RESEARCH NOTE

Deletion of *NSD1* exon 14 in Sotos syndrome: first description

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Introduction

Sotos syndrome (OMIM 11755) is a congenital disorder characterized by overgrowth, advanced bone age, macrocephaly, facial dysmorphic features and variable degrees of mental retardation. Most cases are sporadic (Cole and Hughes 1990), but familial cases with autosomal dominant inheritance have also been described (Tei *et al.* 2006). The major diagnostic criteria established by Cole and Hughes were in force for clinical diagnosis of Sotos syndrome until 2002, when Kurotaki *et al.* (2002) discovered that haploinsufficiency of the nuclear receptor set domain containing protein 1 gene (*NSD1*) causes Sotos syndrome. Tatton-Brown *et al.* (2005) reviewed 239 patients with *NSD1* abnormalities: overgrowth, facial dysmorphisms and learning disability were present in 90% of these patients; the height and head circumference of 10% of the subjects were within normal range. Advanced bone age was found in 76% of individuals. The authors concluded that overgrowth is not obligatory for the diagnosis of Sotos syndrome and advanced bone age should not be considered as mandatory diagnostic criterion, in agreement with other authors (Leventopoulos *et al.* 2009a). However, classic phenotype of Sotos syndrome is conventionally considered as one of the most frequent overgrowth conditions, although its variable expressivity has been clearly noted. Craniofacial abnormalities include macrocephaly, broad forehead, high hairline, downslanting palpebral fissures, prominence of the pointed chin, mild micrognatia. Cardiac and renal anomalies, seizures and scoliosis are described in 15%–30% of cases with variable degrees of severity (Tatton-Brown and Rahman 2007; Leventopoulos *et al.* 2009b). A broad spectrum of other clinical findings can be variably associated (table 1). Psychomotor and intellectual developments are variable, range from normal to severe mental retardation. Developmental delay is characterized by clumsiness, some degree of learning impairment; adaptive problems, autistic features, attention deficit and hyperactivity disorders could be present. Most affected individuals show no specific neuroimaging abnormalities: ventricular dilatation and hypoplasia of the corpus callosum are commonly reported (Leventopoulos *et al.* 2009a). Finally, Sotos syndrome is associated with an increased risk of benign or malignant tumours, recently estimated about 2%–3%. Haematological malignancies are the most common, but the spectrum of tumour types is broad (neuroblastoma, sacrococcygeal teratomas, presacral ganglioneuroma) (Tatton-Brown and Rahman 2004, 2007). Clinical suspicion needs molecular confirmation because of the variable phenotypic expression. Kurotaki *et al.* (2002) reported that Sotos syndrome is caused by *NSD1* haploinsufficiency. The *NSD1* gene on chromosome region 5q35.3 consists of 23 exons (Kurotaki *et al.* 2002). NSD1 contains multiple functional domains and it was proposed that this protein acts as a transcription regulator, able to function both as corepressor and coactivator (Rayasam *et al.* 2003). In most cases *NSD1* intragenic mutations or 5q35 microdeletions are identified, encompassing part or whole of the gene, causing *NSD1* haploinsufficiency (Kurotaki *et al.* 2002; Douglas *et al.* 2003). In Japanese patients, 5q35 microdeletions are the major cause of the syndrome whereas intragenic mutations are more frequent in nonJapanese affected individuals. Even if mutations and deletions of *NSD1* gene are responsible for most cases of Sotos syndrome, rare cases of other chromosomal abnormalities associated with overgrowth and some typical facial gestalts are reported, including a *de novo* apparently balanced t(3;6) (p21;21) and Robertsonian translocation (15q;15q) (qter→p11:: q12or q13→qter) with a consequent deletion of 15q12 or q13 (Schrander-Stumpel *et al.*

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Table 1. A broad spectrum of clinical findings can be associated with variable prevalence among affected patients. Modified from Tatton-Brown *et al.* (2005) and Douglas *et al.* (2005).

Cardinal features (> 90% of patients)	Characteristic facial appearance Learning disability Overgrowth: height and/or head circumference ≥ 98th percentile	
Major features (> 15% of patients)	Advanced bone age Cardiac anomalies Cranial magnetic resonance imaging or CT abnormalities Hyperlaxity/pes planus Maternal pre-eclampsia Neonatal hypotonia Neonatal jaundice Neonatal poor feeding Renal anomalies Scoliosis Seizures	
Other features	Anal fistula Arthrogryposis Astigmatism Behavioural problems Brachydactyly Cataract Cervical ribs Cutis laxa Cholesteatoma Conductive hearing loss Constipation Contractures Craniosynostosis Delayed visual maturation Eleven rib pairs Gastroesophageal reflux Genu valgum Hemangioma Hemihypertrophy Hydrocele Hypercalcemia Hypermetropia Hyperpigmentation Hypopigmentation	Hypoplastic nails Hypospadias Hypothyroid Inguinal hernia Laryngomalacia Myopia Neonatal glaucoma Neonatal hypoglycemia Neonatal thrombocytopenia Neonatal thrombophlebitis Nystagmus Osteoporosis Ovarian cysts Pectus carinatum Pectus excavatum Phimosis Pneumotorax Renal vein thrombosis Strabismus Talipes Two to three toe syndactyly Tumours Umbilical hernia Vertebral anomalies

1990; Wajntal *et al.* 1993). Finally, a variable proportion of patients with a clinical diagnosis do not show *NSD1* abnormalities. It has been suggested that this may be due in part to allelic heterogeneity in some patients with molecular aberrations not identifiable by commonly used screening techniques (Faravelli 2005). About 5% of cases are caused by partial *NSD1* deletions, involving entire exons, identifiable with multiplex ligation dependent probe amplification (MLPA) analysis (Douglas *et al.* 2005). Diagnostic techniques employ hybridation *in situ* fluorescence (FISH) with a single *NSD1* probe to detect 5q35 microdeletions, together with mutational screening of all *NSD1* exons by heteroduplex analysis or direct sequencing; however, these techniques are not always adequate to detect deletion or duplication of one or more exons (Faravelli *et al.* 2003; Raca *et al.* 2003; Douglas *et al.* 2005). We report a case of Sotos syndrome caused by deletion of the entire *NSD1* exon 14, never described previously.

Materials and methods

Case report

Patient, four-year-old male and first child of nonconsanguineous healthy parents. He was born at 37 weeks of gestation by cesarean delivery; his birth weight was 2470 g, length 49 cm and head circumference 34 cm. Some diagnostic investigations were performed since severe hypotonia was noted at birth. Cerebral ultrasound study showed a septum pellucidum cyst, causing mild lateral displacement of the ventricles; in correspondence with the left germinative matrix a 1 cm hyperechogen hemorrhagic spot was seen. Abdominal ultrasound examination showed mild pielectasia (7 mm) on the left side. Standard karyotype was normal. A psychomotor and cognitive delay was seen, for which rehabilitation therapy was started when the infant was 4 years old. An echocardiography showed an open oval foramen and a brain MRI displayed mild dilatation of the lateral left

ventricle, modest expansion of cistern vermiana, perivascular space enlargement of the posterior periventricular white matter bilaterally in the frontal subcortical bilateral region, summit and semioval centre and hypoplasia of the corpus callosum. The skeletal age was estimated at 8 years (when the chronological age was 4 years). As a result of these findings the patient was referred to genetic counselling. At first observation, the patient, 4 years old, weighed 30 kg (> 97th centile), 121.5 cm tall (> 97th centile) and his head circumference was 54 cm (> 97th centile). Physical examination revealed macrocephaly, broad forehead, high hairline, narrow long face, hypertelorism with down slanting palpebral fissures, large ears and pointed chin (figure 1). Cryptorchidism was present on left side. A severe mental retardation was noted. Clinical features supported the suspicion of Sotos syndrome and molecular genetic analysis using MLPA showed a deletion of the entire exon 14 in *NSD1* region. This *NSD1* anomaly has never been described in patients with Sotos syndrome. Informed consent was taken from the parents for genetic analyses followed by the genetic counselling.

NSD1 molecular analysis

The *NSD1* gene consists of 23 exons, the first of which is noncoding. The structure of the gene was deduced from genomic coding NT_037666 and mRNA sequence AF395588 (GenBank database). The routine mutation analysis, consisting in a first screening by denaturing high performance liquid chromatography (DHPLC) followed by the sequencing of the fragments, showed a mobility shift. Sequencing was performed with the BigDye Terminator Cycle Sequencing kit on 3130xl automated sequencer (Applied Biosystems, Foster City, USA). We proceeded with MLPA if no sequence mutations were detected to identify microdeletions involving *NSD1* gene. MLPA SALSA P026 kit (MRC-Holland, Amsterdam, The Netherlands) contains one probe for each *NSD1* exon (two probes for exon 1), two probes for *FGFR4* gene located just before the *NSD1* promotor, as well as two probes close to the 5q telomere. In addition, the kit contains several reference probes on various chromosomes. All reactions (denaturation, ligation and PCR) were performed following the manufacturer's instructions. PCR products were run on 3130xl automated sequencer (Applied Biosystems, Foster City, USA) and data were analysed using Genemapper v 3.2 and Coffalyser v 6.0 software (Applied Biosystems, Foster City, USA). Deletion of an exon was indicated by a ratio of 0.5.

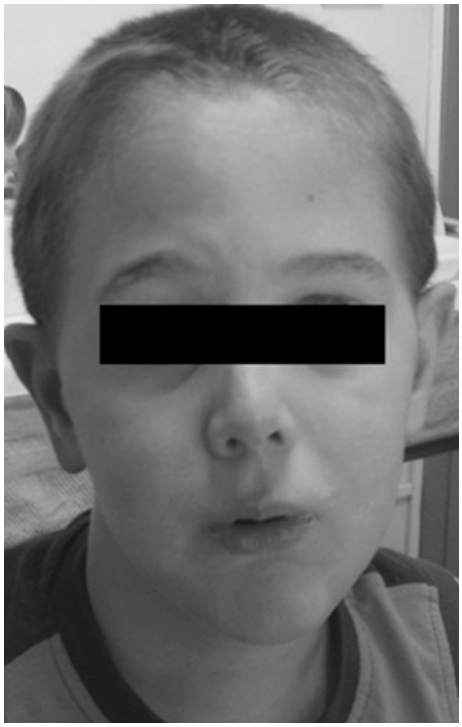

Figure 1. Patient at first observation. Macrocephaly, broad forehead, high hairline, narrow long face, hypertelorism with down slanting palpebral fissures, large ears, pointed chin can be clearly seen.

mRNA analysis

Total mRNA of the patient and of a normal control sample were prepared from lymphoblastoid cell line using TRIZOL reagent (Life Technologies-Gibco BRL, New York, USA). RNA was reverse transcribed into cDNA using Advantage RT-for-PCR kit (Clontech, Mountain View, USA). cDNA was amplified with forward primer (SOT13Fex) 5′-AGAACAAGGGCTTCCGGTGC-3′ (nucleotides 4931–4950 on exon 13) and reverse primer (PTT4Rint) 5′-CTGTCTTAGCTCTTTTTGGGCC-3′ (nucleotides 5613–5592 on exon 17, clone AF395588). The PCRs were performed with 1 μL 10 μM of forward and reverse primers, 2.5 μL of 10× buffer platinum, 0.75 μL 50 mM $MgCl_2$, 3 μL 1.25 mM dNTPs, 0.5 U Platinum *Taq* (Invitrogen, New York, USA) and 30 ng cDNA in a final volume of 25 μL. The amplified fragments were run on a 2% agarose gel (Eurobio, Parigi, France). Sequencing was performed on the smaller fragment eluted from the agarose gel. The cDNA was sequenced using BigDye Terminator Cycle Sequencing kit and 3130xl automated sequencer (Applied Biosystems, Foster City, USA).

Results

We performed a genetic analysis for Sotos syndrome in a 4-year-old patient with suspected phenotype. MLPA SALSA P026 kit was used to analyse the patient case, identified a partial *NSD1* deletion involving exon 14 (figure 2a). MLPA results were confirmed by expression experiments. RNA was extracted from lymphoblastoid cell lines and cDNA was obtained by RT-PCR. By amplification of the coding region spanning exons 13–17, two fragments were detected

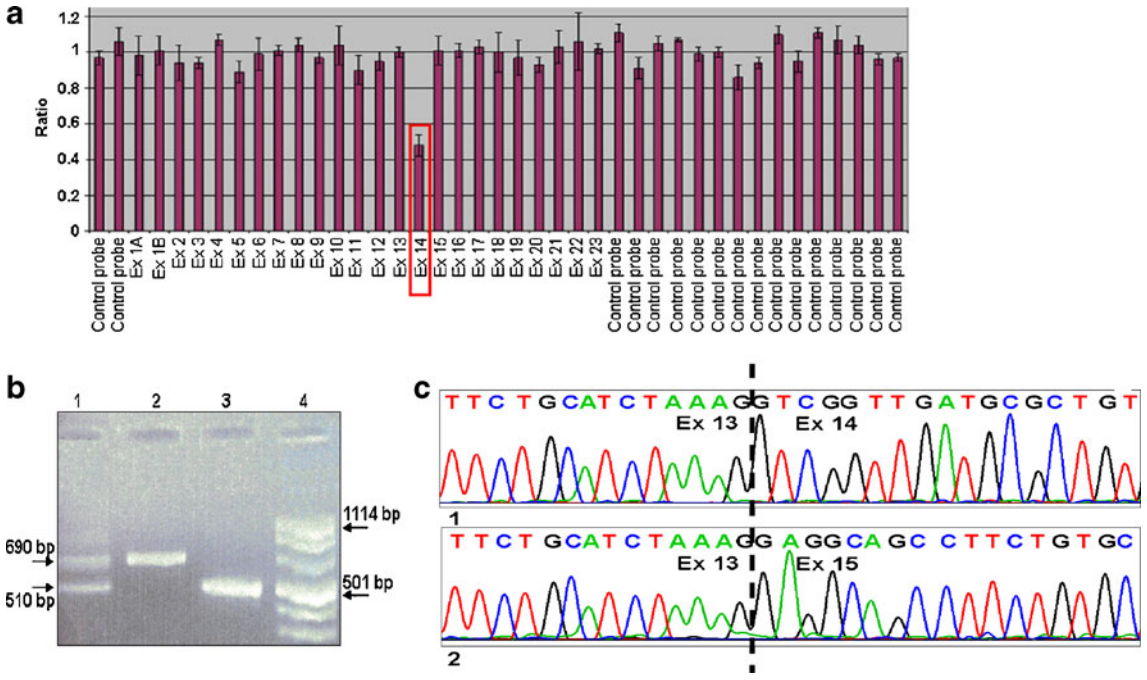

Figure 2. (a) MLPA results showing the apparent deletion of exon 14. (b) Amplification of the coding region spanning exons 13–17: lane 1, patient's cDNA showing normal allele (heavier band) and deleted allele (lighter band); lane 2, normal control's cDNA; lane 3, re-amplification of the purified fragment with the deletion shown in lane 1. (c) Electropherograms of a normal control cDNA (1) and of the smaller cDNA fragment showing the skipping of exon 14 of the *NSD1* gene in the patient (2).

(figure 2b, lane 1), one corresponding to the normal allele, the other, smaller in size, to the deleted allele (figure 2b, lane 3). Sequencing of the smaller cDNA fragment showed the skipping of the exon 14 without frame modification (figure 2c).

Discussion

This is the first case reported of a deletion involving *NSD1* exon 14 (resulting from a '*de novo*' event as demonstrated by family studies). The deletion spanned the PHDIII domain that is critical in chromatin mediated transcriptional regulation. Therefore, the deletion here described must be considered the cause of Sotos syndrome. Partial gene deletions of one or more *NSD1* exons are more recently identified causes of Sotos syndrome and occur in approximately 5% of cases (Douglas *et al.* 2005). Previous studies have shown that most of these deletions are generated through nonallelic homologous recombination (NAHR) between flanking *Alu* repeats, since in the *NSD1* genomic region *Alu* element density is approximately four times higher compared with the whole human genome (Douglas *et al.* 2005; Mochizuki *et al.* 2008). However, other mechanisms can occur and deletions can be generated through nonhomologous end joining (NHEJ). Douglas *et al.* (2005) identified eight partial *NSD1* deletions involving one or more exons in patients with classic phenotype using MLPA. Four cases had deletions of exons 1–2 on different breakpoints. In three of these cases, NAHR between *Alu* elements was the likely mechanism of generation. Saugier-Veber *et al.* (2007), using direct sequencing and a quantitative multiplex PCR of short fluorescent fragments (QMPSF), identified *NSD1* abnormalities in 104 patients, including point mutations, large deletions encompassing the entire *NSD1* gene, partial *NSD1* deletions involving one or more exons (exon 2, exons 6–8, exons 18 and 19 in two patients and exons 22–23), and a partial *NSD1* duplication involving exon 4. In one patient a somatic mosaic for exons 18 and 19 deletion was detected. Partial deletions or duplications were confirmed by using MLPA analysis. Recently, Fagali *et al.* (2009) described total/partial *NSD1* deletions in three patients with clinical diagnosis of Sotos syndrome using MLPA analysis. In one patient, deletion involved the entire *NSD1* gene and neighbouring *FGFR4*. The other patient exhibited *NSD1* exons 13–14 deletion, and another showed deletion involving *FGFR4* and spanning up to *NSD1* exon 17. The facial gestalts of three patients agree with the typical phenotype of Sotos syndrome and postnatal overgrowth was also present. Two patients with *NSD1* and *FGFR4* gene deletions showed congenital heart anomalies (atrial septal defect and patent foramen ovale), which were not present in the patient lacking *NSD1* exons 13–14. These data suggest that there may be many different *NSD1* anomalies leading to Sotos syndrome and the phenotype may not always be predictable on the basis of molecular analysis. Correlations between clinical phenotype and the type of

mutation remain largely undefined, and further studies are needed to clarify this issue. Several studies have attempted to highlight distinctive features in groups showing the same abnormalities of *NSD1* gene: 5q35 microdeletions are associated with less prominent overgrowth, more severe mental retardation and higher frequency of cardiac malformations compared with intragenic mutations (Douglas *et al.* 2005; Fagali *et al.* 2009). On the other hand, the study proposed by Tatton-Brown *et al.* (2005), comparing a large number of subjects with *NSD1* anomalies, showed no difference in the frequency of renal anomalies, scoliosis, or seizures between individuals with mutations and those with microdeletions. Finally, some evidence about the possible dosage effects of genes on 5q35 other than the *NSD1* gene suggested that the deletion of additional genes in patients with 5q35 microdeletions has little specific effect on phenotype (Tatton-Brown *et al.* 2005). In the light of recent literature, the occurrence of many features, variably associated with the main facial gestalt, did not seem to be strongly correlated with genotype and individuals with identical mutations can show different phenotypes. Because of the significant phenotypic variability and the importance of adequate early multidisciplinary therapeutic program, the diagnosis of Sotos syndrome requires molecular confirmation. In more recent times, partial deletions of one or more exons appear to be an emerging cause. Although the sequence 5′ to *NSD1* is particularly enriched with *Alu* elements and partial deletion described by Douglas *et al.* (2005) frequently encompasses exons 1–2, partial deletions may involve all other exons. We reported a new partial deletion of exon 14 in *NSD1* gene in one young patient with dysmorphic features compatible with Soto's syndrome, severe mental retardation, pielectasia and cryptorchidism. This is the first case report of a *de novo* deletion involving only exon 14.

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