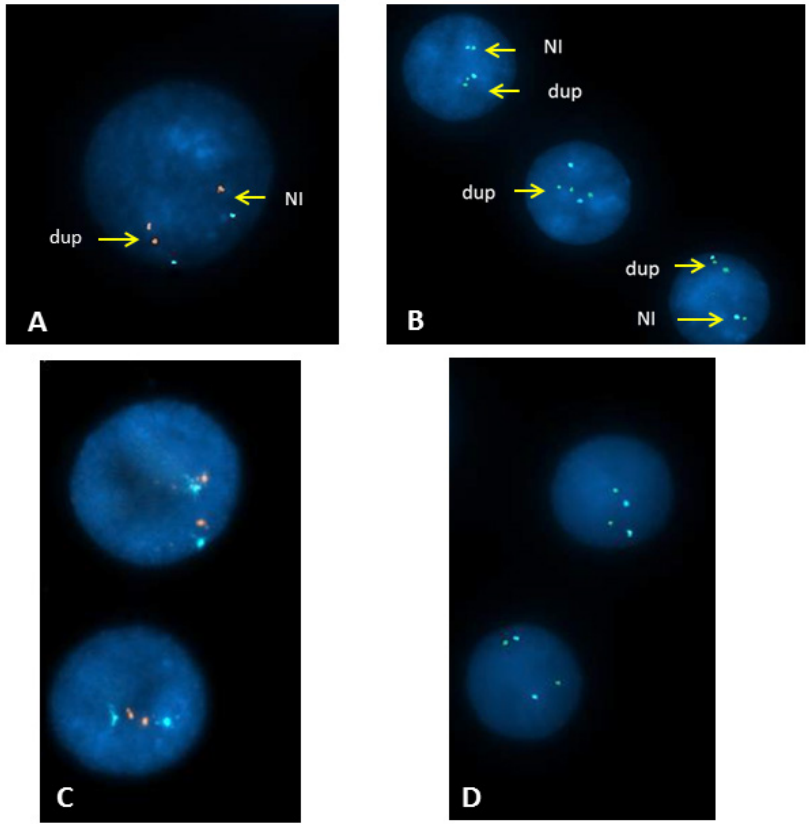

FIG. 3: FISH on interphase cells using 17p11.2 probes specific to RAI1 (green), SHMT1 (red) and control centromeric probe chromosome 17 (aqua). A and B: Hybridization pattern on the patient's interphase cells showing duplication of RAI1 and SHMT1; C and D: A normal hybridization pattern on the parent's interphase cells showing the two normal copies of the probes (two green and two red signals). NI, normal; dup, duplication

DISCUSSION

Array CGH identifies copy-number variations (amplifications or deletions) and single-copy gains and losses across whole chromosomes and genome at high resolution (≤1 Mb), thus allowing precise location information linked to physical and genetic maps of the human genome[7]. The technology has become more cost effective and affordable so that many laboratories including in middle income countries have begun to use it as a first line diagnostic investigation in patients with learning disabilities[8]. However, it is important to note that the technique cannot identify balanced translocations, thus conventional karyotyping must always be included in the investigation of patients with learning disabilities and congenital anomalies.

According to the evidence from the survey done by The International Standard Cytogenomic Array (ISCA) Consortium, chromosomal microarray should be applied as the first-tier cytogenetic diagnostic test on patients with developmental delay/intellectual disability, autism spectrum disorders and multiple congenital anomalies, primarily because of its higher sensitivity compared to the conventional G- banding karyotyping[9]. In addition, a large systematic review of 13,926 subjects with learning disabilities (mental retardation) and congenital anomalies, have provided evidence which support the routine use of array CGH in investigating such patients, in whom karyotyping is normal. However, the study also suggests that caution should be taken in interpreting results as the technique also identifies false positives at a similar rate to causal variants[8].

Using microarray and validation by FISH, we had identified an ~4.2 Mb duplication 17p11.2 in our patient which is associated with PTLS. The duplicated region identified in our patient overlaps the 3.4 Mb common region and the 1.3 Mb critical region of PTLS (Fig. 2). In addition to the common phenotype of PTLS, our patient was also noted to have neonatal hypoglycemia. It is a well-known fact that women with diabetes (gestational diabetes mellitus or pregestational diabetes mellitus) have a higher risk of having a baby with hypoglycemia. Therefore, neonatal hypoglycemia in our patient most probably was due to the mother's gestational diabetes rather than the effect of the duplication. FISH analysis and karyotyping performed on the parents' blood sample showed normal results, indicating that PTLS in our patient occurred as a *de novo* event.

While the majority of PTLS cases reported are *de novo*, to date there are two reports (three families) of inherited PTLS[10,11]. Recently, Lee *et al*[12] discovered a shortest 0.25 Mb (17,575,978–17,824,623) duplication 17p11.2 in a boy with subtle PTLS phenotype. The duplicated region was within the critical PTLS region and contains only four genes – *RAI1*, *SMCR5*, *SREBF1* and *TOM1L2*. In concordance with the previous reports, they suggested *RAI1* as the critical dosage sensitive gene responsible for the predominant clinical features of PTLS phenotype and *SREBF1* as another potential candidate gene. However, it is still unknown whether PTLS can be caused by a duplication of only the *RAI1* or *SREBF1* locus. Studies on mouse models have also provided evidence that *Rai1* is a critical and dosage-sensitive gene involved in the phenotypes of PTLS[13,14] .

In conclusion, our findings demonstrated the importance of array CGH and interphase FISH in the identification and confirmation of 17p11.2 duplication (PTLS) which otherwise would have been missed if only routine Giemsa-banded (G-banded) chromosome were analysed. At 550 banding per haploid resolutions (bph), chromosomal aberrations involving deletion or addition of >5 Megabases (Mb) can be detected but for smaller abnormalities (<5 Mb) a higher banding resolution or alternatively, more specialized technique like FISH, array CGH, sequencing or NGS would the best approach to elucidate this chromosomal rearrangement.

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