



A rare case of a boy with *de novo* microduplication at 5q35.2q35.3 from central Brazil

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ABSTRACT. Genomic disorders are genetic diseases that are caused by rearrangements of chromosomal material via deletions, duplications, and inversions of unique genomic segments at specific

regions. Such rearrangements could result from recurrent non-allelic homologous recombination between low copy repeats. In cases where the breakpoints flank the low copy repeats, deletion of chromosomal segments is often followed by reciprocal duplication. Variations in genomic copy number manifest differently, with duplication and deletions of the same genomic region showing opposite phenotypes. Sotos syndrome is caused by alterations in the dosage of *NSD1* on human chromosome 5 by either deletions or mutations, such as microdeletion of 5q35.2q35.3. In general, patients carrying reciprocal microduplication at 5q35.2q35.3 present no clinical phenotype or milder phenotype than do patients with microdeletion at the same locus. We report the first case of 5q35.2q35.3 microduplication encompassing *NSD1* in a patient from central Brazil. We identified a genomic imbalance corresponding to a *de novo* 0.45 Mb microduplication at 5q35.2q35.3 by chromosomal microarray analysis and study of low-copy repeats. The proband had microduplication in the chromosomal region containing *NSD1*, which resulted in a Sotos syndrome reversed phenotype, and this duplication was associated with microcephaly, short stature, and developmental delay. Analysis of the genomic structure of the rearranged 5q35.2q35.3 chromosomal region revealed two major low-copy repeat families, which caused the recurrent rearrangements. Chromosomal microarray analysis is a potential tool to identify microrearrangements and guide medical diagnosis, which has to be followed by a non-directive genetic counseling approach to improve the quality of life of the patient.

Key words: *NSD1*; Microduplication; LCR; Sotos Syndrome; CMA

INTRODUCTION

Genomic disorders are a group of genetic diseases caused by chromosomal rearrangements such as deletions, duplications, and inversions of unique genomic segments at specific regions. The rearrangements arise by the common mechanism of recurrent non-allelic homologous recombination (NAHR) between low-copy repeats (LCR). (Inoue and Lupski, 2002; Lupski and Stankiewicz, 2005; Franco et al., 2010).

Sotos syndrome (SS) (OMIM117550) is an autosomal dominant genomic disorder caused by microdeletion of region 5q35.2q35.3, which contains *NSD1*; in 90% of the cases, SS involves deletions or mutations (Žilina et al., 2013; Chen et al., 2014; Park et al., 2014). SS is characterized by intellectual disability, facial features with frontal bossing, frontoparietal sparseness of hair, apparent hypertelorism, down-slanted palpebral fissures, pre- and postnatal overgrowth, macrocephaly, and advanced bone age (Türkmen et al., 2003; Dikow et al., 2013; Almuriekh et al., 2015). The incidence of SS is estimated to be 1:14,000-15,000 live births worldwide (Chen et al., 2014; Park et al., 2014).

NSD1 encodes nuclear receptor SET domain-containing protein 1, a histone methyltransferase (Tatton-Brown et al., 2005). *NSD1* regulates the expression of *APC2*, which participates in brain development through its regulation of neuronal migration and axon guidance. Besides, *NSD1* regulates the expression of many other genes required for the normal development of cardiac, skeletal, and renal tissues (Almuriekh et al., 2015).

Patients carrying reciprocal microduplication at 5q35.2q35.3 have no clinical phenotype or have a milder phenotype than do patients with microdeletion of the same region (Kirchhoff et al., 2007; Weise et al., 2012). According to Orphanet Report Series (2016), the number of published cases of the 5q35 microduplication worldwide is 14. Here, we report a rare case of 5q35.2q35.3 microduplication in a patient who was evaluated using chromosomal microarray analysis (CMA) and an analysis of LCRs at a public genetics laboratory of Goiás State, central Brazil.

Case report

The patient is a 4-year-old boy whose weight is 17 kg and height is 95 cm. He was born to non-consanguineous parents at 37 weeks of gestation. At birth, his weight was 2.5 kg and crown-heel length was 45 cm. His mother and father were 25 and 29 years old, respectively, at the time of his birth. He was delivered through a cesarean section procedure.

The patient showed expressive speech and developmental delay, moderate intellectual disability, balanic hypospadias, craniofacial dysmorphisms, pervious foramen ovale without hemodynamic consequences, myopia, low-set ear, hypertelorism, and strabismus. His only elder brother is normal. Family history is unremarkable on both sides.

MATERIAL AND METHODS

The parents signed the informed consent forms approved by the Ethics Committee on Human Research at Pontifícia Universidade Católica de Goiás (CEP-PUC/GO), under the protocol No. 1721/2011. Cytogenetic studies were performed using peripheral blood samples of the patient and his parents by G-banding at approximately 550 band resolution. Short-term lymphocyte cultures were done for the proband and his parents following standard procedures (Verma and Babu, 1995). Genomic DNA was isolated from the peripheral blood of the proband and his parents using QIAamp® DNA mini kit (Qiagen, Germany). Thereafter, genetic analyses were performed on these samples to establish if the origin of DNA rearrangements was *de novo* or inherited. Next, 250 ng DNA of each sample was digested with *NspI*, ligated, PCR amplified and purified, fragmented, biotin-labeled, and hybridized to be used in a GeneChip™ HD CytoScan Array (Affymetrix, Santa Clara, CA, USA), according to manufacturer instructions. The array, designed specifically for cytogenetic research, included approximately 2,696,550 CNV (copy number variation) markers, 743,304 SNP markers, and >1,953,246 non-polymorphic markers. CEL files obtained by scanning the arrays were analyzed using the Chromosome Analysis Suite (ChAS) software (Affymetrix). Gains and losses that affected a minimum of 50 and 25 markers, respectively, in a 100-kb length were initially considered. For determining the LCR structure of 5q35.2q35.3, the segmental duplication track of the <http://genome.ucsc.edu> browser was used. An analysis of the duplicated genomic sequences, including known LCRs, was performed (segmental duplication >1 kb of non-repeat masked sequence with over 99% similarities) by comparing them with the ~3.04-Mb region surrounding the proximal 5q35.2q35.3 locus (chr5:175,085,426-178,129,575).

RESULTS

G-banded karyotyping at approximately 550 band resolution showed a male karyotype (46, XY). CMA detected one genomic imbalance event in the patient's genome,

which corresponds to *de novo* 0.45 Mb microduplication at 5q35.2q35.3 (176,382,009-176,832,993) x3 (Figure 1). This region included 12 genes: *UIMC1*, *ZNF346*, *FGFR4*, *NSD1*, *RAB24*, *PRELID1*, *MXD3*, *LMAN2*, *RGS14*, *SLC34A1*, *PFN3*, and *F12*. Among these genes, *NSD1* is directly involved with SS. Detailed analyses of the rearranged 5q35.2q35.3 region revealed that three major LCR families contributed to the complexity of this region. Two 211-kb segments (211A and 211B) flanking the disease locus are directly repeated and have 99.2% identity. Immediately adjacent and distal to the 211A and 211B direct repeats, there are two 16-kb segments (16A and 16B) that have 99% identity. Furthermore, there are two 95-kb segments (95A and 95B) that share 98.1% identity (Figure 2).

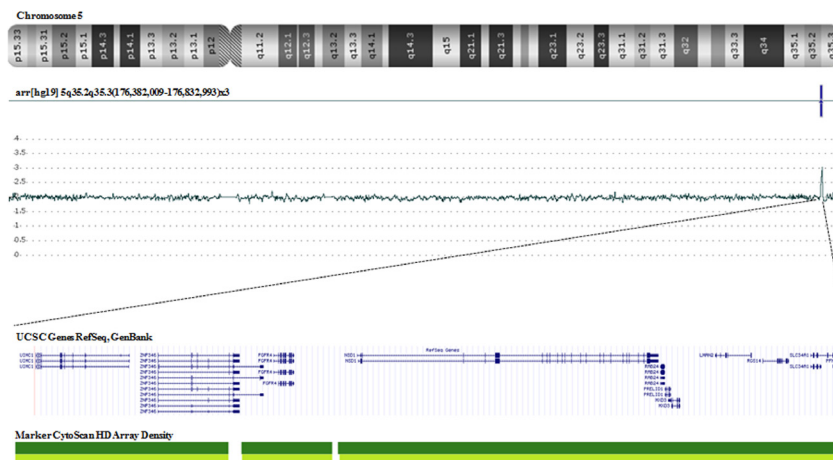


Figure 1. Content of the 5q35.2q35.3 microduplication. CMA showing microduplication of chromosomal segment at 5q35.2q35.3 from the proband.

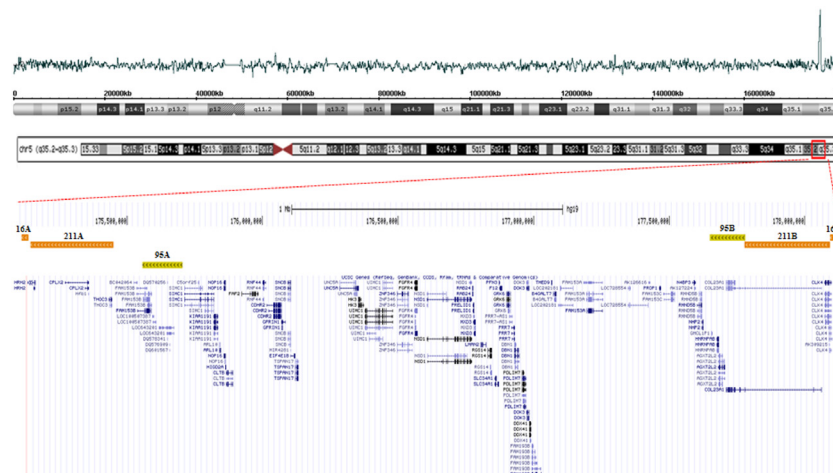


Figure 2. Representation of the three LCR families in the *de novo* duplicated 0.45 Mb chromosomal region at 5q35.2q35.3. There are two LCR families that share 99% identity (211A and 211B; 16A and 16B) and one LCR family that shares 98% identity (95A and 95B).

DISCUSSION

Here we report the first case of a microduplication associated with the Sotos critical region in Goiás State of central Brazil. The proband exhibits short stature, microcephaly, developmental delay, moderate intellectual disability, craniofacial dysmorphisms, and hypertelorism, which resembles the phenotype associated with microduplication of 5q35.2q35.3 in the region containing *NSDI* in SS (Nevado et al., 2014). Our results also suggest that 12 other genes in the microduplicated 5q35.2q35.3 region could be involved in the pathology of the proband's phenotype.

Variations in human phenotype are generated by chromosomal structural rearrangements, which also contribute towards susceptibility to diseases (Franco et al., 2010). Variations in genomic copy number manifest differently, with duplications and deletions of the same genomic region showing opposite phenotypes (Rosenfeld et al., 2013).

Alteration in *NSDI* dosage is responsible for Sotos syndrome, which has an inverted phenotype (Chen et al., 2006; Zhang et al., 2011). However, it is inappropriate to term the phenotype arising from the 5q35 microduplication as a “reversed SS” as there are other chromosomal imbalances that manifest as short stature and microcephaly, but lack the “reversed facial features” that are observed in patients harboring the duplicated 5q35.2q35.3 chromosomal region (Dikow et al., 2013).

Microduplication occurs via NAHR; when the rearrangement breakpoints flank LCRs, recurrent deletion is followed by reciprocal duplication (Novara et al., 2014). Analysis of the genomic structure of the rearranged 5q35.2q35.3 chromosomal region revealed two major LCR families. These LCRs are possibly involved in generating the recurrent deletions and reciprocal duplications by NAHR in SS.

This is the first case report of a microduplication event at 5q35.2q35.3 from central Brazil. Microdeletion in this region occurs with high frequency and is associated with the SS. However, the characterized microduplication has reciprocal rearrangement and the reverse phenotype of microdeletion. We believe that CMA is a potential diagnostic tool that would identify microrearrangements and guide medical diagnosis in the future. The diagnosis has to be complemented by non-directive genetic counseling to improve the quality of life of patients and their family.

Conflicts of interest

The authors declare no conflict of interest.

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