

## ARTICLE

# Mutations in *NSD1* are responsible for Sotos syndrome, but are not a frequent finding in other overgrowth phenotypes

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Recently, deletions encompassing the nuclear receptor binding SET-Domain 1 (*NSD1*) gene have been described as the major cause of Japanese patients with the Sotos syndrome, whereas point mutations have been identified in the majority of European Sotos syndrome patients. In order to investigate a possible phenotype–genotype correlation and to further define the predictive value of *NSD1* mutations, we performed mutational analysis of the *NSD1* gene in 20 patients and one familial case with Sotos syndrome, five patients with Weaver syndrome, six patients with unclassified overgrowth/mental retardation, and six patients with macrocephaly/mental retardation. We were able to identify mutations within the *NSD1* gene in 18 patients and the familial case with Sotos syndrome (90%). The mutations (six nonsense, eight frame shifts, three splice site, one missense, one in-frame deletion) are expected to result in an impairment of *NSD1* function. The best correlation between clinical assessment and molecular results was obtained for the Sotos facial gestalt in conjunction with overgrowth, macrocephaly, and developmental delay. In contrast to the high mutation detection rate in Sotos syndrome, none of the patients with Weaver syndrome, unclassified overgrowth/mental retardation and macrocephaly/mental retardation, harbored *NSD1* mutations. We tested for large deletions by FISH analysis but were not able to identify any deletion

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**cases. The results indicate that the great majority of patients with Sotos syndrome are caused by mutations in NSD1. Deletions covering the NSD1 locus were not found in the patients analyzed here.**

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## Introduction

Sotos syndrome is a well-defined and relatively common overgrowth syndrome characterized by pre- and postnatal overgrowth, developmental delay, advanced bone age, and a typical facial gestalt including macrodolichocephaly with frontal bossing, frontoparietal sparseness of hair, apparent hypertelorism, downslanting palpebral fissures, and facial flushing.

Weaver syndrome, another overgrowth syndrome, exhibits clinical signs that overlap with Sotos syndrome. However, the facial appearance including high and broad forehead, hypertelorism, prominent and long philtrum, and micrognathia is generally thought to be different from that of Sotos syndrome.<sup>1</sup> The existence of rare cases with overlap between both syndromes and many phenotypic similarities have been interpreted as allelic rather than locus heterogeneity.<sup>2</sup>

Recently, the analysis of chromosomal rearrangements involving chromosome 5q35 in two patients with Sotos syndrome has identified a putative locus for the disease.<sup>3,4</sup> Subsequently, deletions encompassing the nuclear receptor binding SET-Domain 1 (*NSD1*) gene as the major cause of Japanese patients with Sotos syndrome and point mutations as the major cause of European patients with Sotos syndrome have been published.<sup>5–7</sup> Miyake *et al*<sup>8</sup> found that microdeletions in Sotos syndrome mostly occurred in the paternally derived chromosome 5.

NSD1 belongs to a family of nuclear receptors (NR) that bind to DNA response elements upon binding of congeneric ligands such as steroid and thyroid hormones, or retinoids. NSD1 is a unique bifunctional cofactor with two distinct NR-interaction domains called NID<sup>-L</sup> and NID<sup>+L</sup>.<sup>9</sup> NSD1 also contains several conserved functional domains, that is, SET, SAC, PWWP, and PHD. The SET domain (*su(var)3-9*, *enhancer-of-zeste*, *trithorax*) was first identified as a motif present in the *Drosophila* proteins *SU(var)3-9*, *E(z)* and *TRx*,<sup>10,11</sup> and was subsequently found in a number of eucaryotic proteins. They were shown to play a role in cell growth and differentiation, to be associated with chromatin, and to function as a transcriptional repressor and/or a transcriptional activator. *NSD1* contains a Cys-rich region, which is composed of different arrangements of three conserved motifs, corresponding to a protein domain that has been called SAC for SET-associated Cys-rich domain.<sup>12</sup> The SAC domain may have a function in chromosome binding. In addition to the SET and SAC domains, *NSD1*

contains six other domains including two proline–tryptophan–tryptophan–proline (PWWP) domains and five plant homeodomain protein (PHD) domains. It has been suggested that the PHD finger domains involve chromatin-mediated transcriptional regulation.<sup>12</sup> The PWWP domain is thought to be involved in protein–protein interactions.<sup>13</sup> Adjacent to the C-terminus of the PHD-V domain is another region rich in cysteines and histidines, possibly corresponding to a zinc-finger-like motif. The function of *NSD1* remains largely unknown; however, the presence of activating as well as silencing domains and its property to bind liganded as well as unliganded NRs suggest that *NSD1* could be a versatile NR intermediary factor controlling transcription either negatively or positively.<sup>12</sup> The observation that haploinsufficiency of *NSD1* induces overgrowth prompted Kurotaki *et al*<sup>5</sup> to suggest that *NSD1* acts as a corepressor of genes that promote growth.

In spite of the well-defined phenotype, Sotos syndrome is frequently difficult to diagnose particularly for the inexperienced clinician. Considerable overlap with other ill-defined overgrowth phenotypes exists, further complicating the situation. The molecular analysis allows an unequivocal classification and a better definition of the range of clinical manifestations in Sotos syndrome. The present study describes the results of mutation and microdeletion analysis of the *NSD1* gene and the phenotypes in 20 patients and one familial case with Sotos syndrome, five patients with Weaver syndrome, six patients with an unclassified overgrowth/mental retardation syndrome, and in six patients with macrocephaly and mental retardation.

## Patients

In all, 37 patients and one familial case with a tentative diagnosis of Sotos or Weaver syndrome or with other childhood overgrowth phenotypes were clinically assessed on the basis of anthropometric measurements, their photographs, bone age, and developmental delay. Phenotypic evaluation was undertaken separately by experienced syndromologists.

The clinical findings of the patients (ages ranging from birth to 37 years) are summarized in Table 1. In our series, three patients originated from one Turkish family (patients 16a, b, c) with an autosomal dominant pattern of

**Table 1** Clinical manifestations and mutational status of our patients with Sotos syndrome (patients 1–21), with Weaver syndrome (patients 22–26), an unclassified overgrowth/mental retardation syndrome (patients 27–32), and macrocephaly (patients 33–38)

| Patient   | Sex | Age at ref. | Height (SD) | Weight >97th cen | Head circumference (SD) | Advanced bone age | Developmental delay | Facial gestalt of Sotos syndrome | Facial gestalt of Weaver syndrome | Additional Symptoms | Mutation |
|---|-----|-------------|-------------|------------------|-------------------------|-------------------|---------------------|----------------------------------|-----------------------------------|---------------------|----------|
| <i>Sotos syndrome</i>                             |     |             |             |                  |                         |                   |                     |                                  |                                   |                     |          |
| 1   | F   | 8.5 years   | +2.2        | NA               | +2.2                    | NA                | +                   | +                                | –                                 | Scoliosis           | +        |
| 2   | M   | 1 year      | +3.7        | +                | +2.6                    | +                 | +                   | +                                | –                                 |                     | +        |
| 3   | F   | 1 year      | +4.2        | +                | +2.7                    | +                 | +                   | +/-                              | +/-                               | Overlap phenotype   | +        |
| 4   | M   | 11 years    | +2.4        | +                | +3.3                    | +                 | +                   | +                                | –                                 |                     | +        |
| 5   | M   | 2 years     | +3.0        | +                | +4.0                    | –                 | ++                  | +                                | –                                 |                     | +        |
| 6   | M   | 2 years     | +3.1        | +                | +6.3                    | –                 | +                   | +                                | –                                 | Retarded bone age   | +        |
| 7   | F   | 30 years    | +1.6        | NA               | +3.6                    | NA                | +                   | +                                | –                                 |                     | +        |
| 8   | M   | 3 years     | +2.9        | +                | +2.1                    | NA                | ++                  | +                                | –                                 | Hyperactivity       | +        |
| 9   | M   | 15 months   | +2.6        | +                | +1.5                    | +                 | +                   | +                                | –                                 |                     | +        |
| 10  | F   | 5 years     | +2.0        | NA               | +3.8                    | +                 | +                   | +                                | –                                 |                     | +        |
| 11  | F   | 9.5 years   | +6.3        | +                | +4.2                    | +                 | +                   | +                                | –                                 |                     | +        |
| 12  | F   | 3 months    | +5.2        | +                | +0.7                    | +                 | +                   | +                                | –                                 |                     | +        |
| 13  | F   | 3 years     | +2.6        | Craniosynostosis | +                       | +                 | +                   | +                                | –                                 |                     |          |
|   |     |             |             | Ventriculomegaly | +1.6                    | +                 | +                   | +                                | –                                 |                     |          |
| 14  | M   | 10 years    | +3.0        | +                | +3.4                    | +                 | +                   | +                                | –                                 |                     | +        |
| 15  | F   | 6.5 years   | +4.0        | +                | +4.4                    | NA                | +                   | +                                | –                                 | A                   | +        |
| 16a   | F   | 37 years    | +2.4        | +                | +2.7                    | NA                | +                   | +                                | –                                 | Familial            | +        |
| 16b   | F   | 10 years    | +3.8        | +                | +5.9                    | +                 | +                   | +                                | –                                 | Familial            | +        |
| 16c   | M   | 5 years     | +1.9        | +                | +2.5                    | –                 | +                   | +                                | –                                 | Familial, B         | +        |
| 17  | F   | 10 years    | +2.3        | +                | +5.1                    | +                 | +                   | +                                | –                                 |                     | –        |
| 18  | F   | 5.5 years   | +2.1        | +                | +3.5                    | NA                | +                   | +                                | –                                 |                     | –        |
| 19  | F   | 4 years     | +5.0        | NA               | +3.5                    | +                 | +                   | +                                | –                                 |                     | –        |
|   |     |             |             | Neuroblastoma    | +                       | +                 | +                   | +                                | –                                 |                     |          |
| 20  | F   | 3 years     | +3.9        | +                | +3.7                    | NA                | +                   | +                                | –                                 |                     |          |
|   |     |             |             | Ventriculomegaly | +                       | +                 | +                   | +                                | –                                 |                     |          |
| 21  | M   | 6.5 years   | +3.8        | +                | +3.3                    | +                 | +                   | +                                | –                                 |                     |          |
|   |     |             |             | Ventriculomegaly | +                       | +                 | +                   | +                                | –                                 |                     |          |
| <i>Weaver syndrome</i>                            |     |             |             |                  |                         |                   |                     |                                  |                                   |                     |          |
| 22  | M   | 19 years    | +3.5        | +                | +3.9                    | +                 | +                   | –                                | +                                 | C, D, lymphoma      | –        |
| 23  | M   | Birth       | +4.2        | +                | +2.4                    | +                 | +                   | –                                | +                                 |                     | –        |
| 24  | F   | 2 years     | +6.4        | +                | +2.9                    | +                 | +                   | –                                | +                                 | C, D                | –        |
| 25  | F   | 3 years     | +4.5        | +                | +2.5                    | +                 | +                   | –                                | +                                 | D                   | –        |
| 26  | F   | Birth       | +3.0        | +                | +3.0                    | +                 | +                   | –                                | +                                 | D                   | –        |
| <i>Unclassified overgrowth/mental retardation</i> |     |             |             |                  |                         |                   |                     |                                  |                                   |                     |          |
| 27  | F   | 3.5 years   | +3.9        | +                | +2.1                    | +                 | +                   | –                                | –                                 |                     | –        |
| 28  | F   | 4 years     | +2.4        | +                | +1.9                    | +                 | +                   | –                                | –                                 |                     | –        |
| 29  | F   | 12 years    | +2.6        | +                | +3.2                    | +                 | +                   | –                                | –                                 |                     | –        |
| 30  | M   | 2.5 years   | +4.3        | +                | –1.1                    | +                 | +                   | –                                | –                                 |                     | –        |
| 31  | M   | 15 years    | +2.7        | +                | NA                      | +                 | +                   | –                                | –                                 |                     | –        |
|   |     |             |             | Ventriculomegaly | –                       | +                 | +                   | –                                | –                                 |                     | –        |
| 32  | M   | 22 years    | +0.3        | +                | NA                      | NA                | +                   | –                                | –                                 | Scoliosis, E        | –        |
| <i>Macrocephaly/mental retardation</i>            |     |             |             |                  |                         |                   |                     |                                  |                                   |                     |          |
| 33  | F   | 11 years    | +0.6        | –                | +5.2                    | NA                | +                   | –                                | –                                 |                     | –        |
| 34  | M   | 9.5 years   | +0.3        | –                | +4.0                    | NA                | +                   | –                                | –                                 |                     | –        |
|   |     |             |             | Ventriculomegaly | –                       | –                 | +                   | –                                | –                                 |                     | –        |
| 35  | F   | 10 years    | +0.4        | –                | +3.5                    | –                 | +                   | –                                | –                                 |                     | –        |
| 36  | F   | 10 years    | +0.2        | –                | +2.4                    | NA                | +                   | –                                | –                                 | Scoliosis           | –        |
| 37  | F   | 7 years     | +1.5        | –                | +1.9                    | NA                | +                   | –                                | –                                 |                     | –        |
| 38  | F   | 2.5 years   | +2.5        | –                | +2.1                    | NA                | –                   | –                                | –                                 |                     | –        |

Note: NA = data not available to us; A = corpus callosum hypoplasia; B = acute lymphoblastic leukemia; C = broad metaphyses; D = carpal bone age more accelerated than phalangeal bone age; E = overgrowth was present in childhood.

inheritance and patient 16c died due to acute lymphoblastic leukemia. The others are sporadic cases of German and Turkish (patients 31, 32) origin. Prior to molecular analysis, the patients were phenotypically scored into four groups. Patients were considered having Sotos syndrome ( $n=21$ ) if four of five major criteria, that is, overgrowth (height and weight  $>2$  SD), macrocephaly (head circumference  $>2$  SD), advanced bone age, developmental delay, and the Sotos facial gestalt, were present. Within this group, one patient (patient 3) showed craniofacial signs of both, Sotos and Weaver syndromes, and was therefore scored as an overlap phenotype. The diagnosis of Weaver syndrome ( $n=5$ ) was established by the presence of the typical facial gestalt, characteristic growth pattern, accelerated bone age, and developmental delay. Patient 22 affected with Weaver syndrome had been reported previously (as patient 1).<sup>1</sup> A group of six patients showed an unclassified phenotype with overgrowth, mental retardation, and a facial aspect different from that of Sotos or Weaver syndrome. The remaining six patients had macrocephaly and mental retardation.

### Molecular analysis

Genomic DNA was extracted from blood lymphocytes using the commercial GenoPrep™ DNA Isolation Kit. All exons of *NSD1* were amplified from genomic DNA with the primers designed by Kurotaki et al.<sup>5</sup> (Matsumoto, personal communication). PCR reactions were performed in 20  $\mu$ l containing 1  $\times$  PCR buffer, 1.5 or 2.5 mM MgCl<sub>2</sub>, 100  $\mu$ M of each dNTP, 1  $\mu$ l of each primer, and 2.5 U of *Taq* polymerase. The PCR conditions included an initial denaturation for 15 min at 96°C, followed by six cycles of 94°C for 30 s, 61–57°C for 45 s, 72°C for 60 s, 31 cycles of

94°C for 30 s, 55°C for 45 s, 72°C for 45 s, and a final extension for 10 min at 72°C. The analysis of PCR products was performed on 1.5% agarose gels.

PCR products were sequenced using Big Dye Terminator on an ABI Prism 3100 Genetic Analyzer (PE Biosystem, USA). Every mutation was confirmed by sequencing of the products from several independent PCRs.

### Metaphase Fluorescence *in situ* hybridization

Metaphase chromosome preparations were obtained from PHA-stimulated lymphocyte cultures according to standard procedures. For fluorescence *in situ* hybridization (FISH) analysis, PAC clone RP1-118m12 was labeled with digoxigenin-11-dUTP by nick translation and preannealed with a 50-fold excess of Cot-1 DNA. Target slides were pretreated using standard methods. Hybridizations were carried out at 37°C overnight. Detection of the probes was achieved by using the fluorochrome-conjugated antibodies mouse-anti-digoxigenin-FITC, rabbit-anti-mouse-FITC, and goat-anti-rabbit-FITC.

Slides were counterstained with 4,6-diamidino-2-phenylindole. Images were obtained using an epifluorescence microscope (Axioscope; Zeiss Germany) equipped with a cooled CCD camera (Photometries) and analyzed using IP Lab Spectrum software.

## Results

### Identification of heterozygous mutations in *NSD1*

Among the patients examined, we were able to detect a total of 19 different heterozygous mutations (Figure 2 and Table 2) in 21 cases with Sotos syndrome. Of these mutations, 17 are novel and two have been reported

**Table 2** *NSD1* mutations identified in the present study

| Exon  | Patient | Mutation         | Protein Change                     | Analysis of Parental Samples |
|-------|---------|------------------|------------------------------------|------------------------------|
| 5     | 1       | 1492C>T          | R498X                              | NA                           |
| 5     | 2       | 3091C>T          | R1031X                             | No mutation in parents       |
| 5     | 3       | 3141delC         | Frame shift                        | No mutation in mother        |
| 5     | 4       | 3160delA         | Frame shift                        | NA                           |
| 5     | 19      | 3541delGAAA      | Frame shift                        | NA                           |
| 5     | 5       | 3536delA         | Frame shift                        | No mutation in parents       |
| 7     | 6       | 4160insC         | Frame shift                        | No mutation in parents       |
| Int8  | 7       | IVS8-2A>G        | Predicted del ex9 and frame shift  | No mutation in parents       |
| 13    | 8       | 4806delTGTTAA    | Frame shift                        | No mutation in parents       |
| 13    | 20      | 4885C>T          | Q1629X                             | NA                           |
| 13    | 9       | 4895delG         | Frame shift                        | NA                           |
| 15    | 10      | 5194G>T          | E1732X                             | NA                           |
| Int15 | 21      | IVS15-1G>T       | Predicted del ex16 and frame shift | NA                           |
| 16    | 11      | 5386G>T          | V1796F                             | No mutation in parents       |
| 16    | 12      | 5398insT         | Frame shift                        | No mutation in parents       |
| 17    | 13      | 5611A>T          | K1871X                             | No mutation in parents       |
| Int19 | 14      | IVS19-2A>G       | Predicted del ex20 and frame shift | No mutation in parents       |
| 20    | 15      | 6013C>T          | R2005X                             | No mutation in parents       |
| 23    | 16a,b,c | 6532delTGCCCCAGC | 2178-2180 del CPS                  | Family                       |

NA = not available.

previously.<sup>5,6</sup> In the patient with the Sotos/Weaver overlap phenotype, we were able to identify a frame shift in exon 5. In the remaining 19 patients (two patients with Sotos syndrome, five patients with Weaver syndrome, six patients with unclassified overgrowth/mental retardation syndrome, and six patients with macrocephaly/mental retardation), no mutation was detected by direct sequencing.

### Polymorphisms

The novel polymorphisms identified in this study were 352C>G (P118A), 1482C>T (C494C), 2242A>G (N748N), 3993T>C (D1331D), 4883T>C (M1628T), 6393C>G (V2131V), and 7636G>A (A2546T). The identified polymorphisms 1749G>A (E583E), 1792T>C (L599L), 1840T>G (V614L), 2071G>A (A691T), 2176T>C (S726P), 3106G>C (L1091I), 3705T>C (N1235N), 6750G>A (M2250I), 6782T>C (M2261T), 6829C>T (L2277L), and 6903G>C (G2301G) have been published previously.<sup>6</sup>

### FISH investigations

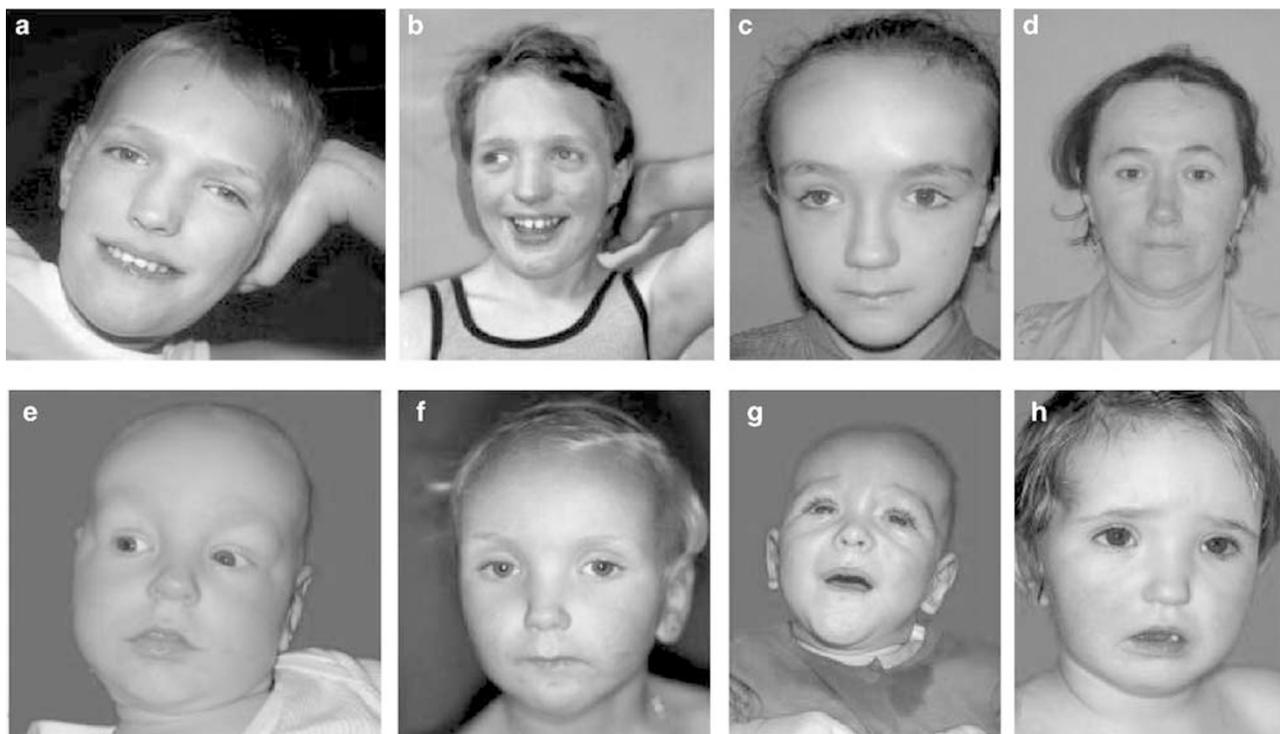
A total of 19 patients (patients 1, 2, 3, 5, 6, 7, 8, 10, 11, 14, 15, 17, 18, 29, 30, 33, 34, 35, and 36) were investigated by FISH for a deletion of *NSD1* locus using PAC RP1-118m12. The 5ptel probe (Appligene Oncor) was used as a control. In each of the 20 metaphases analyzed, FISH signals for the

*NSD1* locus were detected on both chromosomes 5. We tested PAC RP1-118m12 for its specificity by hybridizing digested PAC-DNA with probes specific for exons 2 and 4 of the *NSD1* gene. FISH analysis could not be performed in the remaining patients because of the unavailability of blood samples.

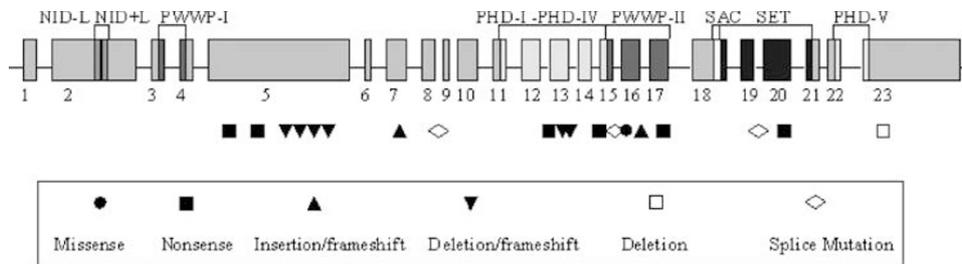
### Clinical data

We compared the clinical findings in the group of patients with Sotos syndrome and in the group of patients with Weaver syndrome, but not in the group of patients with unclassified overgrowth/mental retardation and with macrocephaly/mental retardation because of clinical heterogeneity.

Pre- and postnatal overgrowth, macrocephaly, the typical facial appearance, and development delay were all present in the majority of Sotos syndrome patients. However, in a subgroup of patients, either increased height or macrocephaly were lacking. Patients 10 and 16c were diagnosed as having Sotos syndrome on the basis of their facial gestalt, macrocephaly, and developmental delay, but their heights were still in the normal range at the age of 5 years (+2.0 SD) and of 5 years (+1.9 SD). In patients 9, 12, and 13, overgrowth, typical facial phenotype of Sotos syndrome, and development delay are present but macro-



**Figure 1** Facial photographs of patients harboring *NSD1* mutations. (a–g) Patients with Sotos syndrome (a, patient 14; b, patient 5; c, patient 16b; d, patient 16a; e, patient 2; f, patient 13; g, patient 9). (h) Patient 3 with craniofacial symptoms of both Sotos and Weaver syndromes.



**Figure 2** Schematic representation of *NSD1*, showing the localization of mutations in patients with Sotos syndrome. The 23 exons of *NSD1* are presented by boxes and introns are represented by lines. The domains of *NSD1* are represented with different colored boxes. The mutations identified in the present study are indicated below the diagrammatic structure of *NSD1*.

cephaly was lacking (Table 1). Among our patients with Sotos syndrome, postnatal height varied from +1.9 to +6.3 SD. Head circumference ranged from +0.7 to +6.3 SD. In our series of patients with Sotos syndrome, intellectual impairment varied from very mild (patients 7, 16a) to severe (patients 5, 8).

Three patients with Sotos syndrome did not fulfill the criterion of accelerated bone age, patients 5 and 16c showed a bone age within the normal range at the age of 30 months and 5 years; in patient 6, aged 25 months, a retarded bone age has been determined.

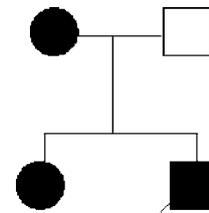
In patient 3, the diagnosis of Weaver syndrome was considered by the presence of the typical facial features during infancy, whereas in childhood the facial anomalies corresponded more to Sotos syndrome. Radiological assessment at 11 months of age showed no differences between the acceleration of carpal bones and phalangeal, radial, and ulnar epiphyses. In our opinion, the facial appearance of this patient draws attention to the existence of an overlapping phenotype between these syndromes (Figure 1h).

Besides overgrowth (ranged from +3.0 to +6.4 SD) and macrocephaly (ranged from +2.4 to +3.9 SD), the typical facial phenotype and developmental delay were present in all five patients with Weaver syndrome. Radiologically, patients 22, 24, 25, and 26 showed that the carpal bone age was more accelerated than the phalangeal bone age further supporting the diagnosis of Weaver syndrome.

The presence of *NSD1* mutations was exclusively demonstrated in the group of patients with Sotos syndrome. In this group, the detection rate was 90%. The best correlation between clinical assessment and molecular results was obtained for the Sotos facial gestalt in conjunction with overgrowth, macrocephaly and developmental delay.

## Discussion

We have conducted mutation and microdeletion analyses of the *NSD1* gene in a series of 21 cases including one familial case with Sotos syndrome, five patients with Weaver syndrome, six patients with an unclassified over-



**Figure 3** Pedigree of the familial case.

growth/mental retardation phenotype, and six patients with macrocephaly associated with mental retardation. All together, 19 mutations were detected that include frame shift, nonsense, missense, splice sites mutations, and in-frame deletion. In total, 17 mutations are described for the first time (Table 2, Figure 2). All were exclusively found in the group of patients with Sotos syndrome, indicating a very good correlation between the presence of *NSD1* mutations and the Sotos phenotype. Mutations occurred throughout the gene, but the majority is clustered between exons 5 and 23. In our series, all mutations were identified only once. Two mutations (3536delA and R498X) were previously described in two patients with Sotos syndrome reported by Kurotaki *et al*<sup>5</sup> and Douglas *et al*.<sup>6</sup>

The proportion of *NSD1* aberrations among the group of patients affected with Sotos syndrome is 90% in comparison to the frequency (77, 76%) reported previously by Kurotaki *et al*<sup>5</sup> and Douglas *et al*.<sup>6</sup> Interestingly, the best correlation between the molecular and the clinical findings was achieved for the facial gestalt in conjunction with overgrowth, macrocephaly, and developmental delay. Mutations were identified in 19 of 21 cases with the typical Sotos syndrome face resulting in a predictive value for this clinical sign of about 90%. This result includes one patient (patient 3) with an overlapping facial phenotype of both, Sotos and Weaver syndromes.

Previous studies have described the overall frequency of 84% for advanced bone age in patients with Sotos syndrome.<sup>14</sup> Our results confirm this finding and show that accelerated bone age is not an obligate symptom of Sotos syndrome.

In the patients with Weaver syndrome studied here, no *NSD1* alterations were identified. Douglas *et al*<sup>6</sup> identified three mutations in Weaver syndrome patients within a 40 amino-acid region encoding for the PHD-V domain and an adjacent domain rich in cysteines and histidines raising the possibility of a phenotype–genotype correlation. However, a mutation in this region was detected in a patient with typical the Sotos syndrome.<sup>6</sup> The molecular results in Weaver syndrome patients reported here and in the overlapping case do not support this hypothesis. Furthermore, the familial case with Sotos syndrome (Figure 3) in our series has an in frame three amino-acid deletion removing one of the Cys residues between the PHD-V and the Cys/His-rich domain. This mutation is in close vicinity (four amino acids) to a published Weaver syndrome (patient COG62)<sup>6</sup> mutation that replaces the cysteine residue 2183 by serine and is likely to have similar functional consequences. The function of the Cys/His-rich domain harboring the mutation is unknown, but sequence comparisons indicate homology to a zinc-finger-like motif. The three amino-acid deletion in our familial case removes one of the Cys residues and would be expected to have critical effects on the functional property of this domain. Alternatively, it may interfere with the function of the nearby PHD-V domain. The PHD-V domain contains a plant homeodomain, also designated as the C4HC3 motif,<sup>15</sup> with a zinc-finger-like motif that predominantly occurs in proteins that function at the chromatin level.

In summary, no consistent relationship has been observed between specific mutations and the severity of the disease or the expression of a particular clinical sign. In two previous studies so far, 37 mutations of the *NSD1* gene have been identified. Results from the present study, coupled with data from previously reported mutations<sup>5,6,16</sup> bring the total number of different mutations to 54. These data demonstrate that the *NSD1* gene is subject of strong allelic heterogeneity, and there appears to be no major mutational hotspot in Sotos syndrome.

Familial cases are rare in Sotos syndrome raising the question of whether an underlying defect in fertility accounts for the paucity of familial cases. In the report of Douglas *et al*,<sup>6</sup> the only patient who has a family history of Sotos syndrome showed a missense mutation in the PWWP-II domain raising the possibility that the observed mutation results in Sotos syndrome but not in reproductive impairment. Our molecular result of the familial case and the early truncating mutation in another familial case reported by Høglund *et al*<sup>16</sup> are not substantiated a correlation between the site and type of the mutation and fertility.

In contrast to the results of Kurotaki *et al*<sup>5</sup> and Douglas *et al*<sup>6</sup> deletions of the *NSD1* gene were not found in the series of the 19 patients analyzed here. Using FISH analysis, Kurotaki *et al*<sup>5</sup> reported a common 2.2-Mb deletion in 19/42 patients (66%) with Sotos syndrome, while in the

studies described by Douglas *et al*<sup>6</sup> a whole gene deletion occurred only in 3/37 patients (8%) with Sotos syndrome. Two of these patients showed deletion breakpoints within the above-mentioned interval detected by microsatellite analysis. The discrepancy between the Japanese and the European findings are difficult to explain and may be due to a selection bias. Using FISH analysis with a PAC clone, as described here, small deletions cannot be detected and may thus have been missed.

*NSD1* aberrations were not identified in six patients with an unclassified overgrowth/mental retardation phenotype, nor six patients with macrocephaly associated with mental retardation. However, additional patients with nonspecific overgrowth syndromes need to be screened before the spectrum of phenotypes associated with *NSD1* mutations becomes clear.

In our series, intragenic *NSD1* mutations cause Sotos syndrome in the vast majority of patients clinically identified to have Sotos syndrome. *NSD1* mutations may also be present in patients with Sotos syndrome lacking either macrocephaly or increased height. Aberrations of the *NSD1* gene were not found associated with other overgrowth phenotypes such as Weaver syndrome. In contrast to prior reports, whole gene deletions could not be detected by FISH analysis.

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