

NSD1 Mutations Are the Major Cause of Sotos Syndrome and Occur in Some Cases of Weaver Syndrome but Are Rare in Other Overgrowth Phenotypes

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Sotos syndrome is a childhood overgrowth syndrome characterized by a distinctive facial appearance, height and head circumference >97th percentile, advanced bone age, and developmental delay. Weaver syndrome is characterized by the same criteria but has its own distinctive facial gestalt. Recently, a 2.2-Mb chromosome 5q35 microdeletion, encompassing *NSD1*, was reported as the major cause of Sotos syndrome, with intragenic *NSD1* mutations identified in a minority of cases. We evaluated 75 patients with childhood overgrowth, for intragenic mutations and large deletions of *NSD1*. The series was phenotypically scored into four groups, prior to the molecular analyses: the phenotype in group 1 ($n = 37$) was typical of Sotos syndrome; the phenotype in group 2 ($n = 13$) was Sotos-like but with some atypical features; patients in group 3 ($n = 7$) had Weaver syndrome, and patients in group 4 ($n = 18$) had an overgrowth condition that was neither Sotos nor Weaver syndrome. We detected three deletions and 32 mutations (13 frameshift, 8 nonsense, 2 splice-site, and 9 missense) that are likely to impair *NSD1* functions. The truncating mutations were spread throughout *NSD1*, but there was evidence of clustering of missense mutations in highly conserved functional domains between exons 13 and 23. There was a strong correlation between presence of an *NSD1* alteration and clinical phenotype, in that 28 of 37 (76%) patients in group 1 had *NSD1* mutations or deletions, whereas none of the patients in group 4 had abnormalities of *NSD1*. Three patients with Weaver syndrome had *NSD1* mutations, all between amino acids 2142 and 2184. We conclude that intragenic mutations of *NSD1* are the major cause of Sotos syndrome and account for some Weaver syndrome cases but rarely occur in other childhood overgrowth phenotypes.

Introduction

Sotos syndrome (MIM 117550), previously known as “cerebral gigantism,” was initially described in 1964, with hundreds of cases subsequently reported (Sotos et al. 1964; Cole and Hughes 1994). The cardinal features are pre- and postnatal accelerated somatic growth, characteristic facial appearance (i.e., macrocephaly, prominent jaw, and a high hairline with sparse hair growth), advanced bone age, and developmental delay (Cole and Hughes 1994). Additional features that may be present include neonatal hypotonia, seizures, scoliosis, strabis-

mus, congenital heart defects, and cancer (Kaneko et al. 1987; Hersh et al. 1992; Cole and Hughes 1994; Noreau et al. 1998; Opitz et al. 1998; Sweeney et al. 2002). To date, the diagnosis of Sotos syndrome has been based primarily on the presence of the characteristic facial appearance in a large child (fig. 1A). However, although the combination of craniofacial features is distinctive, individual components are nonspecific, and diagnosis can be very difficult for the inexperienced clinician. Furthermore, phenotypic overlap with other childhood overgrowth conditions increases the challenge of accurate clinical diagnosis (Cole and Hughes 1994; Cole 1998).

The condition showing greatest phenotypic overlap with Sotos syndrome is Weaver syndrome (MIM 277590). This condition was originally described in 1974, and the cardinal features are accelerated growth, distinctive facies, advanced bone age, and developmental delay (Weaver et al. 1974; Cole et al. 1992). The facial appearance is somewhat similar to that in Sotos syndrome, but experienced dysmorphologists be-

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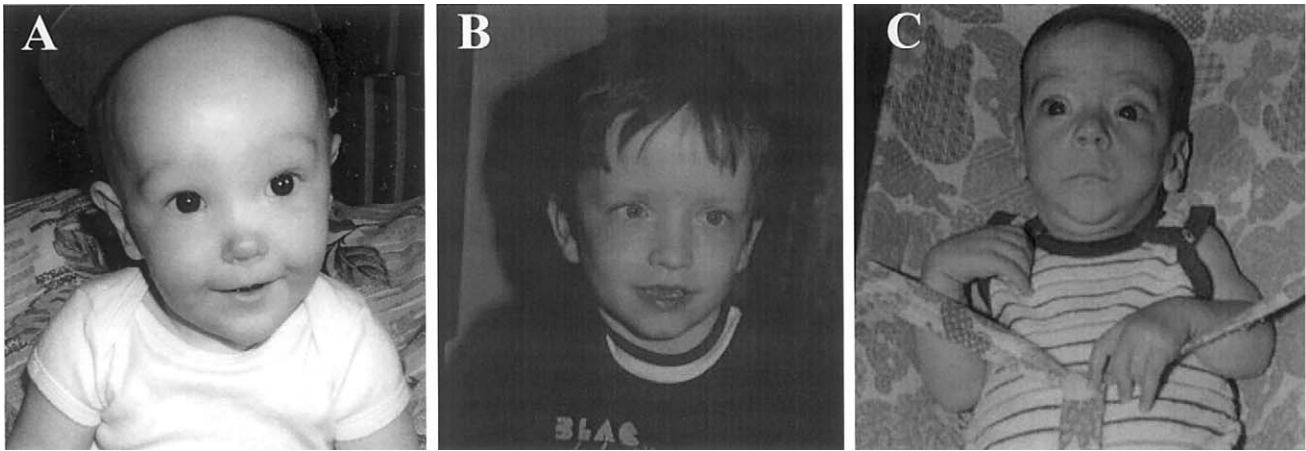


Figure 1 Typical facial phenotype in overgrowth groups 1, 2, and 3. *A*, Group 1 (classic facial gestalt of Sotos syndrome). *B*, Group 2 (patients with similarities to Sotos syndrome but with some atypical characteristics; primarily the facial features were not classical). *C*, Group 3 (Weaver syndrome). Patients in Group 4 did not have a distinctive facial phenotype but were characterized by not having the facial phenotypes present in the other three groups.

lieve they are distinct (fig. 1C). Additional features occurring in Weaver syndrome include a hoarse low-pitched cry, metaphyseal flaring of the femurs, deep-set nails, prominent finger pads, and camptodactyly (Cole et al. 1992; Opitz et al. 1998; Proud et al. 1998). There has been considerable debate as to whether Sotos and Weaver syndromes are representative of locus or allelic heterogeneity (Cole 1998; Opitz et al. 1998).

Both Sotos and Weaver syndromes usually occur sporadically, although occasional families exhibiting autosomal dominant inheritance of these conditions have been reported (reviewed by Cole and Hughes 1990; Opitz et al. 1998; Proud et al. 1998). The absence of multiple-case families has hampered efforts to identify causative gene(s). However, recently a child with Sotos syndrome and a t(5;8)(q35;q24.1) translocation was reported (Imazumi et al. 2002), and the gene disrupted by the 5q35 breakpoint was identified as *NSD1* (nuclear receptor SET-domain-containing protein) (Kurotaki et al. 2002).

The functions of *NSD1* have not been fully elucidated, but it is thought to act as a transcriptional intermediary factor capable of both negatively and positively influencing transcription, depending on the cellular context (Huang et al. 1998; Kurotaki et al. 2001). *NSD1* contains multiple functional domains, including the SET (SU[VAR]3-9, E[Z], trithorax) domain, which was initially identified in *Drosophila* genes involved in chromatin-mediated regulation during development (reviewed by Jenuwein 2001). *NSD1* also contains a SET-associated Cys-rich (SAC) domain adjacent to the SET domain. The combination of SAC and SET domains is present in proteins that function as histone-methyltransferases (HMTases), and both are required for HMTase activity (Rea et al. 2000). Thus, it is possible that *NSD1* is involved in histone modification and

the regulation and maintenance of chromatin states. *NSD1* also contains five plant homeodomain (PHD) domains. The PHD domain is a zinc finger-like motif that predominantly occurs in proteins that function at the chromatin level (Aasland et al. 1995). The consensus motif is C4HC3, and *NSD1* PHD-I and PHD-IV conform to this. However, *NSD1* PHD domains II, III, and V contain a His in place of the final Cys and are thus known as “PHD-H2 fingers” (Huang et al. 1998). *NSD1* contains two PWWP (proline-tryptophan-tryptophan-proline) domains. The role of the PWWP motif has not been established, but it is found in regulatory factors and de novo methyltransferases and is thought to be involved in protein-protein interactions (Stec et al. 2000). Finally, *NSD1* contains two distinct nuclear receptor (NR) interaction domains, NID^{-L} and NID^{+L}, which are found in NR corepressors and coactivators, respectively (Kurotaki et al. 2001).

Analysis of *NSD1* in 42 Japanese patients with sporadic Sotos syndrome revealed 19 with a 2.2-Mb microdeletion of 5q35 encompassing *NSD1* and 4 with intragenic *NSD1* mutations, predicted to inactivate the protein (Kurotaki et al. 2002). We have evaluated a phenotypically characterized series of patients with overgrowth syndrome for deletions and mutations of *NSD1*, to investigate the phenotypic and molecular spectra of *NSD1* aberrations in childhood overgrowth.

Subjects and Methods

Patients

The research was approved by the London Multicentre Research Ethics Committee, and consent was obtained from all patients and/or parents. DNA was extracted by

standard methods. Samples were obtained from two sources: 48 patients were ascertained as part of a previous study and were phenotypically scored independently by three clinical geneticists (Cole and Hughes 1994). Extensive clinical details and serial photographs of these cases have been published elsewhere (Cole and Hughes 1990, 1991, 1994, 1995; Cole et al. 1992, 1995). Twenty-seven patients were ascertained as part of the ongoing Childhood Overgrowth Study. Patients eligible for this study must fulfill four of the following criteria: (a) height >97th percentile, (b) occipito-frontal circumference >97th percentile, (c) bone age >90th percentile, (d) dysmorphic facial features, (e) developmental delay, and (f) congenital anomaly or malformations. The 27 newly ascertained patients were phenotypically scored by three clinical geneticists (T.R.P.C., H.E.H., and I.K.T.). All patients were assigned to one of four groups: patients in group 1 had classical Sotos syndrome (fig. 1A); patients in group 2 had a disease with similarities to Sotos syndrome but with some atypical characteristics, primarily concerning the facial features, which were not fully consistent with the gestalt of Sotos syndrome (fig. 1B); patients in group 3 had Weaver syndrome (fig. 1C); and patients in group 4 had an overgrowth phenotype but did not have either Sotos syndrome or Weaver syndrome. Within group 4, there was one patient considered to have Marshall-Smith syndrome (MIM 602535) and four with a diagnosis of autosomal dominant macrocephaly (MIM 153470; MIM 605309). The remaining group 4 patients did not have a specific recognized overgrowth syndrome. Patients with a confirmed molecular diagnosis of an overgrowth condition, such as Beckwith-Wiedemann syndrome (MIM 130650) or Simpson-Golabi Behmel syndrome (MIM 312870), were excluded. In cases where all three scores were not concordant, the majority score was accepted. No patient was scored into three different categories, and no patient was scored into both groups 1 and 4 or groups 3 and 4 by the assessors. There were high levels of concordant scoring for patients in groups 1, 3, and 4. For group 2, the level of concordant scoring was lower, with several patients scored as either group 1 or 4 by one assessor. Parental DNA was obtained wherever possible. The numbers of patients and parental samples analyzed in each group are shown in table 1.

NSD1 Microdeletion Analyses

To identify microdeletions encompassing *NSD1*, we analyzed polymorphic microsatellite markers within and surrounding *NSD1*. We developed new microsatellite markers through use of the UCSC Human Genome Project Working Draft sequence (UCSC Genome Bioinformatics Web site). We searched the 2-Mb interval encompassing *NSD1* for dinucleotide, trinucleotide, and tetranucleotide repeat elements. Flanking amplifying

primers were designed using Primer3 software (Primer3 Web site). All markers were amplified using a touchdown protocol, cycling from 68°C to 50°C, with the exception of SOT19 and SOT20, for which the PCR was performed at a single annealing temperature of 55°C. Of the 22 markers designed, 9 were highly informative and worked reliably in the analyses. The forward primer for each marker was end-labeled with γ [³²P]-ATP, and the PCR products were electrophoresed on denaturing polyacrylamide gels and were exposed to x-ray film. The positions of the markers relative to *NSD1* were calculated using the UCSC Working Draft sequence. Two markers, SOT3 and SOT17, are intragenic within intron 2 and intron 17 of *NSD1*, respectively. All the markers are well within the 2.2-Mb interval reported as commonly deleted in patients with Sotos syndrome (Kurotaki et al. 2002). All patients ($n = 75$) and parents ($n = 87$) were analyzed at all markers. The physical locations relative to *NSD1*, primer sequences, and sizes of the nine markers are given in table 2.

Patients who were homozygous at both intragenic markers and at all intragenic polymorphisms detected in the mutation screen were additionally screened for a whole gene deletion, through use of a method of multiplex PCR amplification of short fluorescent fragments, using *MLH1* as a control (Charbonnier et al. 2002). 6-Fam-labeled primers for *NSD1* exons 6, 8, and 21 and *MLH1* exons 18 and 4 were simultaneously PCR amplified. The resulting product was electrophoresed on an ABI 3100 sequencer (ABI Perkin Elmer) and analyzed with GENOTYPER software. All experiments were repeated six times. A consistent, reproducible 0.5 reduction (i.e., a reduction by one half) in peak height of all three *NSD1* exons, compared with *MLH1* exons, was taken as indicative of a deletion of one copy of *NSD1* in that individual.

NSD1 Mutation Analyses

NSD1 contains 23 exons, the first of which is noncoding. Primers were designed to amplify the remaining 22 exons and intron-exon boundaries of *NSD1*, using conformation-sensitive gel electrophoresis (CSGE) (Ganguly et al. 1993). In our laboratory, we estimate the sensitivity of CSGE to detect small deletions/insertions and point mutations to be $\geq 90\%$. Primer sequences were designed using the *NSD1* genomic sequence (GenBank accession number AF395588) and Primer 3 software. Exons >400 bp were amplified using overlapping primer pairs. The gene was screened in 40 fragments. The primer sequences and sizes for the *NSD1* mutation screen are shown in table 3. All fragments were amplified using a touchdown 68°C–50°C protocol. Genomic DNA from patients showing mobility shifts on CSGE was bidirectionally sequenced using the BigDyeTerminator Cycle Sequencing Kit and a

Table 1

Phenotypic Delineation of Patients with Childhood Overgrowth, Indicating Numbers of Affected Individuals and Parents Analyzed and the Number of Patients with *NSD1* Deletions and Mutations

GROUP	PHENOTYPE	NO. OF PATIENTS ANALYZED	NO. OF PATIENTS WITH			
			<i>NSD1</i> Deletions	<i>NSD1</i> Mutations	Both Parents Analyzed	One Parent Analyzed
1	Classic Sotos syndrome	37	2	26	19	6
2	Sotos-like, but with atypical features	13	1	3	6	0
3	Weaver syndrome	7	0	3	5	2
4	Childhood overgrowth, but not Sotos or Weaver syndrome	18	0	0	4	11

3100 automated sequencer (ABI Perkin Elmer). All 75 patients were screened through all 40 fragments. In mutation-positive patients, parental DNA (if available) was analyzed by direct sequencing for the specific mutation identified in the proband. Mutations were considered pathogenic if they were likely to result in premature truncation of the protein or in exon skipping (small insertions and deletions, nonsense and splice-site mutations). To decide whether missense alterations were likely to be pathogenic, we considered whether they occurred de novo, whether they were present in 200 control individuals, and whether they were at conserved residues in the mouse orthologue and human paralogues. For comparison of *NSD1* missense mutations with mouse *nsd1* and human *NSD2* and *NSD3*, the BLAST program was used (NCBI BLAST Home Page).

Results

NSD1 Microdeletion Analyses

We analyzed DNA from 75 patients and 87 parents at nine microsatellite markers in the 1.5-Mb interval encompassing *NSD1*, including two intragenic markers (SOT3 and SOT17). In 69 patients, a microdeletion encompassing *NSD1* was excluded by the presence of two alleles of differing sizes at one or both intragenic markers and/or by heterozygosity for *NSD1* mutations or polymorphisms detected by CSGE. Three patients (COG25, COG44, and COG70) are highly likely to have hemizygous deletions resulting in the loss of one whole copy of *NSD1* (and surrounding genes), since they carry a single allele at a minimum of eight of the nine markers analyzed (fig. 2A). For COG25, DNA from both parents was available and confirmed a maternal deletion telomeric to SOT12 in the affected child (fig. 2A). In addition, the multiplex PCR demonstrated a consistent 0.5 reduction in peak height of *NSD1* exons (fig. 2B). COG70 has a single allele at all nine SOT markers and is homozygous for all 15 intragenic polymorphisms. Moreover, the multiplex PCR demonstrated a 0.5 reduction in *NSD1* exon peak height in six separate experiments. Thus, although we do not have parental DNA or cells for FISH analysis, our results very strongly sug-

gest that COG70 has a deletion of one copy of *NSD1*. Similarly, COG44 has a single allele at eight consecutive markers, is homozygous for all intragenic polymorphisms, and consistently shows a 0.5 reduction in peak height of *NSD1* exons in the multiplex PCR (fig. 2). Only maternal DNA was available and was consistent with transmission of a maternal allele at all SOT markers (fig. 2A). Thus, it is very likely that COG44 has a deletion of the paternal copy of *NSD1*. Three patients (COG7, COG23, and COG50) were homozygous at the two intragenic markers and were homozygous for the common alleles at the 15 *NSD1* polymorphisms, but they did not show reduction of *NSD1* exon peak height in the multiplex PCR and are thus very unlikely to have a whole gene deletion.

NSD1 Mutation Analyses

Genomic DNA from all 75 patients was screened for intragenic *NSD1* mutations by CSGE. In total, 32 mutations were identified (table 4). Twenty-one mutations were predicted to result in premature truncation of the protein because of frameshift or nonsense alterations. Two splice-site mutations were identified and are also likely to be pathogenic, although the precise effect of these mutations was not determined. The truncating mutations were spread fairly evenly through the gene, between exons 4 and 23 (fig. 3). One mutation (R604X) was identified in two separate patients, COG22 and COG54. Analysis of parental samples for COG22 demonstrated that the mutation had occurred de novo and, hence, these two identical mutations have arisen separately. The mutation is at a CpG dinucleotide, which is known to be particularly susceptible to mutation (Cooper and Krawczak 1989). All other mutations were identified only once. Samples from both parents were available for eight patients with truncating *NSD1* mutations, and all were wild type, indicating the mutations had occurred de novo in the affected child.

We identified nine missense mutations, and these were clustered in the C-terminal half of *NSD1*, between exons 13 and 23 (fig. 3). For seven patients with missense mutations, analysis of parental samples indicated the mutations had occurred de novo and, hence, are highly

Table 2**Primer Sequences for Chromosome 5q35 Microsatellite Markers and Positions Relative to *NSD1***

MARKER	POSITION RELATIVE TO <i>NSD1</i> ^a (kb)	PRIMER SEQUENCE (5'→3')		SIZE (bp)
		Forward	Reverse	
SOT12	645 cen	GATAAACCACAACCCCAACC	ACGTAGCTAGGCACCACCAT	172
SOT11	391 cen	CAGGCTCGTTCATTCACAAA	AGGCAAAATTCCTCCCATC	203
SOT10	383 cen	GACTGCAAGGAGCTTGAACC	GGGCCACCATACTTGTTC	148
SOT1	181 cen	GGGAAAGTTGACAGGATTTTGA	GCAAATAGGGCATCTGCAAG	226
SOT4	26 cen	GGTCCTCCACACATTCTGCT	ACATGCCCTATGACCTGGAA	201
SOT3	Intragenic	GCACCGTTTTACAGTCTACTT	CTGCAGTGAGCCAAGACCAT	216
SOT17	Intragenic	GGCATTGTTCTGGATGAGA	GGAGATGGATGTTGCAGTGA	201
SOT19	284 tel	CCCCTTTGTATGGGGTCTTT	CCTGGGTGACACAGTGAGACT	201
SOT20	290 tel	CCAGTTCATCCAAGACACA	CATTTGATCCAGAAATCCCACT	267

^a The position relative to the first base of *NSD1* is given for markers centromeric (cen) to *NSD1*. The position relative to the last base of *NSD1* is given for markers telomeric (tel) to *NSD1*. Marker positions are based on the UCSC Human Genome Project Working Draft.

likely to be pathogenic (table 4). Samples from both parents were not available for the remaining missense alterations; however, these mutations (C1925R and C1674W) were not present in the other 74 patients or 200 control individuals. Further evidence supporting a pathogenic role for the missense alterations was obtained from analysis of their position within *NSD1*. All occur at residues within functional domains and are conserved not only in mouse *Nsd1* but also in the two known human paralogues of *NSD1*, *NSD2* (also known as *MMSET* [Chesi et al. 1998] and *WHSC1* [Stec et al. 1998]) and *NSD3* (Angrand et al. 2001). Four missense mutations (H1616L, L1637P, C1674W, and H2143E) occur within the PHD domains, and three of these occur at consensus Cys/His residues and would be expected to have critical effects on substrate binding by these domains (fig. 4A). Two further missense mutations also occur at Cys residues: C2183S, in a Cys/His-rich region adjacent to PHD-V, which may correspond to another zinc finger-like motif and is present in mouse *Nsd1* and in human *NSD2* and *NSD3* (fig. 4B); and C1925R, which is in the consensus motif of the SAC domain (fig. 4B). The R2005Q and R2017Q mutations occur within the highly conserved SET domain (fig. 4B), and G1792V is in the PWWP-II domain (fig. 4B).

We identified 15 polymorphisms (table 5). These were considered nonpathogenic, since they were identified in multiple individuals, both affected and unaffected, and were present in patients with a separate pathogenic *NSD1* mutation. Moreover, several are intronic or result in synonymous amino acid changes.

Correlation of *NSD1* Aberrations and Phenotype

There was a strong correlation between the presence of *NSD1* mutation and phenotype (table 1; fig. 3). Twenty-six of 32 mutations and two of three deletions

were identified in group 1 (classic Sotos) patients. Thus, we identified a pathogenic *NSD1* aberration in 28 of 37 group 1 patients, a frequency of 76%. Of 13 group 2 (Sotos-like) patients, 3 harbored *NSD1* mutations, and 1 group 2 patient had an *NSD1* deletion. The overall frequency of *NSD1* alterations in the group 2 patients was thus 4 of 13 patients (30%). In contrast, none of the 18 group 4 (non-Sotos, non-Weaver overgrowth) patients harbored either *NSD1* mutations or deletions. Of 7 patients with Weaver syndrome, 3 (42%) harbored *NSD1* mutations (fig. 3). Intriguingly, these were all within a 40-amino acid region between 2142 and 2184 in exons 22 and 23 and were the most C-terminal mutations identified (fig. 3).

Discussion

Recently, deletions and mutations in *NSD1* were reported in a series of Japanese patients with Sotos syndrome (Kurotaki et al. 2002). To evaluate the contribution of *NSD1* aberrations to childhood overgrowth syndromes, we have undertaken an analysis of *NSD1* in a phenotypically characterized series of childhood overgrowth syndrome cases from Britain. Our results demonstrate that 76% of patients with Sotos syndrome harbor deleterious alterations of *NSD1*. Thus, functional abrogation of one copy of *NSD1* is the major—and possibly the only—cause of Sotos syndrome. We did not identify mutations or deletions in nine patients with classic Sotos syndrome. Although it is possible that mutations of another gene can lead to Sotos syndrome in a minority of cases, it is equally plausible that the patients with unexplained Sotos syndrome in our series have underlying aberrations in *NSD1* that were not detected, either because of lack of sensitivity of the screening technique or because they result in alterations that are not

Table 3**Primer Pairs Used to Amplify the *NSD1* Coding Sequence, and Sizes of PCR Products**

EXON	PRIMER SEQUENCE (5'→3')		SIZE (bp)
	Forward	Reverse	
2A	AGAGTCGAGTCAGATGGCCTA	GATCCATCAGCAGACCCATT	355
2B	GTGGAACATCCCAAATGCT	TCTGTGACTGGCTGTTCTGG	367
2C	TGGCTTTCTGCACTTTGAGA	GAAGGGCTGCTTTTTTCATTG	317
2D	GCCATTCTTGCCATTAGCTC	TTTCCCTTTAAGTGGCCTGT	323
3	TGCTTTTTCAGAAGGCTAATAGG	TCATTCACAAAATGTTCCAAGG	332
4	GCAATGATGTGGCTGTTCTC	TCCAATCTGGGAAACAGAGC	364
5A	TCTGATTTTCATCTCCCTTTTCC	GGCTTTTCCTTCTCATCTGC	315
5B	ATGCCATTGAAGACTGCAC	TCCACAGGAAGAAAACAGAAAA	360
5C	TCCACAGGAAGAAAACAGAAAA	TATGGGATCCAGGTCACCTGC	323
5D	GGAAAAGCGAAGTGATTCCA	TCTGACTGGGGTTTGTGAAC	348
5E	GGGTTGTACTAAGAGTGCAGAGC	TTAGAAATGCTGGCCAAAAGG	348
5F	TATGGCAGAACCCCCAGTTA	CGCTGCTCCTTCGTCTTACT	381
5G	TGGAACATCAAAGCCATCAA	CGCCAGATAATGCAGAGTCA	332
5H	GGCTCCACACACAATTCAGA	CTCCCTGCAGTACAGCATCA	334
5I	ATGCTTTTTTCAGCCCAAATG	CTGGGCCTTTTCCGTTTT	312
5J	GATGTGCATTTTCGATAGCAAG	GCTCTGTCAAGTGGTTCCTCA	343
5K	GGTCTTACTTCCCTAGTGACC	TATCACATTTAGATGTCCTTAC	255
6	ATGTGGTTTTCCCATCTGGTT	TGACATTGAAGATAAAAATTGCAG	300
7	AACAATTTTGGCCTGTGGAC	TCAAATACTGAGACCCCAACC	345
8	TTGTGCCCAGTTTCTAAATCA	TGCAAAACAGCCTTTCATGT	327
9	TGGCAGCTGACAATTCAGAC	CTCACTGGTCGGGCTTACAC	268
10	CCCGTTTTCTAATCCACAA	CCTCTGGCGTGAAAAGTAGC	310
11	AGGGGGTCAAATGGAAGAGA	GATGGAGTGGGTTTCCCTTT	273
12	TCACCTCCTTTTCTGCCACT	CCCAGTGTTGCCACAAAATA	337
13	TGGGTTCCAGACGATGTCAA	TCTGTTGCCAATTAACTGAGG	383
14	TCCATCATCTTAGTGGTCATTCC	TCCAGTGGCAATATGATGAAA	411
15	TGGATGTACACATACATGACTTGC	AAGAGGGGAGGAGTACCATGA	342
16	ATTTTCTAATGCCTTGCAG	GGCAGTTTCAAAAATGGAGCA	364
17	TCTCCAACCTTAAAGGGGAAAAA	AGAGTGGGAAGAGCCAGCTA	273
18	GGACGTGAATTGTCTTCTGCT	TCAAGCAACTGCAAAGAGGA	381
19	CTGCTGCTGACAGTGGTAGG	TATGGCTGGGACAACACAAA	386
20	AAATTTAATCCACAGCAGAGGTC	GTGGTGATGGTTGCACAAAA	356
21	TCTTGGGAGTTGGTATCCTTTG	AACACTGTTAGGGAGGGAGGA	257
22	GAATGAGGCTCAGAGAGGGTA	AAATGGCATGAGACCCTGAG	360
23A	GGAAGGTCATCATCCACACC	GATTGCTCTGCCAGGTGAGT	356
23B	TGGGGAGATCCGTGAGTATG	CTGGTCACTGGAGAGGGTTT	369
23C	AATCCCAATCCTTGGTTTCC	GGTCTGGACCACAGCTGATA	380
23D	CCTACTGACAAACCCCATGC	CTGCTGCTTTCCAGATGTC	346
23E	AGCAAAGGTCTGGGGCATA	CAGGGACTTTGCTCTGTGGT	395
23F	TTTTAGGTCTCTCGGGAAGG	GGGGCAGCTTGTGTTGTTT	331

detectable by our methods, such as genomic rearrangements of *NSD1* or regulatory mutations.

The frequency (76%) of pathogenic *NSD1* aberrations in our series of patients with Sotos syndrome is very similar to the 77% reported by Kurotaki et al. (2002). However, the spectrum of *NSD1* aberrations is rather different in the two series. In our series, whole gene deletions occurred in only 8% (3/37) of patients with Sotos syndrome, compared with 66% (20/30) of patients in the Japanese study. Furthermore, Kurotaki et al. (2002) reported a common 2.2-Mb deletion in 19 patients. Although we have not defined the precise breakpoints in our three patients with Sotos syndrome who have deletions, the centromeric breakpoint in COG25 must be

telomeric to SOT12, and the telomeric breakpoint in COG44 must be centromeric to SOT21. This positions these breakpoints well within the 2.2-Mb interval reported, and, thus, these two patients are very unlikely to carry the common deletion found in the Japanese patients with Sotos syndrome. Conversely, we detected intragenic mutations in 70% (26/37) of our patients with Sotos syndrome, whereas the Japanese group identified *NSD1* mutations in only 10% (4/38). Clinical evaluation of our three patients with 5q35 microdeletions suggest they have more-severe learning difficulties, a coarser facial gestalt, and less pronounced overgrowth than is typical for patients with intragenic *NSD1* mutations.

The reasons for the disparity in *NSD1* pathogenic

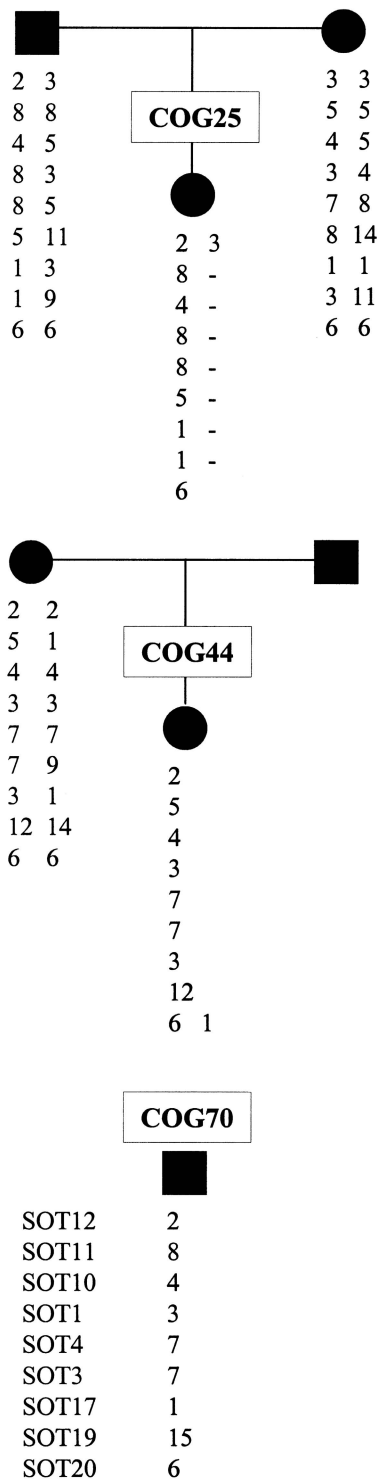
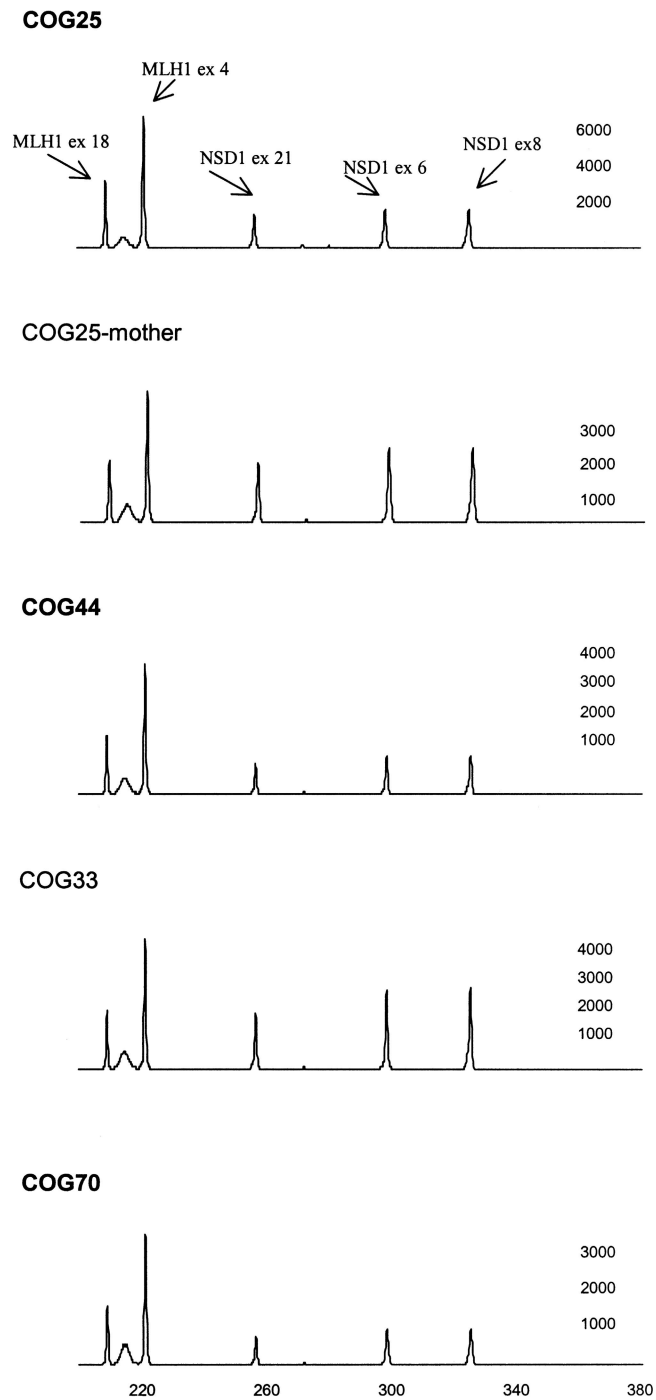
A**B**

Figure 2 *NSD1* deletions in three patients with childhood overgrowth—COG25, COG44, and COG70. **A**, Marker allele haplotypes for nine microsatellite markers spanning *NSD1*, demonstrating maternal deletion in COG25, probable paternal deletion in COG44, and deletion of unknown origin in COG70. **B**, Fluorescent multiplex PCR demonstrating a 0.5 reduction of *NSD1* exons relative to *MLH1*, in COG25, COG44, and COG70, and a normal ratio in two control samples: (1) the mother of COG25 and (2) COG33 (who has an intragenic *NSD1* mutation). The Y-axis shows fluorescence (in arbitrary units), and the X-axis indicates the size (in bp).

Table 4
Pathogenic *NSD1* Mutations Identified in 75 Patients with Childhood Overgrowth

Mutation	Protein Change ^a	Exon	Patient	Phenotypic Group	Results of Mutation Analysis of Parental Samples ^b
Deletion:					
1171delC	Q391fsX418	4	COG14	1	No mutation in parents
1727delA	N576fsX598	5	COG57	1	...
2576delAT	H859fsX873	5	COG69	2	...
3383delCT	S1128fsX1129	5	COG67	1	...
4883delT	M1628fsX1641	13	COG45	1	No mutation in mother
6001delC	L2001fsX2001	19	COG77	1	...
6302delA	K2101fsX2149	22	COG29	1	No mutation in parents
Insertion:					
2807-8insA	Y936fsX936	5	COG75	1	...
3549-50insT	E1184fsX1184	5	COG73	2	...
5008-9insG	A1670fsX1672	14	COG66	1	...
5744-5insT	M1915fsX1919	18	COG30	1	No mutation in parents
6431-2ins17	A2144fsX2155	22	COG17	1	No mutation in parents
6450-1insC	K2151fsX2165	22	COG6	3	No mutation in mother
Nonsense:					
1492C→T	R498X	5	COG51	2	...
1810C→T	R604X	5	COG22	1	No mutation in parents
1810C→T	R604X	5	COG54	1	...
2323C→T	Q775X	5	COG74	1	No mutation in parents
4411C→T	R1471X	10	COG33	1	No mutation in mother
4417C→T	R1473X	10	COG21	1	No mutation in parents
5332C→T	R1778X	16	COG63	1	...
5861G→A	W1954X	18	COG28	1	No mutation in parents
Splice-site:					
IVS15-1G→C		Int15	COG72	1	...
IVS16-2delA		Int16	COG35	1	No mutation in mother
Missense:					
4847A→T	H1616L	13	COG24	1	No mutation in parents
4910T→C	L1637P	13	COG31	1	No mutation in parents
5022C→G	C1674W	14	COG52	1	...
5375G→T	G1792V	16	COG76	1	No mutation in parents
5773T→C	C1925R	18	COG34	1	No mutation in mother
6014G→A	R2005Q	20	COG5	1	No mutation in parents
6050G→A	R2017Q	20	COG27	1	No mutation in parents
6429C→G	H2143E	22	COG15	3	No mutation in parents
6548G→C	C2183S	23	COG62	3	No mutation in parents

^a Frameshift mutations are designated according to the following example: Q391fsX418 refers to a frameshift mutation in which Q391 is the first amino acid altered, with termination of the ORF at residue 418.

^b If the result of a parental analysis is not given, the sample was not available for analysis.

spectra in the Japanese and British populations are not clear. It could simply reflect the particular patients analyzed in these initial studies, and, as more cases are screened, the frequencies of mutations and deletions in the different populations may become more comparable. Indeed, it is quite likely that the difference in *NSD1* mutation frequency is explicable by a relative enrichment of true Sotos syndrome in our group 1, since many of the 75 patients we analyzed were initially referred with a potential diagnosis of Sotos syndrome (i.e., group 1) but were rescored as group 2, 3, or 4 by clinical geneticists with expertise in overgrowth conditions.

The disparity in the incidence of *NSD1* deletions in the two series is more difficult to resolve. We cannot be missing many deletions, since 69 of 75 patients had *NSD1* mutations and/or polymorphisms that were identified by a heteroduplex assay (CSGE) and, hence, were only detectable if two copies of the gene were present. A possible explanation is a specific Japanese overgrowth phenotype, caused by a 5q35 microdeletion, which is very uncommon in Britain. A separate “Japanese Sotos” phenotype has been postulated in the past, to account for the higher incidence of congenital heart disease in Japanese patients with Sotos syndrome (Kaneko et al.

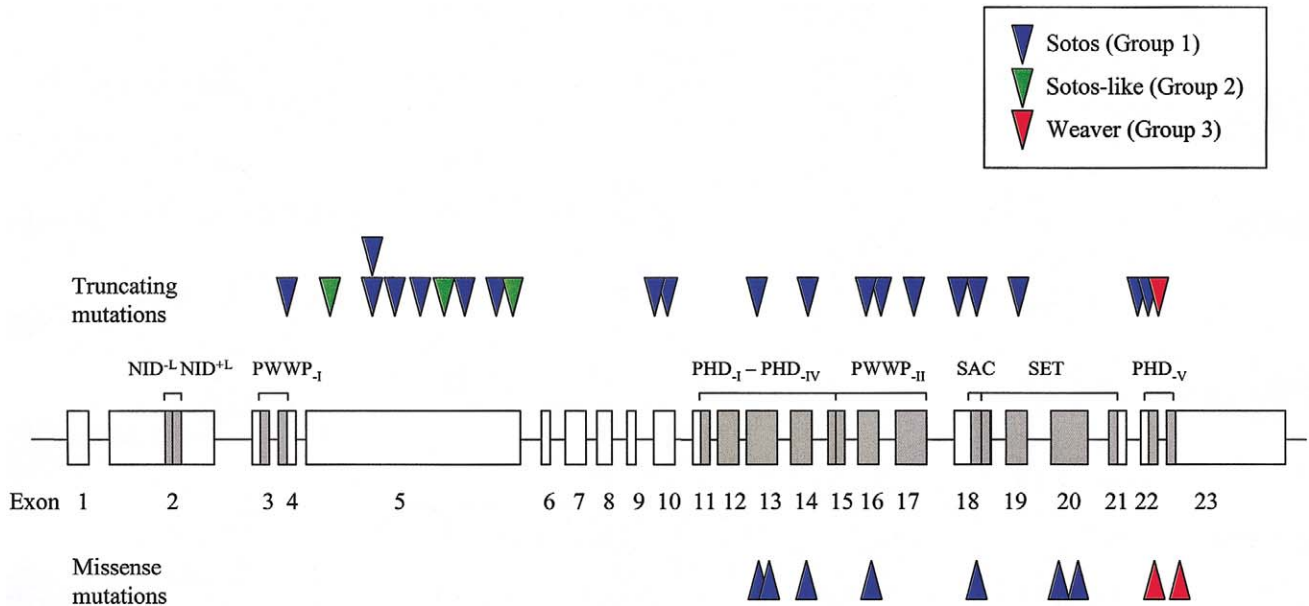


Figure 3 Schematic representation of *NSD1*, showing the distribution of intragenic mutations identified in the present study and the associated phenotype. Exons of *NSD1* are shown as boxes with the exon number underneath. Functional domains of *NSD1* are shown as shaded boxes, with the domain name above. Mutations are shown as triangles, with truncating mutations (insertions, deletions, nonsense, and splice-site) above the gene and missense mutations below the gene. One mutation (R604X) was identified twice and is depicted as two triangles, one above the other.

1987). However, the Japanese *NSD1* deletions occurred in patients with sporadic Sotos syndrome and, thus, seem unlikely to be caused by a single Japanese founder.

Whatever the reason(s) for these apparent differences, the very high frequency of mutations in our study clearly indicates that intragenic *NSD1* mutations are the major cause of Sotos syndrome in the British population. We therefore believe that Sotos syndrome should be considered a single-gene disorder analogous to neurofibromatosis type 1, in which the majority of cases are due to *NF1* mutations, with a minor proportion (5%–10%) attributable to chromosomal aberrations that result in deletion of one copy of the gene (Lopez-Correa et al. 2000). We do not consider Sotos syndrome a genomic disorder, comparable to syndromes such as Williams or Smith-Magenis, in which the predominant pathogenetic mechanism is a recurrent chromosomal microdeletion (Stankiewicz and Lupski 2002).

Given that Sotos syndrome is generally caused by hemizygous mutations in an autosomal nonimprinted gene, it is of interest that it does not behave as an autosomal dominant trait and that familial cases are so rare. The mental impairment in Sotos syndrome is usually in the mild/moderate range and is not sufficiently severe to account for the extreme paucity of familial Sotos cases. All of the 15 mutations in which we could assess inheritance occurred de novo, and the 5q35 microsatellite analyses confirmed paternity in all cases.

Furthermore, of the 37 group 1 patients with classic Sotos syndrome, only one, COG76, has a family history of the disease, in that her daughter also has Sotos syndrome. Unfortunately, DNA from the daughter was not available for analysis, but she is very likely to carry the G1762V mutation that has occurred de novo in her mother.

The most likely reason for the lack of familial cases is an underlying defect in fertility associated with *NSD1* mutations. Delayed menarche, oligomenorrhea, and increases in the rate of spontaneous abortions and stillbirths have been reported in patients with Sotos syndrome (Opitz et al. 1998). Since the diagnostic criteria for the syndrome were reported only relatively recently, it is only now that a generation of children with diagnosed Sotos syndrome are reaching reproductive age, and, thus, the incidence and nature of fertility defects may become clearer in the near future. It is possible that rare cases of familial Sotos syndrome may be explicable by *NSD1* mutations that result in Sotos syndrome but not in reproductive impairment. It is noteworthy, in this regard, that the only patient in our series who has a family history of Sotos syndrome has a missense mutation in the PWWP-II domain, and functions of other *NSD1* domains may be intact in carriers of this mutation. However, further investigation of patients with familial Sotos syndrome and of the functions of *NSD1* are required before conclusions about the postulated

A

PHD domains

NSD1 PHD-I	1545	V	Q	N	C	E	K	L	G	E	L	L	-----	C	E	A	Q	-	C	G	A	T	H	L	E	--	C	L	G	L	T	E	M	P	R	G	K	F	I	---	C	N	E	C	R	1587												
NSD1 PHD-II	1592	T	C	F	V	C	K	Q	S	G	E	D	V	K	R	-----	C	L	L	P	L	C	G	K	P	Y	H	K	E	--	C	V	Q	K	Y	P	P	T	V	M	Q	N	K	G	F	R	C	S	T	H	I	1639						
NSD1 PHD-III	1639	I	C	I	T	C	H	A	N	P	A	N	V	S	A	S	K	G	R	L	M	R	C	V	R	--	C	P	V	A	Y	H	A	N	D	F	C	L	A	A	G	S	K	I	L	A	S	N	S	I	I	-	C	P	N	H	F	1693
NSD1 PHD-IV	1709	W	C	F	V	C	S	E	G	G	S	L	L	C	-----	C	D	S	--	C	P	A	A	F	H	R	E	--	C	L	N	I	D	I	P	E	G	N	W	Y	---	C	N	D	K	1749												
NSD1 PHD-V	2120	E	G	F	S	C	G	D	A	G	Q	L	V	E	-----	C	K	K	P	G	C	P	K	V	Y	H	A	D	--	C	L	N	L	T	K	R	P	A	G	K	W	E	---	C	P	W	H	Q	2163									

B

PWWP II domain

NSD1	1790	D	V	G	E	F	P	V	L	F	F	G	S	N	D	Y	1804
Nsd1	1688	D	V	G	E	F	P	V	L	F	F	G	S	N	D	Y	1805
NSD2	914	E	I	G	E	F	P	V	F	F	G	S	K	D	Y	928	
NSD3	994	D	L	G	D	F	P	V	F	F	G	S	H	D	Y	1008	

SAC domain

NSD1	1910	E	C	I	N	R	M	L	L	Y	E	C	H	P	T	V	C	P	A	G	G	R	C	Q	N	Q	1934
Nsd1	1808	E	C	I	N	R	M	L	L	V	E	C	H	P	T	V	C	P	A	G	G	R	C	Q	N	Q	1935
NSD2	1031	E	C	L	N	R	M	L	M	F	E	C	H	P	Q	V	C	P	A	G	E	F	C	Q	N	Q	1055
NSD3	1113	E	C	L	N	R	M	L	Q	Y	E	C	H	P	Q	V	C	P	A	G	D	R	C	Q	N	Q	1137

SET domain

NSD1	2000	T	L	D	K	R	I	I	D	A	G	P	K	G	N	Y	A	R	F	M	N	H	2021	
Nsd1	1898	T	L	D	K	D	R	I	I	D	A	G	P	K	G	N	Y	A	R	F	M	N	H	2022
NSD2	1120	T	I	D	K	D	R	I	I	D	A	G	P	K	G	N	Y	S	R	F	M	N	H	1141
NSD3	1203	T	V	T	K	D	R	I	I	D	A	G	P	K	G	N	Y	S	R	F	M	N	H	1224

C/H rich region

NSD1	2173	S	F	C	E	M	C	P	S	S	F	C	K	Q	H	R	E	G	M	2190
Nsd1	2071	S	F	C	E	M	C	P	S	S	F	C	K	Q	H	R	E	G	M	2088
NSD2	1294	S	F	C	H	L	C	P	N	S	F	C	K	E	H	Q	D	G	T	1311
NSD3	1376	S	F	C	E	F	C	P	H	S	F	C	K	D	H	E	K	G	T	1393

Figure 4 *NSD1* missense mutations in patients with childhood overgrowth. Residues altered by missense mutations are shown in red boxes. Consensus residues in the PHD and SAC domains are shown in black boxes. Conserved residues in *NSD1*, mouse *Nsd1*, *NSD2*, and *NSD3* are shown in shaded boxes. A, The alignment of five PHD domains in *NSD1* and the position of the missense mutations identified in the present study. B, Missense mutations in PWWPII, SAC, SET, and C/H-rich domains and in corresponding regions in mouse *Nsd1*, *NSD2*, and *NSD3*.

reproductive impairment in Sotos syndrome can be drawn.

We analyzed seven patients with Weaver syndrome and identified three *NSD1* mutations. Weaver syndrome is a childhood overgrowth syndrome that shares many phenotypic features with Sotos syndrome but is much less common. Identification of *NSD1* mutations in patients with Weaver syndrome demonstrates that the two conditions are allelic. However, we have investigated only a small number of patients with Weaver syndrome, and *NSD1* mutations were not identified in over half of them, all of whom have phenotypes typical of the syndrome. It remains possible, therefore, that a separate, second Weaver syndrome gene exists. Additional patients with Weaver syndrome need to be screened, to more accurately assess the frequency of *NSD1* mutations in this condition.

Our results revealed a strong correlation between clinical phenotype and presence of a deleterious *NSD1* mutation or deletion. Most striking was the absence of

any mutations or deletions in the 18 group 4 patients with a childhood overgrowth syndrome that was definitely not either Sotos syndrome or Weaver syndrome. The group 4 patients are likely to have a heterogeneous group of conditions, and the majority of patients in this group were felt to have an overgrowth condition that did not correspond to any recognized syndrome. Four of 13 group 2 (Sotos-like, but with some atypical features) patients harbored pathogenic *NSD1* alterations. The individual phenotypic scores for three of these were available, and, for each, one clinician (who had actually met the patient) scored the patient as group 1, whereas the other two (who only reviewed photographs) scored the patient as group 2. The fourth mutation-positive group 2 patient was part of the original study that established the diagnostic criteria of Sotos syndrome (Cole and Hughes 1994). This patient was initially scored as group 1 on the basis of the facial gestalt alone but was rescored as group 2 because his bone age was not advanced. Thus, our results suggest that *NSD1* aberrations

Table 5
Polymorphisms in the *NSD1* Gene

Location and Nucleotide Change	Protein Change
Exon 5:	
1749G→A	E583E
1792T→C	L599L
1840T→G	V614L
2071G→A	A691T
2176T→C	S726P
3106G→C	A1036P
3271C→A	L1091I
3705T→C	N1235N
Intron 10:	
IVS10-9delT	...
Intron 17:	
IVS17-22G→A	...
Intron 18:	
1VS18-132G→A	...
Exon 23:	
6750G→A	M2250I
6782T→C	M2261T
6829C→T	L2277L
6903G→C	G2301G

are rather specific for Sotos and Weaver syndromes, and the frequency in other phenotypes is likely to be very low. However, given the challenges of accurate clinical diagnosis of Sotos and Weaver syndromes, a low threshold for diagnostic *NSD1* screening in overgrowth conditions would be prudent, until the associated range of phenotypes becomes clearer.

Our data provide preliminary indications of possible genotype-phenotype associations. The majority of known *NSD1* functional domains are in exons 11–23, and all missense mutations we identified were clustered in these domains, within highly conserved regions in mouse *Nsd1* and human *NSD2* and *NSD3*. It is possible that missense mutations outside these domains do not cause Sotos syndrome. Perhaps the most intriguing finding is the clustering of the three Weaver syndrome mutations within a 40–amino acid region in exons 22 and 23. This region is in the PHD-V domain and the adjacent Cys/His-rich region, and all are 5' to the SET domain, which may retain some function. It is possible that the position of mutations in the patients with Weaver syndrome is not coincidental and reflects a genotype-phenotype correlation, whereby mutations within the C-terminal region of the gene, after the SET domain, give rise to a Weaver phenotype. However, a mutation within this 40–amino acid region was also identified in a patient with classic Sotos syndrome, indicating that the correlation, if substantiated, cannot be completely specific.

Both Sotos and Weaver syndromes are associated with a wide spectrum of additional abnormalities, in-

cluding congenital heart disease, scoliosis, and cancer. Investigation of mutational spectra in relation to the phenotype of individual patients may reveal further genotype-phenotype associations and may also be helpful in clarifying the complex functions and interactions of *NSD1*. The reason why functional abrogation of *NSD1*, a bifunctional transcriptional regulator with a postulated role in histone and chromatin modification, results in overgrowth and the wide phenotypic spectrum of Sotos and Weaver syndromes remains to be elucidated.

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Electronic-Database Information

Accession numbers and URLs for data presented herein are as follows:

- GenBank, <http://www.ncbi.nlm.nih.gov/Genbank/> (for the *NSD1* genomic sequence [accession number AF395588])
 NCBI BLAST Home Page, <http://www.ncbi.nlm.nih.gov/blast/> (for comparison of human *NSD1* [GenBank accession number AF395588; RefSeq protein sequence NP_071900] with mouse *Nsd1* [GenBank accession number AF419220, RefSeq protein sequence NP_032765] and human *NSD2* [GenBank accession number AF083389, RefSeq protein sequence NP_579890] and human *NSD3* [GenBank accession number AF332469, RefSeq protein sequence NP_075447])
 Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim/> (for Sotos syndrome [MIM 117550], Weaver Syndrome [MIM 277590], Marshall Smith syndrome [MIM 602535], autosomal dominant macrocephaly [MIM 605309, MIM 153470], Beckwith-Wiedemann syndrome [MIM 130650], Simpson-Golabi Behmel syndrome [MIM 312870], Neurofibromatosis type 1 [MIM 162200], Williams syndrome [MIM 194050], Smith-Magenis syndrome [MIM 182290])
 Primer3, http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi (for designing microsatellite markers amplifying repetitive elements in the vicinity of *NSD1* and *NSD1* mutation screening primers)
 UCSC Genome Bioinformatics, <http://genome.ucsc.edu/> (for identification and positioning of repetitive sequence elements flanking *NSD1*)

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