



Short report

MLPA analysis in 30 Sotos syndrome patients revealed one total *NSD1* deletion and two partial deletions not previously reported

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ABSTRACT

Sotos syndrome (MIM #117550) is an autosomal dominant condition characterized by pre and postnatal overgrowth, macrocephaly and typical facial *gestalt* with frontal bossing, hypertelorism, antimongoloid slant of the palpebral fissures, prominent jaw and high and narrow palate. This syndrome is also frequently associated with brain, cardiovascular, and urinary anomalies and is occasionally accompanied by malignant lesions such as Wilms tumour and hepatocarcinoma. The syndrome is known to be caused by mutations or deletions of the *NSD1* gene.

To detect both 5q35 microdeletions and partial *NSD1* gene deletions we screened 30 Brazilian patients with clinical diagnosis of Sotos syndrome by multiplex ligation dependent probe amplification.

We identified one patient with a total deletion of *NSD1* and neighbouring *FGFR4*, other with missing *NSD1* exons 13–14 and another with a deletion involving *FGFR4* and spanning up to *NSD1* exon 17. All deletions were *de novo*. The two *NSD1* partial deletions have not been previously reported.

The clinical features of the three patients included a typical facial *gestalt* with frontal bossing, prominent jaw and high anterior hairline; macrocephaly, dolichocephaly, large hands; neonatal hypotonia and jaundice. All presented normal growth at birth but postnatal overgrowth. Two patients with *NSD1* and *FGFR4* gene deletions presented congenital heart anomalies.

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1. Introduction

Sotos syndrome (MIM #117550) (SoS) is an overgrowth condition characterized by pre and postnatal overgrowth, macrocephaly and a typical facial *gestalt* with frontal bossing, hypertelorism, palpebral fissures, antimongoloid slant, prominent jaw, large ears, high and narrow palate and large hands and feet. The syndrome is also frequently associated with brain, cardiovascular, and urinary anomalies and is occasionally accompanied by malignant lesions such as Wilms tumour and hepatocarcinoma [1,2,6,9].

SoS is caused by haploinsufficiency of the *NSD1* gene (Nuclear receptor binding Su-var, enhancer of zeste, and trithorax domain protein 1) located at chromosome region 5q35 [7]. The *NSD1* gene has 23 exons and encodes at least six functional domains possibly related to chromatin regulations (SET, PWWP-I, PWWP-II, PHD-I, PHD-II and PHD-III) [9]. *NSD1* mutations cause SoS in ~76% of

non-Japanese individuals, whereas large deletions involving this same gene are responsible for ~10% of cases [12]. The large majority of *NSD1* abnormalities are *de novo* events, but there are several reports of autosomal dominant inheritance [3].

Herein, we present the results of multiplex ligation dependent probe amplification (MLPA) screening of *NSD1* gene as a primary genetic test for 30 Brazilian patients with clinical diagnosis of SoS. This test was optimized to detect 5q35 microdeletions as well as partial *NSD1* gene losses. We were able to detect one patient with a total deletion of *NSD1* and neighbouring *FGFR4* genes, other with missing *NSD1* exons 13–14 and another with a deletion involving *FGFR4* and spanning up to *NSD1* exon 17. The two *NSD1* partial deletions have not been previously reported.

2. Materials and methods

2.1. Subjects

We investigated 30 Brazilian patients of non-Japanese ancestry with clinical diagnosis of SoS (facial *gestalt*, macrocephaly,

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overgrowth, poor coordination and/or learning disabilities) referred to our Center by physicians for SoS genetic testing. They were examined by at least one of the authors, following a standard protocol that included physical and behavioral characteristics determination. This research was approved by the Institutional Ethics Committee and informed consent was obtained on behalf of all patients.

2.2. Methods

Patients and parental genomic DNA were extracted from peripheral blood leukocytes by Autopure LS (Gentra Systems).

All 30 patients were investigated using the MLPA technique with the Salsa MLPA kit P026B (MRC-Holland, Amsterdam, Netherlands). MLPA is a high throughput, sensitive technique for detecting copy number variations in genomic sequences [11]. The MLPA kit P026B contains 24 probes that cover all 23 exons of *NSD1* gene, two probes for *FGFR4*, one probe for *FLT4*, one for *TRIM52* and 16 control probes for other chromosomes. The results were analyzed using the Fragment Profile program (Amersham Bioscience). Chromatograms were initially visually checked and then samples peak areas were exported to an Excel template developed by NGRL, Manchester Analysis Sheets (Manchester, UK). Parents from patients with deletion were also investigated.

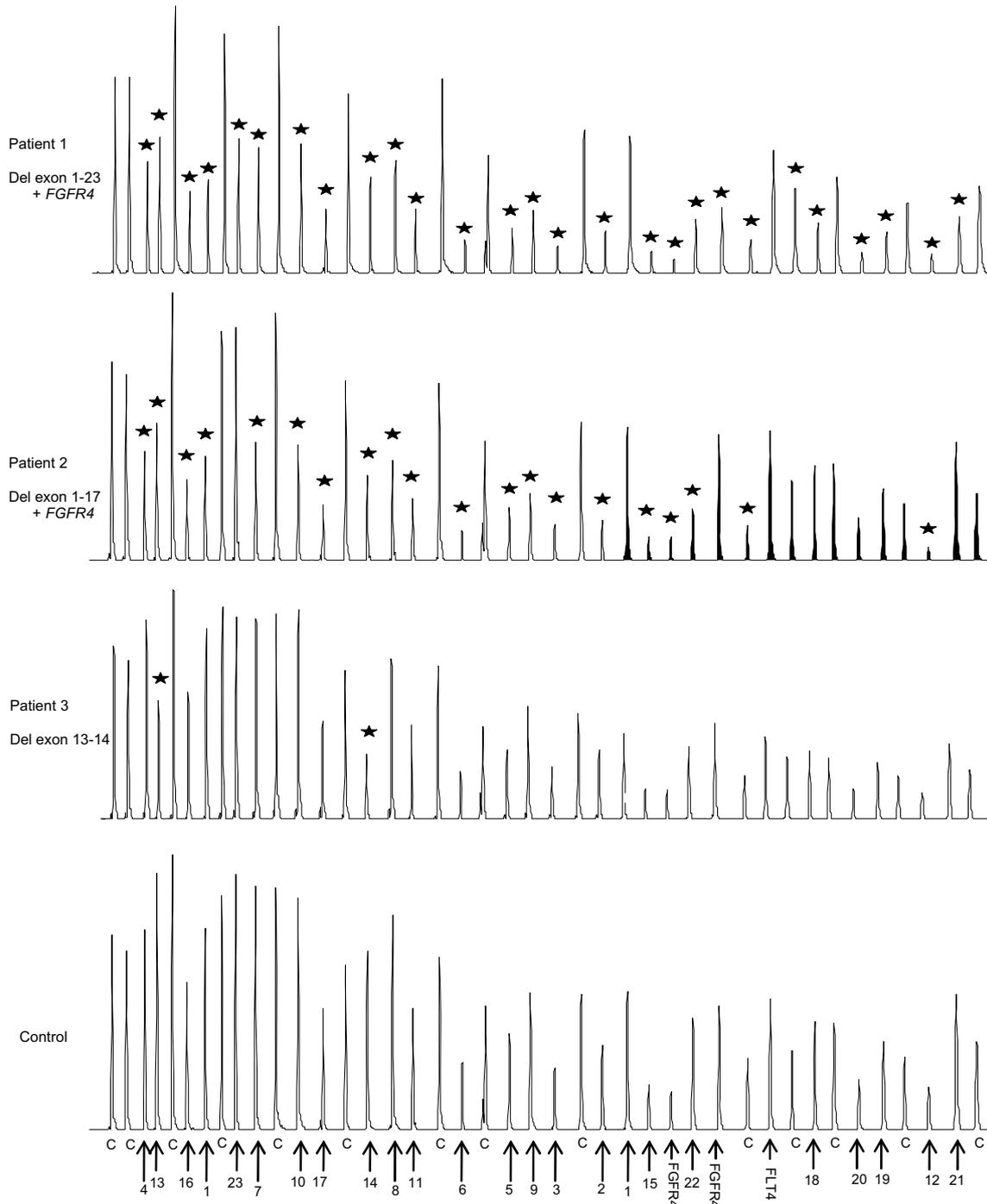


Fig. 1. Multiplex ligation dependent probe amplification (MLPA) analysis in Sotos syndrome cases with partial/total *NSD1* deletions and a control. The arrows indicate deleted exons. Numbers at the bottom of the figure refer to the *NSD1* exons; C indicates a control probe.

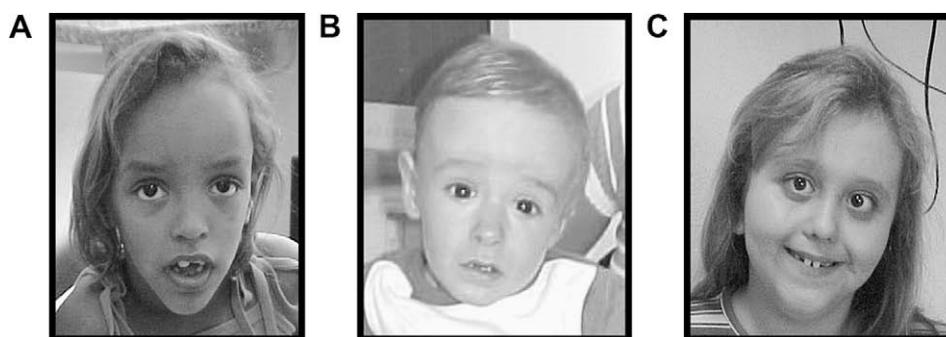


Fig. 2. A. Patient 1 with total deletion of *NSD1* and *FGFR4* genes. B. Patient 2 with deletion spanning from *FGFR4* up to exon 17 of *NSD1* gene. C. Patient 3 with deletion of *NSD1* exons 13–14.

3. Results

Three patients with deletions were identified: in patient 1 a complete deletion for *NSD1* and *FGFR4* genes, in patient 2 a deletion spanning the *FGFR4* gene up to exon 17 of *NSD1* and in patient 3 *NSD1* exons 13–14 (Fig. 1). No deletions/duplications for *TRIM52* and *FLT4* genes located telomeric to *NSD1* were found. The parents of all three patients were normal. No *NSD1* copy

number variations were identified amongst the remaining 27 patients.

3.1. Patients

The three patients had frontal bossing, prominent jaw and high anterior hairline (Fig. 2); macrocephaly, dolichocephaly, large hands; neonatal hypotonia and jaundice. Developmental delay, poor coordination, and learning disabilities were present in patients 1 and 3. Patient 2 was too young to have a definite developmental evaluation, but at 8 months he was able to sit without support. In all of them, growth at birth was normal but at evaluation, height was at or above P97 (Table 1). Patients 1 and 3 had behavioral problems, including hyperactivity, anxiety and aggressiveness. Two patients presented cardiovascular anomalies: patient 1 had atrial septal defect and patent foramen ovale and patient 2 had atrial septal defect.

Table 1
Phenotypic and behavioral characteristics of SoS patients with deletions.

Phenotypic characteristics	Patient 1	Patient 2	Patient 3
Age at diagnosis (years)	5	8/12	7
Maternal age at birth (years)	25	25	39
Paternal age at birth (years)	32	23	41
Birth length (centile)	10 > p > 3	p = 90	75 > p > 50
Birth weight (centile)	25 > p > 50	10 > p > 3	p < 2.5
Current height (centile)	p97	p > 97	p > 97
Current weight (centile)	75 > p > 50	p > 97	p > 97
Macrocephaly at birth	n.k.	+	n.k.
Macrocephaly	+	+	+
Dolichocephaly	+	+	+
Frontal bossing	+	+	–
High anterior hairline	+	+	+
Hypertelorism	+	+	–
Palpebral fissures antimongoloid slant	+	+	–
Strabismus	+	–	+
Large ears	–	+	–
Prominent jaw	+	+	–
Advanced bone age	–	n.k.	+
Joint laxity	–	n.k.	+
Large hands (p>97th)	+	+	+
Large feet (p>97th)	–	–	+
Pes planus	+	+	–
Neonatal hypotonia	+	+	+
Neonatal poor feeding	–	+	+
Neonatal jaundice	+	+	+
Sitting without support	10 months	8 months	8 months
Independent gait	24 months	n.a	25 months
Poor coordination	+	n.k.	+
Acquired language delay	+	+	+
Learning disabilities	+	n.k.	+
Drooling	+	–	–
Other	Atrial septal defect and patent foramen ovale	Atrial septal defect	Nystagmus
Behavioral characteristics			
Temper tantrums	–	n.k.	+
Aggressiveness	+	n.k.	+
Hyperactivity	+	n.k.	+
Anxiety	+	n.k.	+
Anti-social behaviour	+	n.k.	+

Legend: + presence; – absence; n.a.: not applicable; n.k.: not known.

4. Discussion

In order to detect both 5q35 microdeletions and partial *NSD1* gene deletions, 30 patients with clinical diagnosis of SoS were investigated using MLPA technique. Three patients with *NSD1* haploinsufficiency were detected. In patient 1, the *NSD1* and *FGFR4* whole gene deletions were paternally derived (data not show); patient 2 had a deletion spanning from *FGFR4* gene up to *NSD1* exon 17; and in patient 3, *NSD1* exons 13–14 were missing. The two partial deletions (Fig. 3) have different breakpoints and were not previously reported. As no deletions were found in their parents, mutations occurred as a result of *de novo* events. The ~2 Mb common deletion in patient 1 is probably the result of non-allelic homologous recombination between LCRs (Low Copy repeats) one proximal (SoS-PTR) and the other distal (SoS-DREP) found in the breakpoints [8]. In patients 2 and 3 the non-homologous end joining (NHEJ) between Alu elements is the most likely mechanism since *NSD1* is enriched for these elements with a density of 40.2% compared with 10.6% for the entire genome [4].

In a previous study of 18 SoS patients negative for *NSD1* mutations and large 5q35 microdeletions, screening by MLPA assay for exonic deletions/duplications of the *NSD1* gene revealed eight unique partial deletions involving exons 1–2, exons 3–5, exons 9–13, exons 19–21 and exon 22 [4].

Furthermore, Saugier-Verber et al. [10], in a series of 116 patients with SoS, detected six *NSD1* gene small exonic rearrangements, including five partial deletions (exon 2, exons 6–8, exons 22–23, and exons 18–19 in two patients) and a duplication involving exon 4. Interestingly, for patient with exons 18–19 deletions, a somatic mosaicism was suggested.

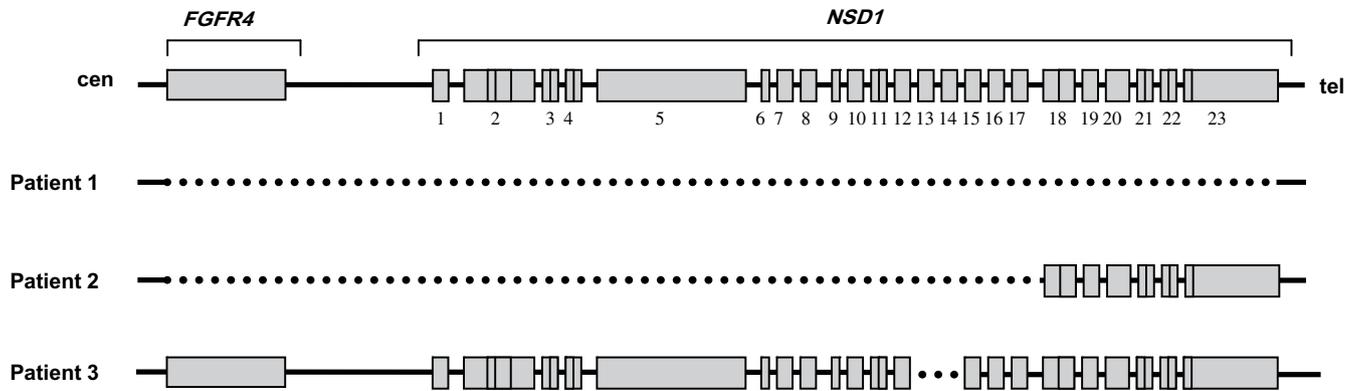


Fig. 3. Schematic representation of *NSD1* and *FGFR4* genes showing size and position of partial deletions. The dot lines indicate the position and extent of the deletions.

Congenital heart defects are relatively frequent in SoS, being reported in 8.7% of patients with intragenic mutations and in 55.5% of patients with microdeletions. Also variability in the prevalence of congenital heart defects has been noted in different populations [5,10]. Previously, Nagai et al. [9] reported in Japanese patients that cardiovascular and kidney anomalies are caused by dosage effects of genes on 5q35 other than the *NSD1* gene. By the other hand, Tatton-Brown et al. [12] studying a cohort of 266 non-Japanese individuals with *NSD1* molecular abnormality, observed only a trend towards an increased frequency of congenital heart defect in conjunction with 5q35 microdeletions. It is possible that *NSD1* haploinsufficiency contributes to the presence of congenital heart defects in SoS patients [5].

Our patients (1, 2) with *NSD1* and *FGFR4* gene deletions presented cardiovascular anomalies, which was not observed in patient 3 whose molecular abnormality was restricted to *NSD1* gene. Interestingly, one of the eight patients studied by Douglas et al. [4] also presented *NSD1* exon 1–2 combined to a *FGFR4* deletion. This patient had the characteristic facial *gestalt*, macrocephaly and also cranio stenosis, but cardiovascular anomalies were absent.

The spectrum of *NSD1* intragenic mutations are wide and can be recognized only by sequencing [10]. MLPA technique provides the detection of partial or whole gene deletions/duplications and could be used as a primary genetic test in individuals with clinical diagnosis of SoS, independently of ethnic background.

In summary, MLPA screening of 30 Brazilian patients with a clinical diagnosis of SoS detected *NSD1* microdeletions in three individuals: in one of them, it involved exons 13–14, in other missing fragment ranged from *FGFR4* gene up to exon 17 of *NSD1* and in another comprised whole *NSD1* and the neighbouring *FGFR4* gene.

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