Mutations in the NSD1 gene in patients with Sotos syndrome associate with endocrine and paracrine alterations in the IGF system

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Abstract

Objective: To investigate the effect of nuclear receptor Su-var, 3-9, enhancer of zeste, trithorax (SET) domain-containing protein 1 (NSD1) gene alteration in patients with Sotos syndrome on plasma IGFs and IGFBPs, as well as on the IGF/IGFBP system activity at the tissue level.

Design: Twenty-nine patients suspected of Sotos syndrome were divided into two groups: patients with heterozygous deletions or mutations in the NSD1 gene (NSD1⁺/⁻) (n = 11) and subjects without (NSD1⁺/⁺) (n = 18). Plasma samples (n = 29) and skin fibroblasts (n = 23) were obtained. The results of both groups were compared and related to reference values.

Methods: IGF-I, IGF-II, IGFBP-2, IGFBP-3, IGFBP-4 and IGFBP-6 levels were determined by RIAs. The mitogenic response of fibroblasts to IGFs was investigated by [methyl-³H]thymidine incorporation. IGFBP-3 levels in the culture media were measured by RIA. IGFBP-3 mRNA expression was determined by real time RT-PCR.

Results: NSD1⁺/⁻ patients showed significantly altered levels of IGF-I (mean 2 1.2 SDS), IGF-II (2 1.2), IGFBP-3 (2 1.7), IGFBP-4 (2 0.4), IGFBP-2 (2 0.8) and IGFBP-6 (2 1.5). The NSD1⁺/⁺ patients did not differ from the reference, with the exception of the mean IGFBP-3 level (2 1.3).

Basal proliferation and mitogenic response to IGFs was diminished in NSD1⁺/⁻ fibroblasts compared with NSD1⁺/⁺ (basal, P = 0.02; IGF-I, P < 0.001; IGF-II, P = 0.02). Compared with control fibroblasts, only the mitogenic response was diminished (basal, P = 0.07; IGF-I, P = 0.04; IGF-II, P = 0.04). A trend of higher IGFBP-3 secretion after IGF-I stimulation (P = 0.09) and 3.5–5 times higher mRNA expression of IGFBP-3 in basal conditions was found in NSD1⁺/⁻ fibroblasts in comparison to controls.

Conclusions: NSD1⁺/⁻ patients show endocrine and paracrine changes in the IGF system. These changes may contribute to the abnormal growth pattern.

European Journal of Endocrinology 151 333–341

Introduction

Sotos syndrome (cerebral gigantism; OMIM 117550) is an overgrowth syndrome that was first described in 1964 (1). Patients show such typical facial characteristics as frontal bossing, dolichocephaly, high hairline, prominent chin and antimongoloid slant of palpebral fissures. Other important clinical features are overgrowth with large size at birth, rapid growth in the first 4 years (2) and tall stature in childhood, advanced bone age, macrocephaly, mental retardation and delayed motor development (1, 3). Heterozygous deletions and inactivating mutations involving the gene for nuclear receptor Su-var, 3-9, enhancer of zeste, trithorax (SET) domain-containing protein 1 (NSD1) have been proposed as a major determinant in the aetiology of Sotos syndrome (4–7).

In Sotos syndrome prenatal overgrowth is often present. Both insulin-like growth factors (IGFs) I and II are important growth factors in utero. They exert their growth-promoting effects at both the endocrine and para-/autocrine levels (8, 9). Mice carrying null mutations for the IGF-I, IGF-II or IGF-I receptor (IGF-IR) genes are smaller at birth (10, 11). In humans, cases of intrauterine growth retardation have been reported in association with a homozygous partial deletion of the IGF-I gene leading to total IGF-I deficiency (12) and more recently with absence of one copy of the IGF-IR gene (13). The latter study also describes a patient with three copies of the IGF-IR
gene, showing intrauterine and postnatal overgrowth and a head circumference on the 98th percentile at birth. This patient showed a normal serum IGF-I level with increased cell proliferation and response to IGF-I by skin fibroblasts. The Sotos-like phenotype of this patient would suggest that changes in the IGF system may contribute to the abnormal growth pattern in Sotos syndrome.

In studies of patients clinically suspected of Sotos syndrome, biochemical growth parameters such as growth hormone (14–17) and somatomedin activity (biological activity of IGFs) (2, 16, 18–21) have been measured. Levels were in the normal range for most cases, but elevated or decreased levels were also described. In a recent study of plasma IGFs and IGF-binding proteins (IGFBPs), we found decreased levels of IGF-II, IGFBP-3 and IGFBP-4 in the circulation of patients clinically suspected of Sotos syndrome (22). However, no data are available on IGFs and IGFBPs in Sotos patients with or without a heterozygous mutation or deletion in the NSD1 gene.

The function of NSD1 has not yet been fully understood. The protein, a transcriptional intermediary factor, may act as either a nuclear receptor co-repressor or co-activator by interacting with the holo- or apo-forms respectively, of the ligand-binding domain of different subsets of nuclear hormone receptors (23). It has been postulated that NSD1 acts as a co-repressor of growth promoting genes (4). NSD1 expression in human tissue has been detected in foetal/adult brain, kidney, skeletal muscle, spleen and thymus (24). It is unknown whether NSD1 influences the expression of IGFs and IGFBPs.

In this study patients clinically suspected of Sotos syndrome were divided into a group with heterozygous deletions or mutations in the NSD1 gene (NSD1+/−) and a group with only wild-type alleles (NSD1+/+). In these two categories of patients we studied systemic levels of IGFs and IGFBPs and the responses of cultured skin fibroblasts to IGFs. Skin fibroblasts express IGF-I and IGF-II receptors and therefore represent a suitable model for studying cellular responses to IGFs and IGFBP-3 expression in growth disorders (25–27). We intended to answer the following questions: (i) do NSD1+/− patients show a distinct pattern of plasma IGF/IGFBP levels, i.e. being different from those of the NSD1+/+ subjects and the reference population? and (ii) similarly, do NSD1+/− skin fibroblasts respond differently to IGFs in terms of mitogenic response and IGFBP-3 secretion?

### Patients and methods

#### Patients

The study was conducted with the prior consent of the Medical Ethical Committee of the Leiden University Medical Center. All subjects or their parents included in the study gave informed consent to participate. Twenty-nine patients clinically suspected of Sotos syndrome were divided in an NSD1+/− group and an NSD1+/+ group based on the results of fluorescence in situ hybridization (FISH) and mutation analysis on blood samples. The NSD1 mutation analysis is described in a separate paper (28). Auxological and clinical characteristics of the patients are shown in Table 1. In one patient a deletion was detected and in 10 patients, including three members of one family, pathogenic mutations were detected. Skin fibroblasts were obtained by punch biopsy of the forearm skin of 23 patients. Skin fibroblasts of 12 normal donors, mean age 10.5 years (range 3.7–17.1 years), three females and nine males, were obtained from the fibroblast bank of the Wilhelmina Children’s Hospital, Utrecht, The Netherlands.

#### Measurements of IGFs and IGFBPs in plasma

Plasma IGF-I, IGF-II, IGFBP-2, IGFBP-3, IGFBP-4 and IGFBP-6 levels were determined by specific RIAs. For each parameter and the IGF-I/IGFBP-3 ratio normative range values were available and plasma levels were expressed as SDS. Assays, their validation and normative range values have been described in detail in previously published studies (22, 29–34).

### Table 1 Characteristics of the patients suspected of Sotos syndrome.

<table>
<thead>
<tr>
<th>NSD1+/−</th>
<th>NSD1+/+</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of blood samples</td>
<td>11</td>
<td>18</td>
</tr>
<tr>
<td>Male/female</td>
<td>7/4</td>
<td>13/5</td>
</tr>
<tr>
<td>Mean age (range) in years</td>
<td>15 (2.1–36.3)</td>
<td>14.8 (4.6–48.4)</td>
</tr>
<tr>
<td>Mean height SDS corrected for TH SDS (95% CI)</td>
<td>1.70 (1.05–2.36)</td>
<td>0.92 (0.27–1.56)</td>
</tr>
<tr>
<td>Mean birth length SDS (95% CI)</td>
<td>1.18 (−0.16–2.51)</td>
<td>0.84 (0.14–1.54)</td>
</tr>
<tr>
<td>Mean head circumferences SDS (95% CI)</td>
<td>2.91 (1.98–3.83)</td>
<td>2.28 (1.47–3.08)</td>
</tr>
<tr>
<td>Number of cells lines</td>
<td>10</td>
<td>13</td>
</tr>
<tr>
<td>Male/female</td>
<td>6/4</td>
<td>10/3</td>
</tr>
<tr>
<td>Mean age (range)</td>
<td>16.2 (2.1–36.3)</td>
<td>11.3 (4.6–36.3)</td>
</tr>
</tbody>
</table>

TH, target height.
**Cell culture**

Skin fibroblasts were maintained in 9 cm culture dishes in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% (vol/vol) foetal calf serum (FCS; Gibco BRL), penicillin and streptomycin. The cultures were established in a humidified atmosphere with 5% (vol/vol) CO₂ at 37°C. The cultures were split (1:4) upon reaching confluence. In all experiments, cells between passages 2 and 15 were used. Cells were counted and seeded at a density of 1.5 × 10⁴ cells/ml. For [methyl-³H]thymidine (84 Ci/mmol; Amersham International) experiments, cells were cultured in either 24-well plates (0.5 ml/well) or 6-well plates (1.5 ml/well), as indicated.

**Mitogenic response to IGF-I/IGF-II**

Mitogenic response after treatment of fibroblasts with IGF-I or IGF-II (Pepro Tech, Rocky Hill, NJ, USA) was assessed by the cellular incorporation of [methyl-³H]thymidine. For these experiments cells were cultured in 24-well dishes in DMEM containing 10% FCS for 48 h. This was followed by incubation in serum-free DMEM containing 0.1% (wt/vol) BSA for 72 h. Subsequently cells were exposed to DMEM containing 0.1% BSA with increasing concentrations of IGF-I (0.3, 1, 6, 10 and 20 ng/ml) or IGF-II. After 20 h of incubation, [methyl-³H]thymidine was added in a final concentration of 0.5 μCi/ml. After 4 h the incorporation of [methyl-³H]thymidine was terminated by aspirating the medium. Plates were washed twice with 1 ml PBS and once with 0.5 ml 10% (vol/vol) trichloric acid for 30 min at 4°C, cell lysates were solubilized overnight in 0.25 ml 0.1 M NaOH and 0.2% SDS. Aliquots of 250 μl in 1 ml instagel (2 + 1) (Packard, Downers Grove, IL, USA) were counted in a Packard 1500-tri-carb-liquid scintillation analyser. Measurements were done in quadruplicate and all experiments were performed at least twice. The mean was calculated from the experiments. Data were expressed as counts per min (c.p.m.).

**Analysis of IGFBP-3 release into the medium**

Conditioned media of fibroblasts from Sotos patients and controls were analysed. Cells were cultured in 6-well dishes in DMEM containing 10% FCS for 48 h. This was followed by incubation in serum-free DMEM containing 0.1% (wt/vol) BSA for 72 h. Subsequently cells were cultured in DMEM containing 0.1% BSA with or without IGF-I (10 ng/ml) for 48 h. Media of each well (1.5 ml) were collected and stored at −20°C. Media were concentrated 10 times and immunoreactive IGFBP-3 levels were measured in duplicate by a specific RIA (32). All experiments were performed twice; means from two experiments are reported. Using Western ligand blotting (with [¹²⁵]IIGF-II as a probe) IGFBPs secreted in the culture media were studied (35).

**RNA extraction and real-time quantitative RT-PCR**

To quantitate IGFBP-3 mRNA levels, real-time quantitative RT-PCR was performed in triplicate using SybrGreen. First, total RNA was prepared from the fibroblast cultures using RNAzol B followed by clean-up using the RNeasy Mini Kit (Qiagen). RNA was quantified by measuring its absorbance at 260 and 280 nm (Ultrospec 2000 spectrophotometer; Pharmacia Biotech), and its quality was checked by gel electrophoresis and ethidium bromide staining of the 28 and 18 S rRNA bands. RNA was reverse transcribed into cDNA using the SuperScript First-Strand Synthesis System for RT-PCR (Gibco BRL). The following primer sets were used for the PCR: IGFBP-3; forward 5’-GCT TGA TCC CAA GTT CC-3’ (nucleotides 476–495) and reverse 5’-AGA GGC TGC CCA TAC TTA TTC-3’ (795–815); glyceraldehyde-phosphate dehydrogenase (GAPDH): forward 5’-TTA GCA CCC CTC GGC AAG G-3’ (469–487) and reverse 5’-CTT ACT CCT TGG AGG CCA TG-3’ (989–1008); B-2-microglobulin: forward 5’-CCA GCA GAG AAT GGA AAG TC-3’ (100–119) and reverse 5’-GAT GCT GCT TAC ATG TCT CG-3’ (341–360). All PCR amplicons spanned exon–intron boundaries. The PCR were performed in the presence of 5 μl Taq Gold buffer, 6 μl 25 mM MgCl₂, 8 μl 1.25 mM dNTPs, 1.25 U AmpliTaq Gold DNA polymerase (all from Applied Biosystems, Nieuwekerk ad Ijssel, The Netherlands), 2 μl 10 μM stock solution of sense and antisense primers (Eurogentec, Maastricht, The Netherlands), 0.15 μl SybrGreen (Molecular Probes, Leiden, The Netherlands) and 0.6 μl 2 μg/μl cDNA in a final volume of 50 μl. Water was used as a negative control. PCR amplification reactions were performed in an ABI Prism 7700 spectrophluorometric thermal cycler (Applied Biosystems). Fluorescence spectra were recorded and the threshold cycle number (Ct) was calculated with the accompanying software, after checking the specificity of the end products of the PCR by gel electrophoresis. The angle of inclination of the linear part of the amplification curve was identical using the three different primer combinations. For each cell line Ct values for IGFBP-3 were subtracted from the Ct values of GAPDH or B-2-microglobulin (ΔCt values). The mean ΔCt value of an individual sample was based on three independent measurements.

**Statistical analysis**

Data were analysed with SPSS for Windows version 10.0. To compare plasma SDS values with the reference
population a Student’s t test was used. Values of IGFBP-3 before and after stimulation of IGF-I were compared with the Wilcoxon signed ranks test. The Mann–Whitney U test was used to compare all other measurements. P values of < 0.05 were considered significant.

Results

Plasma levels of IGFs and IGFBPs

Mean values of IGF and IGFBP SDS are shown in Table 2. Individual SDS values are shown as scatter plots in Fig. 1. Within the NSD1+/− group, no differences were found between the patient with a deletion and the patients with mutations, except for IGFBP-2, which was higher in the former patient. Mean serum levels of IGFs and IGFBPs in the NSD1+/− group differed from the NSD1+/+ group in most parameters, except in IGFBP-3 and -4. The largest difference between the NSD1+/− group and the NSD1+/+ group was found for IGFBP-6, which was significantly higher in the first group. The mean values of all IGFs and IGFBPs in the NSD1+/− patients differed from the reference values. IGFBP-3 was the only parameter that was significantly decreased in comparison with the reference population in both groups. The IGF-II/IGFBP-3 ratio SDS was significantly elevated in the NSD1+/− group (mean +1.27, 95% confidence interval (CI) 0.42–2.12), whereas for the NSD1+/+ group this parameter was within the normal range (mean −0.31, 95% CI −0.88–0.26).

Mitogenic response

No correlation was found between the age of a particular patient at the moment of skin biopsy and both unstimulated and IGF-stimulated [methyl-3H]thymidine incorporation by the cultured fibroblasts. In a series of pilot experiments, a dose–response curve of two NSD1+/− and three control cultures clearly revealed a decreased sensitivity to IGF-I in NSD1+/− fibroblasts (mean ED50: NSD1+/− cells, 10.8 ng/ml; control cells, 3.2 ng/ml: data not shown). Subsequently, the responses to 10 ng/ml IGFs I and II were studied in all fibroblast cultures. Mean values of basal and IGF-I- and IGF-II-stimulated rates of [methyl-3H]thymidine incorporation are depicted in a bar chart (Fig. 2). Basal values of [methyl-3H]thymidine incorporation by NSD1+/− cells were lower compared with those for NSD1+/+ fibroblasts (P = 0.02), but the difference with controls did not reach significance (P = 0.07). Both IGF-I and IGF-II stimulated growth of NSD1+/− fibroblast cultures was less pronounced than encountered for either NSD1+/+ (P < 0.001, P = 0.02) or control (P = 0.04) cells. No differences were found between fibroblasts from the patient with an NSD1 deletion and cells derived from patients with an NSD1 mutation.

IGFBP-3 secretion

Western ligand blots with labelled IGF-II of the media of six NSD1+/− fibroblasts and eight controls showed no significant differences in IGFBP patterns between NSD1+/− and controls (data not shown). Only overall levels of IGFBP-3 tended to be higher in NSD1+/− fibroblasts. To substantiate this observation IGFBP-3 secretion in medium was quantified by RIA. For each of the three groups no significant increase of IGFBP-3 secretion was detected after stimulation with IGF-I (controls, P = 0.65; NSD1+/+, P = 0.35; NSD1+/−, P = 0.13). No significant differences were found between the three groups of fibroblasts for IGFBP-3 secretion, but a trend of higher IGFBP-3 secretion was detected in the NSD1+/− cell lines in comparison with controls (basal, P = 0.17; after IGF-I, P = 0.09) as shown in Fig. 3.

IGFBP-3 mRNA expression

Expression levels of IGFBP-3 mRNA by the NSD1+/− and control fibroblasts were compared. The mean difference in Ct between the two groups was 1.9 when corrected for GAPDH and 2.3 when corrected for β2-microglobulin. This corresponds to approximately 3.5–5-fold more IGFBP-3 mRNA in NSD1+/− fibroblasts than in controls. A representative amplification curve of pooled cDNA derived from NSD1+/− and of control fibroblasts using IGFBP-3 primers is shown in Fig. 4.

Discussion

In this study, we show for the first time that several parameters of the IGF system in Sotos patients, harbouring a heterozygous mutation or deletion of the NSD1 gene, differ from those in patients clinically suspected of Sotos syndrome who have two normal NSD1 alleles, and the reference population.

Based on the essential role of IGF-I in intrauterine and postnatal growth we postulated that NSD1 mutations would result in increased IGF-I bioactivity,
leading to overgrowth in Sotos syndrome. Additional support for this hypothesis comes from a patient with three copies of the IGF-IR (13), who exhibits a Sotos-like phenotype, suggesting that overactivation of the IGF-IR can indeed provide an explanation for the overgrowth observed in Sotos syndrome. However, our studies did not show evidence for increased IGF-I bioactivity in serum, nor for increased responsiveness

Figure 1 Scatter plots of the individual SD scores of plasma IGF and IGFBP levels of patients suspected of Sotos syndrome with and without an aberrant NSD1 gene. Three outliers did not fit in the plots: IGFBP-3: NSD1^{+/2} 2.1 years, −5.4 SDS; NSD1^{+/+} 36.3 years, +5.4 SDS; IGFBP-4: NSD1^{+-} 8.2 years, +6.7 SDS. The encircled dots depict the values of the patient with a deletion of the NSD1 gene. • NSD^{+/2}, × NSD^{+/+}. 

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of the IGF-IR in fibroblasts of NSD1<sup>+/−</sup> patients. Instead, serum values of IGF-I, IGF-II, IGFBP-3 and IGFBP-4 were decreased and IGFBP-2 and IGFBP-6, generally considered as inhibitors of IGF bioactivity (36), were increased compared with the reference population. In addition, the IGF-I/IGFBP-3 ratio was normal. Furthermore, skin fibroblasts of NSD1<sup>+/−</sup> patients had a reduced mitogenic response both under basal conditions and after IGF-I stimulation when compared with cells derived from NSD1<sup>+/+</sup> patients and controls (only after IGF-I stimulation) and they express more IGFBP-3 mRNA than controls.

In the past limited reports on the expression of components of the IGF/IGFBP family in Sotos syndrome have appeared in the literature, but the results were not related to the presence or absence of NSD1 mutations. The studies were inconclusive concerning somatomedin activity or IGF-I values. Normal values have been reported, but so have elevated or decreased levels (2, 16, 18–21). Remarkably, the NSD1<sup>+/−</sup> group is distinguishable from the NSD1<sup>+/+</sup> group as well as from other patient groups with growth disorders based on the serum levels of IGFs and IGFBPs. For example, in constitutionally tall children normal IGF-I values, high IGF-II values and an increased IGF/IGFBP ratio have been described (37) and in subjects with intrauterine growth retardation low IGF-I levels and normal IGF-II levels have been reported (38). NSD1<sup>+/−</sup> patients, who were Sotos-like clinically, resemble the reference population in all studied endocrine and paracrine parameters, except for lower IGFBP-3 values and a higher IGF-I/IGFBP-3 ratio. Although we cannot completely rule out that some of these patients may have an aberrant NSD1 gene not detected by the mutation screening (e.g. splicing variants due to deletions or mutations in introns or mutations in the promoter region of NSD1), our data are strongly suggestive for a non-NSD1-related growth disorder with Sotos-like features in the majority of these patients. These patients differ from constitutionally tall patients by the absence of elevated serum IGF-II levels, but have in common an increased IGF/IGFBP ratio. This suggests that an increase in bioavailability of IGFs in these patients may at least partly contribute to the overgrowth phenotype.

A striking finding was that plasma levels of IGFBP-2 and especially IGFBP-6 in NSD1<sup>+/−</sup> patients were elevated. Both IGFBP-2 and IGFBP-6 have a higher affinity for IGF-II than for IGF-I and are therefore thought to
inhibit IGF-II bioactivity preferentially (39, 40). IGFBP-2 and IGFBP-6 are the two major binding proteins present in cerebrospinal fluid and are locally produced in the central nervous system. Developmental delay as well as increased head circumference is a characteristic finding in Sotos syndrome.

We found a diminished basal proliferative activity and a diminished response to IGF-I and IGF-II by NSD1+/− fibroblasts. These findings are in contrast to observations in fibroblasts of a Sotos-like patient with tall stature and an enhanced expression of the IGF-IR (13). Altered expression of the IGF-IR is therefore unlikely to play a key role in the aetiology of Sotos syndrome. IGFBP-3 is the most abundant IGFBP secreted by human fibroblasts (41). A trend of higher secretion of this IGFBP after stimulation with IGF-I was found for NSD1+/− fibroblasts compared with controls. Also a higher IGFBP-3 mRNA expression was found. IGFBP-3 can inhibit IGF effects by competing for binding with their specific receptors (42). Elevated secretion of this peptide has been found for fibroblasts of patients with idiopathic short stature (ISS) (26) and Turner syndrome (27), indicating that this phenomenon is seen in distinct growth disorders characterized by tall as well as short stature.

How do the modest endocrine and paracrine changes in the IGF system relate to NSD1 gene aberrations? It is postulated that NSD1 encodes a protein that can act both as a co-repressor and activator of a subset of nuclear hormone receptors for growth-promoting genes (i.e. the thyroid hormone receptor, the oestrogen receptor and the retinoic acid receptor) through interaction with the ligand-binding domain in the presence or absence of the ligand respectively (4, 43–45). In a knockout mouse model, NSD1 has been shown to be important for post-implantation development (46). Homozygous NSD1 mutant mice were severely growth retarded, showed a high incidence of apoptosis and died before day 10.5 in utero. Mice heterozygous for an NSD1 mutation were viable and showed normal growth apparently lacking the typical features of Sotos syndrome. It is presently unclear whether these mice represent a suitable animal model for Sotos syndrome. Although detailed studies are currently lacking, there are some indications that the receptors for oestrogen and retinoic acid are involved in the regulation of transcription of IGF-I, IGF-II, IGFBP-3 and IGFBP-6 (47–50). Of particular interest is the presence of a functional retinoic acid-response elements in the IGFBP-6 promoter suggesting that elevated IGFBP-6 serum levels in NSD1+/− patients could be due to alterations in retinoic acid-mediated gene transcription of the IGFBP-6 promoter. The endocrine and paracrine aberrations in the IGF-I system of NSD1+/− Sotos patients may therefore be a direct consequence of disturbed gene transcription by nuclear hormone receptors. In this respect it is noteworthy to mention that IGFBP-3 can accumulate in the nucleus where it can interact with the retinoid X receptor α (RXRα). This interaction can modulate retinoid-mediated gene transcription and apoptosis in an IGF-I-independent manner (51–53). Whether NSD1 and IGFBP-3 compete for binding to RXRα is presently unclear. However, the increased IGFBP-3 mRNA expression in NSD1+/− fibroblasts may also contribute to disturbed nuclear hormone signalling via retinooids.

The relation between the alterations in the IGF/IGFBP family and overgrowth are less clear. In fact, the observed aberrations would be more in line with short stature (low IGFs, high inhibitory IGFBPs, blunted mitogenic response to IGF-I) rather than with tall stature. It could be that the impact of NSD1 haploinsufficiency on the expression of IGFs and IGFBPs varies between organs, resulting in tissue-specific changes in availability of bioactive IGF-I. In this respect, the effect of NSD1+/− exerted on the growth plate is relevant. NSD1 is expressed by chondrocytes in the human growth plate (M Karperien, unpublished observations) and may affect the equilibrium in the IGF system favouring increased proliferation at the tissue level. Animal studies have shown that chondrocytes express IGFBPs 2–6 (54). Alternatively, the observed endocrine and paracrine changes may be the result of a compensatory physiological reaction of the body to decelerate the increased growth induced by NSD1 aberrations. In that case, NSD1 haploinsufficiency would induce overgrowth by processes being largely independent of the IGF system. However, then one should expect that fibroblasts of NSD1+/− patients have a higher intrinsic proliferative capacity compared to controls. Our data do not support such a phenomenon.

In conclusion, NSD1 gene mutations in Sotos patients are reflected in modest endocrine and paracrine alterations in the IGF/IGFBP system. Based on these alterations, the group of Sotos patients with NSD1+/− can be distinguished from the NSD1+/+ subjects, the reference population as well as from patients with other growth disorders. The mechanism by which NSD1 gene mutations induce overgrowth and the involvement of the IGF system in this process requires further study.

**Acknowledgements**

We would like to thank the patients and their families for participating in the study. Saskia le Cessie for statistical advice. Niels van Lelyveld for his help with the Western ligand blotting. Sylvia van Buul-Offers for her help and comments and STINAFO and LUMC for their financial support. This work was supported by the ‘Dutch fund for disabled children’ (STINAFO) and the Leiden University Medical Center.
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Received 10 March 2004
Accepted 10 June 2004