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Genotype-Phenotype Associations in Sotos Syndrome: An Analysis of 266 Individuals with *NSD1* Aberrations

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We identified 266 individuals with intragenic *NSD1* mutations or 5q35 microdeletions encompassing *NSD1* (referred to as "*NSD1*-positive individuals"), through analyses of 530 subjects with diverse phenotypes. Truncating *NSD1* mutations occurred throughout the gene, but pathogenic missense mutations occurred only in functional domains ($P < 2 \times 10^{-16}$). Sotos syndrome was clinically diagnosed in 99% of *NSD1*-positive individuals, independent of the molecular analyses, indicating that *NSD1* aberrations are essentially specific to this condition. Furthermore, our data suggest that 93% of patients who have been clinically diagnosed with Sotos syndrome have identifiable *NSD1* abnormalities, of which 83% are intragenic mutations and 10% are 5q35 microdeletions. We reviewed the clinical phenotypes of 239 *NSD1*-positive individuals. Facial dysmorphism, learning disability, and childhood overgrowth were present in 90% of the individuals. However, both the height and head circumference of 10% of the individuals were within the normal range, indicating that overgrowth is not obligatory for the diagnosis of Sotos syndrome. A broad spectrum of associated clinical features was also present, the occurrence of which was largely independent of genotype, since individuals with identical mutations had different phenotypes. We compared the phenotypes of patients with intragenic *NSD1* mutations with those of patients with 5q35 microdeletions. Patients with microdeletions had less-prominent overgrowth ($P = .0003$) and more-severe learning disability ($P = 3 \times 10^{-9}$) than patients with mutations. However, all features present in patients with microdeletions were also observed in patients with mutations, and there was no correlation between deletion size and the clinical phenotype, suggesting that the deletion of additional genes in patients with 5q35 microdeletions has little specific effect on phenotype. We identified only 13 familial cases. The reasons for the low vertical transmission rate are unclear, although familial cases were more likely than nonfamilial cases ($P = .005$) to carry missense mutations, suggesting that the underlying *NSD1* mutational mechanism in Sotos syndrome may influence reproductive fitness.

Introduction

The nuclear receptor SET domain–containing protein 1 (*NSD1*) gene encodes a histone methyltransferase and is located at chromosome 5q35. NSD1 was initially isolated in a search for proteins that interact with the ligand-binding domain of retinoic acid receptor α and subsequently was shown to be the fusion partner of NUP98 in some cases of childhood acute myeloid leukemia (Huang et al. 1998; Jaju et al. 2001). NSD1 contains multiple functional domains, including SU(VAR)3–9,E(Z),trithorax (SET) and SET-associated (SAC) domains that together mediate the histone methyltransferase activity of NSD1; a C5HCH and five plant homeodomains (PHDs), which are implicated in chromatin regulation and are zinc finger–like motifs characterized by cysteine and histidine residues; and two proline-tryptophan-tryptophan-proline (PWWP) domains that may mediate protein-protein interactions and that are often found in proteins that act at the chromatin level (Aasland et al. 1995; Stec et al. 2000; Rayasam et al. 2003). NSD1 also contains two nuclear-receptor interaction domains, NID^{-L} and NID^{+L}, which are typical of those found in nuclear-receptor corepressors and coactivators, respectively (Huang et al. 1998).

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The functions of NSD1 are not known, but it has been shown to methylate both H4 K20 and H3 K36, modifications that are individually associated with transcriptional repression (Rayasam et al. 2003). The presence of two distinct nuclear-receptor interaction domains (NIDs) has given rise to the hypothesis that differential ligand binding to NID^{-L} and NID^{+L} allows NSD1 to both negatively and positively regulate transcription (Huang et al. 1998).

In 2002, NSD1 haploinsufficiency was reported in patients with Sotos syndrome (MIM 117550), a condition characterized by facial dysmorphism, learning disability, and childhood overgrowth (Kurotaki et al. 2002). Subsequently, >100 individuals with *NSD1* abnormalities were reported in the literature (Douglas et al. 2003; Kurotaki et al. 2003; Rio et al. 2003; Türkmen et al. 2003; de Boer et al. 2004; Cecconi et al. 2005). The range and relative contributions of *NSD1* abnormalities and the spectrum of clinical phenotypes associated with *NSD1* have been unclear. In particular, the prevalence of 5q35 microdeletions in Japanese and non-Japanese individuals has been controversial. In Japan, 5q35 microdeletions are the most common cause of Sotos syndrome and are predominantly mediated by nonallelic homologous recombination between flanking low-copy repeats (Visser et al. 2005). Outside Japan, 5q35 microdeletions are uncommon, accounting for only 10% of affected individuals, and multiple mechanisms are implicated in their generation (Tatton-Brown et al. 2005). It was postulated that this difference in microdeletion frequency was due to variation in patient-selection criteria for *NSD1* analyses (Kurotaki et al. 2003). However, it seems more likely that differences in sequence architecture are responsible, since an inversion polymorphism that predisposes to microdeletions appears to be common in Japan (Tatton-Brown et al. 2005; Visser et al. 2005).

The range of clinical features associated with Sotos syndrome is broad, and it has been suggested that this variability is related to the underlying mutational mechanism. Specifically, it was proposed that the phenotypes of individuals with *NSD1* mutations and of those with 5q35 microdeletions differ because some features of Sotos syndrome, such as overgrowth and learning disability, are attributable to *NSD1,* whereas other features, such as cardiac and renal anomalies, occur only in individuals with microdeletions and are due to deletion of other genes (Nagai et al. 2003; Niikawa 2004). It has also been suggested that *NSD1* mutations can cause other overgrowth conditions such as Weaver syndrome (MIM 277590) and Beckwith-Wiedemann syndrome (BWS [MIM 130650]) (Douglas et al. 2003; Rio et al. 2003; Baujat et al. 2004).

In the present study, we screened a large series of subjects with diverse phenotypes for mutations and deletions of *NSD1,* to clarify the range and contribution of different *NSD1* abnormalities and the spectrum of associated clinical features. We compared the clinical features of individuals with mutations and microdeletions, as well as the clinical features of individuals with different-sized microdeletions, to investigate whether the deletion of other genes contributes to the phenotype of patients with 5q35 microdeletions. Additionally, we investigated the heritability and phenotypic variability of *NSD1* abnormalities, to provide information for counseling on recurrence and offspring risks and to evaluate whether prognostic information can be provided by identification of the underlying mutational mechanism in individuals with *NSD1* abnormalities.

Subjects and Methods

Patients

The research was approved by the London Multicentre Research Ethics Committee, and consent was obtained from participating subjects and/or their parents. Through analyses of 530 individuals, 266 patients with *NSD1* aberrations (referred to as "*NSD1*-positive individuals") were identified. This included 179 *NSD1*-positive individuals identified through analyses of 443 subjects in the Childhood Overgrowth Study. Patients recruited into this study have a broad range of phenotypes, including a clinical diagnosis of Sotos syndrome, overgrowth and/or macrocephaly (but without the typical facial features of Sotos syndrome), and facial features similar to Sotos syndrome (but no overgrowth). Of 179 subjects, 132 *NSD1*-positive individuals ascertained through these analyses were from the United Kingdom, and the remainder had diverse origins. NSD1 analyses of 73 of these individuals have been published elsewhere (Douglas et al. 2003, in press; Tatton-Brown et al. 2005). The remaining 87 *NSD1*-positive individuals were identified in collaborating centers in France, Germany, Italy, and the United States, and descriptions of 54 have been published elsewhere (Rio et al. 2003; Türkmen et al. 2003; Cecconi et al. 2005; Waggoner et al., in press). A standard clinical questionnaire was requested from all *NSD1*-positive individuals and was obtained from 239 of the individuals. Facial photographs of 290 of the 530 patients were evaluated independently by three clinical geneticists (T.R.P.C., H.E.H., and I.K.T.), who were unaware of the *NSD1* status, as described elsewhere (Douglas et al. 2003). The patients were categorized into four phenotypic groups: (1) "typical Sotos syndrome," (2) "possible Sotos syndrome," (3) "Weaver syndrome," and (4) "definitely not Sotos or Weaver syndrome." If another diagnosis, such as BWS, was thought possible, this was noted.

Table 1

Summary of the Number of Patients with Different *NSD1* Abnormalities

Category and Type of *NSD1* Aberration	No. of Patients
Intragenic mutations:	233
Frameshift	91
Nonsense	59
Missense	64
Splice site	11
Partial-gene deletions	8
5q35 microdeletions	33
Total	266

NSD1 *Analyses*

The 443 subjects collected through the Childhood Overgrowth Study were screened for intragenic mutations by use of conformational sensitive gel electrophoresis and sequencing, as described elsewhere (Douglas et al. 2003). To identify 5q35 microdeletions, all subjects were initially analyzed at the highly polymorphic intragenic microsatellite marker SOT3 (Douglas et al. 2003). Individuals who were homozygous at SOT3 were further analyzed by multiplex ligation-dependent probe amplification (MLPA) with the use of the SALSA P026B *NSD1* kit to identify 5q35 microdeletions, which were confirmed by quantitative fluorescent PCR and/or microsatellite analyses, as described elsewhere (Douglas et al. 2003; Tatton-Brown et al. 2005). Individuals with the classic facial features of Sotos syndrome whose mutation screening was negative were also analyzed by MLPA, even if they were heterozygous at SOT3, to identify partial-gene deletions/duplications. This revealed eight partial-gene deletions involving one or more exons (Douglas et al., in press). *NSD1* mutations in patients identified in collaborating centers were detected by sequencing and/or denaturing high-performance liquid chromatography, and 5q35 microdeletions were identified by FISH, as described elsewhere (Rio et al. 2003; Türkmen et al. 2003; Cecconi et al. 2005; Waggoner et al., in press).

Statistical Analyses

Statistical analyses were performed with the program R 2.0.1 (R Project for Statistical Computing). The statistical significance of the clustering of pathogenic mutations in the functional domains of NSD1, comprising 23% of the whole protein, was evaluated with the exact binomial test. Comparisons of proportions in two-way contingency tables were performed with the Fisher's exact test. Height, head circumference, and learning disability were compared among groups with the Wilcoxon rank-sum test. The co-occurrence of dichotomous clinical features—cardiac anomalies, renal anomalies, seizures, and scoliosis—was tested with Pearson's correlation and the Bonferroni correction. All reported *P* values are two-sided.

Results

Spectrum of NSD1 *Abnormalities*

We identified *NSD1* abnormalities in 266 of the 530 subjects, of whom 122 were girls and 144 were boys. The mean parental ages for 46 U.K. *NSD1*-positive individuals born between the years 1997 and 2003 were 30.2 years for mothers and 33.2 years for fathers. National averages for a comparable time period are 29.1 years for mothers and 32.3 years for fathers.

Of the 266 *NSD1*-positive individuals, 233 had 180 different intragenic *NSD1* mutations, and the remaining 33 individuals had 5q35 microdeletions encompassing *NSD1* (fig. 1*A* and tables 1 and 2). We identified 117 different truncating *NSD1* mutations, including 50 deletions of 1–10 bp, 23 insertions of 1–17 bp, 6 in/del mutations, and 37 nonsense mutations generating stop codons (table 2). One mutation was an insertion of ~190 bp from an *Alu*-Y element into exon 11, which likely results in premature protein truncation (Douglas et al., in press). We detected 10 different mutations affecting consensus splice-site residues. The precise effect of these is not known, but they likely result in premature protein truncation or exon skipping. The truncating and splice-site mutations were positioned evenly through the gene (fig. 1*B*).

To investigate the pathogenicity of missense alterations, we compared 34 de novo mutations, which we assumed were disease causing, with 24 sequence variants detected in unaffected individuals, which we assumed were nonpathogenic polymorphisms (table 2). The de novo missense mutations all occurred within functional domains that comprise 622 of the 2,696 amino acids of the protein, and this distribution was significantly different from that expected by chance (exact binomial test, $P < 2 \times 10^{-16}$) (fig. 1*B*). Furthermore, the pathogenic base substitutions were all nonsynonymous, whereas only 14 of 24 polymorphisms were nonsynonymous (Fisher's exact test, $P = 4 \times 10^{-5}$), and none of these was in a functional domain (Fisher's exact test, $P = 2 \times 10^{-12}$). Eleven de novo mutations targeted critical cysteine/histidine residues implicated in chromatin regulation (fig. 1*C*).

On the basis of these results, we considered 30 other individuals to have pathogenic missense mutations. These included 4 individuals with known mutations (i.e., mu-

Table 2

Intragenic Mutations and Exonic Polymorphisms in *NSD1*

The table is available in its entirety in the online edition of *The American Journal of Human Genetics*.

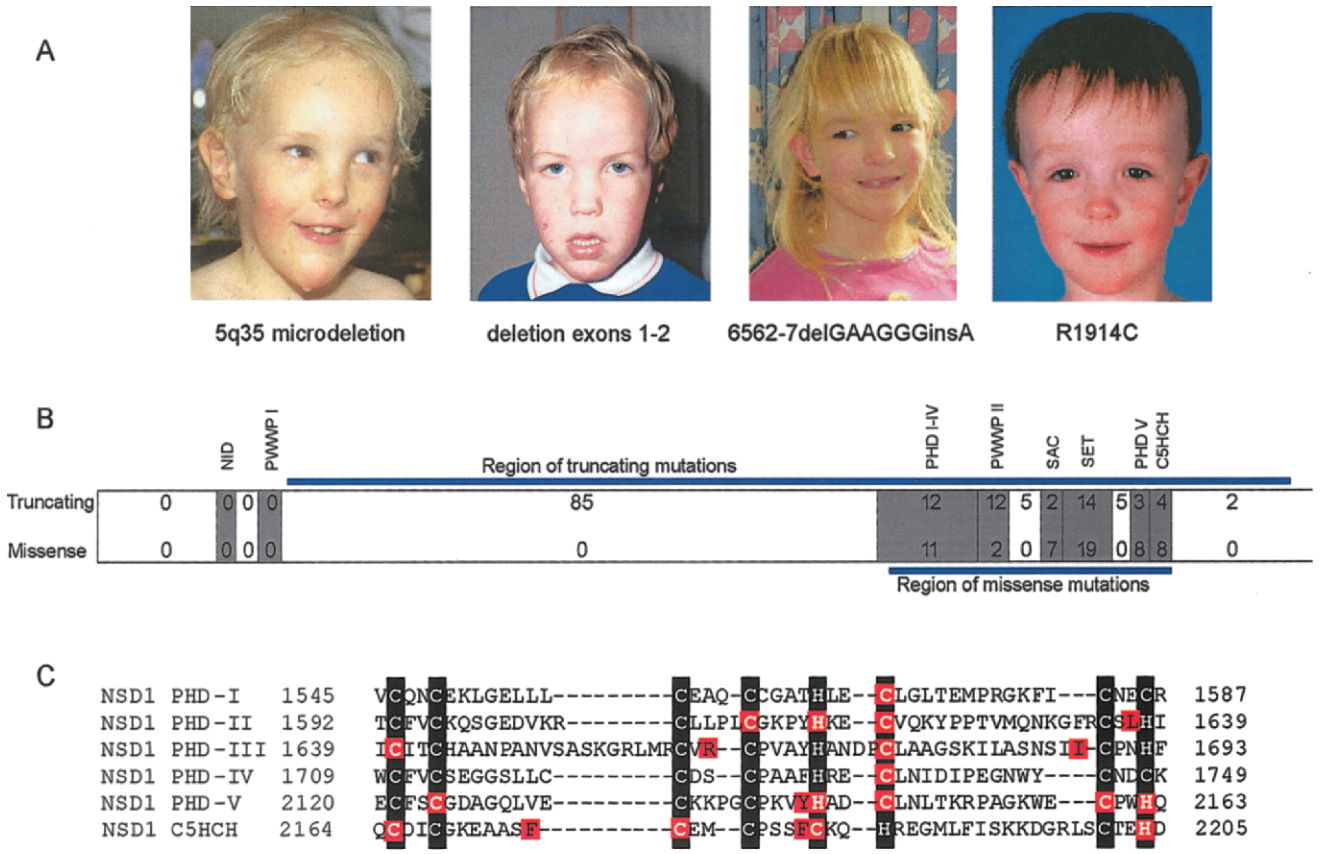

Figure 1 Facial features and *NSD1* mutations in patients with Sotos syndrome. *A,* Typical facial features of patients with different *NSD1* abnormalities: 5q35 microdeletion, partial *NSD1* deletion, truncating mutation, and missense mutation. *B,* Number of truncating and missense mutations identified in different NSD1 domains, demonstrating that missense mutations occur only within functional domains. The number of truncating mutations is at the top of each section of *NSD1,* and the number of missense mutations is shown at the bottom. Functional domains are shown as shaded boxes.*C,* Position of missense mutations (*red*) in PHD and C5HCH domains, showing bias toward mutations at consensus cysteine and histidine residues (*black*).

tations that had occurred de novo in other unrelated individuals), 10 with mutations that targeted consensus cysteine/histidine residues in functional domains, and 1 with an in-frame 3-bp deletion within PHD-V; in addition, six families (15 patients) were identified in which nonsynonymous mutations of conserved residues in functional domains segregated with Sotos syndrome in the family. Three mutations (F1177del-2, V1595A, and Y2058C) that were present in patients with classic Sotos syndrome were classified as variants of unknown significance and were excluded from further analyses. We strongly suspect that Y2058C, which alters a conserved residue in the SET domain, and F1177del-2, which results in the in-frame deletion of two amino acids, are pathogenic, but we did not have parental DNA to confirm that they arose de novo. V1595A is located in a PHD finger but does not affect a highly conserved residue. Moreover, it was present in both the affected individual and his unaffected mother and therefore may not be pathogenic.

We identified eight individuals with distinct exonic deletions of exons 1 and 2 (four individuals), exons 3–5, exons 9–13, exons 19–21, and exon 22 (Douglas et al., in press). Additionally, we identified 33 individuals with 5q35 microdeletions encompassing *NSD1* (Tatton-Brown et al. 2005). The deletion size in these individuals was very variable and ranged from 482 kb, in which only *NSD1* was deleted, to 5 Mb, in which at least 54 genes were deleted. In 18 individuals, microdeletions may have been mediated by nonallelic homologous recombination between flanking low-copy repeats. However, in 15 individuals, microdeletions cannot be mediated by recombination between these repetitive elements, and at least seven different deletion sizes were present among the 33 individuals (Tatton-Brown et al. 2005).

Familial and Recurrent NSD1 *Abnormalities*

Thirteen *NSD1*-positive individuals had affected relatives, and, in 12 families, we were able to demonstrate

Table 3

Clinical Features of Patients with Recurrent *NSD1* Mutations

Mutation and Patient ID	Clinical Feature(s)[a]
2386-9delGAAA:	
COG300	Severe LD, seizures, scoliosis, scaphocephaly
COG514	Moderate LD, seizures, ASD, VSD, PDA
COG523	Moderate LD, scoliosis
3549-50insT:	
COG073	Severe LD, seizures, complex cardiac anomaly, duplex kidney, hypercalcemia
COG095	Moderate LD, seizures, scoliosis
5279-82delTCTG:	
COG252	LD[b], ASD, scoliosis
COG288	Mild LD, conductive hearing loss
COG455	Mild LD
6596delG:	
COG265	Moderate LD, scoliosis, constipation, fixed flexion deformity of knees
COG272	Mild LD, seizures
R440X:	
COG196	No LD, bicuspid aortic valve, left pyelectasia
COG494	Moderate LD, strabismus, hyperlaxity, pectus excavatum
R604X:	
COG022	Mild LD, seizures, ASD
COG054	Mild LD, cardiac anomaly, scoliosis, strabismus
COG154	Severe LD, VSD, VUR, GOR, laryngomalacia
R611X:	
COG088	Mild LD, seizures, VSD
COG223	Moderate LD, scoliosis
R1031X:	
COG469	Moderate LD
COG502	Mild LD, scoliosis, cataracts, nystagmus
R1072X:	
COG133	Severe LD, seizures
COG497	Moderate LD
S1269X:	
COG365	Moderate LD, strabismus
COG394	Mild LD
R1322X:	
COG538	Mild LD
COG630	Moderate LD
R1811X:	
COG103	Mild LD, strabismus
COG251	Moderate LD, seizures
COG321	Mild LD, seizures, ASD, heart conduction defect, scoliosis
COG465	Moderate LD
COG495	Moderate LD, PUJ obstruction, neonatal glaucoma
R1984X:	
COG190	Mild LD, ASD, hypoplastic thyroid
COG311	Mild LD
COG458	LD[b], ASD, VUR
R2005X:	
COG323	LD[b], sacrococcygeal teratoma
COG482	Moderate LD
R1914C:	
COG131	Moderate LD
COG389	Mild LD
C1925R:	
COG034	Moderate LD, scoliosis
COG144	Mild LD, scoliosis, pectus carinatum, strabismus
R1952W:	
COG371	Mild LD, hemihypertrophy, hepatomegaly
COG434	Severe LD, TGA, scoliosis, cutis laxa
R1984Q:	
COG463	Mild LD, seizures, cardiac anomaly, chiari malformation
COG464	Moderate LD, ASD
COG490	Moderate LD, seizures, scoliosis
COG628	LD[b], cryptorchidism, phimosis
Y1997C:	
COG233	Moderate LD, scoliosis, pectus excavatum, hypospadias
COG491	Severe LD
R2017W:	
COG308	Mild LD, scoliosis, strabismus
COG500	Moderate LD, scoliosis, VUR
IVS9+3-6delGAGT:	
COG134	Moderate LD, VUR
COG227	Moderate LD, seizures, scoliosis, inguinal hernia, scaphocephaly

NOTE.—ASD = atrial septal defect; GOR = gastroesophageal reflux; PDA = patent ductus arteriosus; PUJ = pelviureteric junction; TGA = transposition of the great arteries; VSD = ventricular septal defect; VUR = vesicoureteral reflux.

[a] In addition to dysmorphism and overgrowth. LD = learning disability.

[b] Degree of LD not specified.

that the mutation segregated with the disorder (table 2). Only one of the families, with affected MZ twin boys, had a 5q35 microdeletion. Of the 12 families with inherited mutations, 7 harbored missense alterations, and this was significantly greater than the proportion of missense mutations in nonfamilial cases (48/240; Fisher's exact test, $P = .005$). The mutation was transmitted from the mother in nine individuals and from the father in three individuals. We analyzed 303 unaffected parents of *NSD1*-positive individuals, none of whom carried the mutation/deletion that was present in their children. For 141 nonfamilial cases, samples were available from both unaffected parents, and analyses confirmed that all cases had arisen de novo.

Twenty-eight mutations occurred independently in at least two unrelated individuals and are referred to as "recurrent" mutations (tables 2 and 3). Twenty-one of the recurrent mutations were base substitutions, and 18 occurred at CpG dinucleotides.

Clinical Features Associated with NSD1 *Abnormalities*

Facial dysmorphism, learning disability, and childhood overgrowth were each present in at least 90% of

NSD1-positive individuals and were designated as cardinal features.

Cardinal Features (present in ⩾90% of patients)
- Characteristic facial appearance
- Learning disability
- Overgrowth: height and/or head circumference ⩾98th percentile

Major Features (present in ⩾15% of patients)
- Advanced bone age
- Cardiac anomalies
- Cranial magnetic resonance imaging or CT abnormalities
- Hyperlaxity/pes planus
- Maternal pre-eclampsia
- Neonatal hypotonia
- Neonatal jaundice
- Neonatal poor feeding
- Renal anomalies
- Scoliosis
- Seizures

Other Features
- Astigmatism
- Behavioral problems
- Cataract
- Cholesteatoma
- Conductive hearing loss
- Constipation
- Contractures
- Craniosynostosis
- Cryptorchidism
- Gastroesophageal reflux
- Genu valgum
- Hemangioma
- Hemihypertrophy
- Hydrocele
- Hypercalcemia
- Hypermetropia
- Hyperpigmentation
- Hypopigmentation
- Hypoplastic nails
- Hypospadias
- Hypothyroid
- Inguinal hernia
- Myopia
- Neonatal hypoglycemia
- Nystagmus
- Pectus excavatum
- Phimosis
- Strabismus
- Talipes
- 2-3 toe syndactyly
- Tumors
- Umbilical hernia
- Vertebral anomalies

The strongest phenotypic feature associated with *NSD1* abnormalities was dysmorphism (fig. 1*A*). All *NSD1*-positive individuals were dysmorphic, although this was often mild. Only 2 of 166 *NSD1*-positive individuals from whom photographs were available were phenotypically scored as "definitely not Sotos or Weaver syndrome," both of whom had truncating *NSD1* mutations. Even after reviewing the subjects together with the *NSD1* results, we did not consider the facial features of these two individuals to be consistent with Sotos syndrome. The remaining 164 *NSD1*-positive individuals had been phenotypically scored as either "typical Sotos syndrome" or "possible Sotos syndrome." Only 2 of 66 individuals who were phenotypically scored as "definitely not Sotos or Weaver syndrome" had *NSD1* mutations.

Learning disability was very common (present in 97% of affected individuals). However, the degree of cognitive impairment was variable, with 21% of affected individuals considered to have "severe" impairment, 46% with "moderate" impairment, and 30% with "mild" impairment. Only seven individuals had normal intellectual development, but there is likely to be a bias toward referral of children with learning disability and, as a result, *NSD1*-positive individuals with normal development may be underrepresented in our analyses. Childhood overgrowth was also common, with 90% of affected individuals having height and/or head circumference at least 2 SDs above the mean (table 4). For these analyses, we included only individuals with height measurements taken between the ages of 1 and 10 years, to limit potential bias resulting from inaccuracies of measurement in neonates and/or the effects of puberty. In 60% of affected individuals, the height and head circumference were both above the 99.6th percentile. However, many individuals had growth

Table 4

Number of Patients with Height and Head Circumference within Each Growth Percentile, for Individuals with *NSD1* Mutations and 5q35 Microdeletions

	No. of Patients, by Measure and Mutation Type			
	Height[a]		Head Circumference[b]	
Growth Percentile	Intragenic Mutations	5q35 Microdeletions	Intragenic Mutations	5q35 Microdeletions
<.4	0	0	0	0
⩾.4	1	1	0	0
⩾2	0	0	1	0
⩾9	0	0	0	0
⩾25	2	0	2	0
⩾50	5	1	6	1
⩾75	6	6	14	0
⩾91	12	4	33	6
⩾98	27	3	31	10
⩾99.6	98	9	105	13
Total	151	24	192	30

[a] At age 1–10 years.
[b] At all ages.

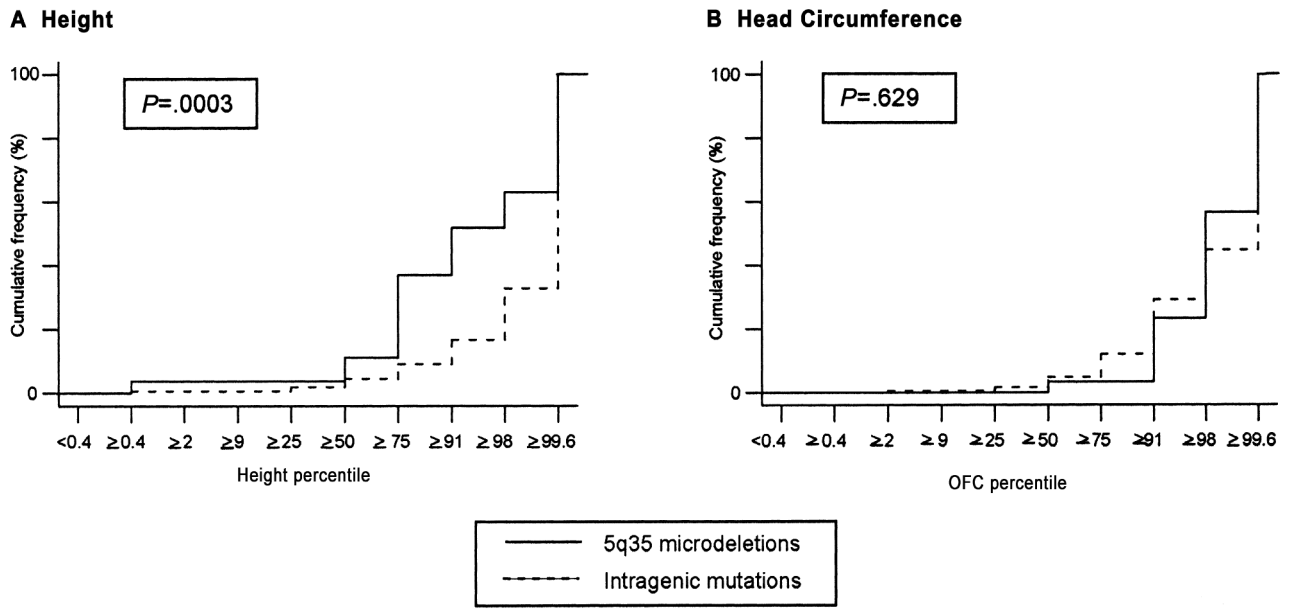

Figure 2 Cumulative frequency distribution graphs for patients with *NSD1* mutations or 5q35 microdeletions. *A*, Height distribution. *B*, Head circumference distribution. The distributions are significantly different for height (Wilcoxon rank-sum test) but not for head circumference.

parameters within the normal range, and, in four individuals, the height was below the median (table 4). We have limited data on adult growth parameters. However, the height of only 8 of 32 *NSD1*-positive adult individuals was at or above the 99.6th percentile, and 10 adults had height at or below the 50th percentile.

Eleven clinical features were present in ⩾15% of affected individuals and were designated major features of Sotos syndrome (see list above). Advanced bone age was the most common major feature and was present in 76% of affected individuals in whom it was measured. However, bone age was not advanced in nearly one-quarter of patients, and, therefore, advanced bone age should not be considered to be an essential feature of Sotos syndrome. Abnormalities on cranial imaging were also common, although these were generally nonspecific, such as dilated ventricles, prominence of the trigone and occipital horns, and/or hypoplasia of the corpus collosum. Neonatal problems, such as hypotonia, poor feeding, and jaundice, were all common (present in ~70% of patients). Scoliosis occurred in one-third of patients and was very variable in severity. Similarly, a broad spectrum of cardiac anomalies and different seizure types was present in 21% and 25% of patients, respectively. Maternal preeclampsia was reported in 17% of patients, although it was not previously known to be associated with Sotos syndrome. Renal anomalies, particularly vesicoureteral reflux, were reported for 15% of patients, but this is likely an underestimate, since relatively few patients had undergone renal investigations. More information about the clinical spectrum of associated clinical features of *NSD1*-positive individuals with Sotos syndrome is given in the study by Tatton-Brown and Rahman (2004).

Several other features were reported in at least two individuals (see list above). Apart from tumors, information about these additional features was not specifically requested on the clinical questionnaire, and their true frequency in individuals with Sotos syndrome is therefore uncertain. It is likely that some features, such as constipation or self-limiting conductive hearing loss in early childhood, occur in ⩾15% of patients and could be considered major features of Sotos syndrome. We requested information from all subjects on whether benign or malignant tumors had occurred. Only eight patients developed tumors, including one neuroblastoma, three sacrococcygeal teratomas, one presacral ganglioneuroma, two acute lymphocytic leukemias, and one small-cell lung cancer. There were many additional features that were reported in only one case, and it is unclear whether these were coincidental or related to functional NSD1 abrogation (data not shown).

Genotype-Phenotype Analyses

We compared the clinical features of individuals with intragenic *NSD1* mutations with those of individuals with 5q35 microdeletions. We compared seven features: height distribution, head circumference distribution, learning disability (scored as "none," "mild," "moderate," or "severe"), cardiac anomalies, renal anomalies, seizures, and scoliosis (all scored as either present or absent). There were highly significant differences in the

height distribution and severity of learning disability. Patients with *NSD1* intragenic mutations were significantly taller than patients with 5q35 microdeletions (Wilcoxon rank-sum test, $P = .0003$) (table 4 and fig. 2*A*). By contrast, there was no difference in the head circumference distribution between patients with mutations and those with deletions (table 4 and fig. 2*B*). The individuals with 5q35 microdeletions had more-severe learning disability (Wilcoxon rank-sum test, $P = 3 \times 10^{-9}$ (fig. 3), and there was also a trend toward more cardiac anomalies (Fisher's exact test, $P = .01$) in individuals with microdeletions. However, there was no difference in the frequency of renal anomalies, scoliosis, or seizures between individuals with mutations and those with microdeletions. There were also no differences in the severity of learning disability or the frequency of associated features in patients with truncating mutations compared with patients with missense mutations. Pairwise comparisons of combinations of major features demonstrated that the occurrence of cardiac anomalies, renal anomalies, seizures, and scoliosis were all mutually independent.

All of the clinical features shown in the list above were present in at least two of the patients with mutations, but some were not seen in the patients with microdeletions, probably because only 31 individuals with microdeletions were included in these analyses, compared with 208 individuals with mutations. Conversely, nephrocalcinosis was the only feature present in individuals with microdeletions that was not reported in the individuals with mutations. Two individuals with 5q35 microdeletions, with different-sized deletions, had nephrocalcinosis. However, relatively few patients with mutations had undergone renal investigations, and it is probable that nephrocalcinosis can also occur in patients with mutations but that it has yet to be reported. The microdeletions varied in size from 482 kb to 5 Mb and included a minimum of seven different-sized deletions. There was no correlation between the clinical phenotype and the size of deletion. Additional details about the clinical features of the individuals with microdeletions are given in the study by Tatton-Brown et al. (2005).

We compared the clinical phenotypes of patients with identical mutations that had arisen independently. This revealed considerable variability in the degree of learning disability and associated clinical features in patients with recurrent mutations (table 3).

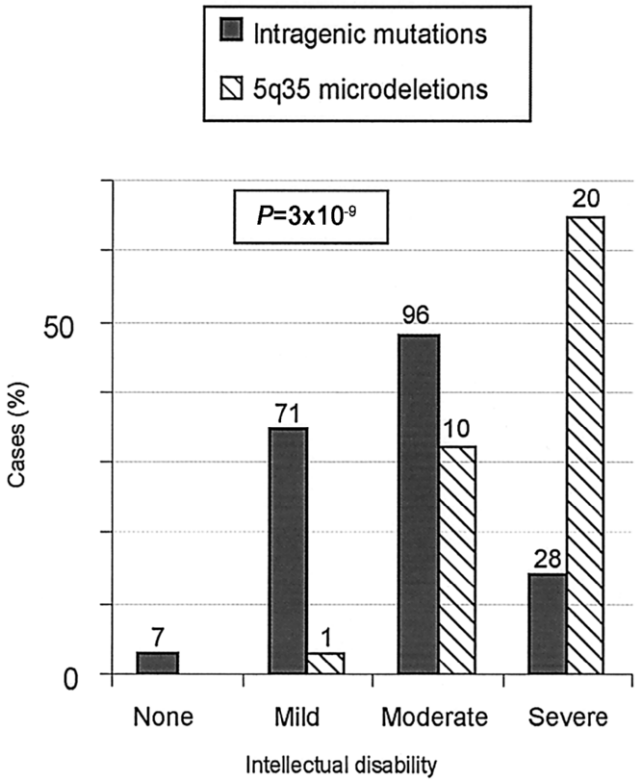

Figure 3 Bar graph showing the frequency and severity of learning disability in patients with *NSD1* mutations (*blackened*) and in those with 5q35 microdeletions (*hatched*). The actual number of patients is given above each bar. The distributions are significantly different (Wilcoxon rank-sum test).

Discussion

Multiple Mutational Mechanisms Abrogate NSD1 *Function*

Multiple mutational mechanisms—including small intragenic insertions and deletions, nonsense mutations, splicing defects, missense mutations, partial-gene deletions, and whole-gene deletions—can abrogate NSD1 function and can result in human disease. Intragenic truncating mutations occur throughout the gene, and most are unique. Sequence architecture influences both the partial- and whole-gene deletions, with several partial-gene deletions arising through nonallelic homologous recombination between *Alu* repeats (Douglas et al., in press). Similarly, a proportion of whole-gene deletions are 2-Mb 5q35 microdeletions that are mediated by nonallelic homologous recombination between low-copy repeats flanking *NSD1*. This recurrent microdeletion is particularly common in Japan, where a prevalent inversion polymorphism predisposes to the microdeletion (Tatton-Brown et al. 2005; Visser et al. 2005).

Missense *NSD1* mutations are pathogenic only if they occur within functional domains implicated in chromatin regulation. Within the PHD, SAC, and C5HCH domains, mutations predominantly occurred at the consensus cysteine and histidine residues that define these domains. Nineteen of the missense mutations were within the SET domain and may therefore result in altered methylation of K20 on H4 and/or K36 on H3. We did not identify missense mutations in the two NIDs at the 5′ end of the gene. It is uncertain whether this was because they are

not pathogenic or because the NIDs together consist of only 34 amino acids. We did not have sufficient mutations to formally evaluate whether the phenotype differed in accordance with the domain affected. However, the phenotypes appeared broadly similar, suggesting that missense alterations in different domains may have comparable effects on NSD1 function. Nonsynonymous base substitutions in the central portion of *NSD1,* which is devoid of recognized functional domains, do not appear to be deleterious. The function of this part of the gene is unknown, but it is noteworthy that exon 5, which is 2.56 kb in length, is not present in the NSD1 paralogs, NSD2, or NSD3, although the functional domains are all present (Douglas et al. 2005).

NSD1 *Abnormalities Are Specific to Sotos Syndrome*

We identified 266 individuals with *NSD1* abnormalities. Of the 166 individuals for whom we had photographs, 164 were given a clinical diagnosis of "typical Sotos syndrome" or "possible Sotos syndrome," independent of the molecular analyses. Conversely, only 2 of 66 individuals given a clinical diagnosis of "definitely not Sotos or Weaver syndrome" had *NSD1* abnormalities. We analyzed >500 subjects with a broad range of phenotypes, and it is unlikely that there exists a major unidentified group of cases attributable to *NSD1* that was not included in these analyses. These data therefore indicate that *NSD1* abnormalities are essentially specific to Sotos syndrome.

It has been proposed that *NSD1* may cause other overgrowth phenotypes, such as Weaver syndrome and BWS. In a previous study, we reported three individuals with atypical Weaver syndrome and with *NSD1* mutations and proposed that *NSD1* may cause Weaver syndrome in some patients (Douglas et al. 2003). In the current analysis, these three individuals were included in the phenotypic review of 290 subjects, but instead of a single photograph in infancy, multiple pictures at different ages were available. This resulted in two of the individuals being classified as having "typical Sotos syndrome" and one as having "possible Sotos syndrome." Moreover, none of the patients with classic Weaver syndrome had *NSD1* mutations. These data suggest there is considerable clinical overlap between Sotos and Weaver syndromes, particularly at young ages, but that classic Weaver syndrome is not due to *NSD1* abnormalities. We therefore believe a diagnosis of Weaver syndrome should be given only if the presence of *NSD1* abnormalities has been excluded. Our practice is to perform *NSD1* testing for all individuals for whom a diagnosis of Weaver syndrome is being considered. If this reveals a mutation, the child is managed as for other *NSD1*-positive individuals and a diagnosis of Sotos syndrome is given.

One of the two *NSD1*-positive individuals whose facial features were not consistent with Sotos syndrome was reported elsewhere as having BWS (patient BA [Baujat et al. 2004]). The child is not typical of patients with BWS since he has learning disability and no macroglossia. The diagnosis of BWS was based on the presence of neonatal hypoglycemia, umbilical hernia, and persistent vesicoureteral reflux. However, our data demonstrate that these features are all associated with Sotos syndrome and that vesicoureteral reflux is more commonly associated with Sotos syndrome than with BWS. Since overgrowth is a cardinal feature of both conditions, they will both be included in the differential diagnosis of many children. Macroglossia is a common feature of BWS that has never been seen in an *NSD1*-positive individual, whereas characteristic facial dysmorphism and learning disability are almost universal in Sotos syndrome but are rare in BWS. For most children, these conditions should therefore be distinguishable by use of clinical criteria. For rare instances of children with overlapping phenotypes, investigations of 11p15 and *NSD1* may be required. The clinical management of such children should be in accordance with the underlying molecular cause.

The Clinical Features of Individuals with 5q35 Microdeletions Are Explicable by NSD1 *Haploinsufficiency*

It was proposed, on the basis of a comparison of only 5 individuals with mutations and 21 individuals with microdeletions (Nagai et al. 2003), that the clinical features of Sotos syndrome should be classified into two categories: those caused by *NSD1* haploinsufficiency (overgrowth and learning disability) and those ascribed to other genes in the deleted interval (such as cardiovascular and renal anomalies). Our findings, which are based on 208 individuals with mutations and 31 individuals with microdeletions, are not consistent with this model. The cardinal and major features of Sotos syndrome were identified in similar proportions of individuals with microdeletions and intragenic mutations, strongly suggesting that these features are due to functional abrogation of NSD1. Overall, individuals with microdeletions had more-severe learning disability and less-pronounced overgrowth. Although it is possible that these differences are attributable to specific genes within the deleted interval, it is more likely to be a nonspecific result of the larger chromosomal insult in patients with microdeletions, since learning disability and growth retardation are very common nonspecific features of microdeletions throughout the genome (Devriendt and Vermeesch 2004). Moreover, despite the large variability in deletion size (0.4–5 Mb), there was no correlation between deletion size and clinical phenotype. Indeed, no additional clinical features were present in the three largest deletions that were not present in at least one of the three smallest deletions (Tatton-Brown et al. 2005). Overall, our data suggest the clinical phenotype of individuals

with 5q35 microdeletions is primarily attributable to haploinsufficiency of NSD1 and that the deletion of other genes exerts little phenotypic effect.

The Phenotypic Variability of Sotos Syndrome Is Largely Independent of Genotype

The phenotype of *NSD1*-positive individuals was extremely variable. The three cardinal features—characteristic facial dysmorphism, learning disability, and childhood overgrowth—were present in 90% of affected individuals, but many other features were also variably present. The presence of these additional features was not correlated with and did not appear to be strongly influenced by the underlying mutational mechanism. This was most clearly demonstrated by the differing phenotypes of individuals with identical mutations. It is currently unclear what determines the clinical variability of Sotos syndrome. Stochastic factors, intrauterine environment, functional polymorphisms in genes that interact with *NSD1,* and intrinsic variability in the regulation of downstream targets of NSD1 may all be implicated.

It is often assumed that Sotos syndrome with severe learning disability and/or multiple additional features is due to 5q35 microdeletions (Nagai et al. 2003). Although the phenotypes of individuals with microdeletions tend to be at the severe end of the spectrum, Sotos syndrome in most-severely affected individuals is attributable to intragenic mutations, which are a much more common cause of Sotos syndrome, accounting for the disorder in >80% of patients. Our data also suggest that missense and truncating *NSD1* mutations are similar with respect to degree of overgrowth, spectrum of learning disability, and nature and frequency of additional features.

Sotos Syndrome Is a Fully Penetrant Disorder That Is Usually Nonfamilial

We screened >300 parents of *NSD1*-positive individuals but identified mutations in only 11, all of whom were clinically diagnosed with familial Sotos syndrome before the molecular analyses. Thus, we have not seen a case of nonpenetrance for a confirmed pathogenic *NSD1* mutation. Neither have we seen a family with affected siblings of unaffected parents that would suggest germinal mosaicism. These data indicate that the recurrence risk for unaffected parents of a child with Sotos syndrome is extremely low and that such cases are almost always the result of de novo mutations. Familial cases had a markedly increased proportion of missense mutations, compared with nonfamilial cases, and it is interesting that all familial missense mutations occurred outside the SET domain. This suggests that certain types of mutations may be more likely to be heritable. However, affected families with truncating mutations were also identified.

The reasons for the low vertical transmission rate are currently unclear. One-third of patients with Sotos syndrome have only mild learning disability, and many of these patients are likely to consider having children in the future. Our very small series of adult patients with Sotos syndrome who have been trying to have children has not revealed any obvious problems during puberty, conception, pregnancy, or childbirth. Moreover, the outcomes have been consistent with the expected 50% offspring risk of an autosomal dominant disorder. Long-term prospective follow-up of patients with Sotos syndrome will likely be required to clarify these apparently paradoxical observations.

To date, we have undertaken all *NSD1* testing in the United Kingdom. This includes 124 U.K. subjects who were clinically judged to have Sotos syndrome by three clinical geneticists who were unaware of the molecular results. Of these 124 individuals, 96 have intragenic *NSD1* mutations, 12 have 5q35 microdeletions, 7 have partial-gene deletions, and 9 have no identified cause. These data suggest that, in at least 93% of patients, Sotos syndrome is attributable to *NSD1*—with 77% of patients having small intragenic mutations, 6% with partial-gene deletions, and 10% with whole-gene deletions. Only 7% of patients did not have an identifiable abnormality in *NSD1*. Such patients are clinically very similar to *NSD1*-positive patients with Sotos syndrome, and we suspect they also have abrogated NSD1 function. It is likely such patients harbor either mutations that were not detected by our heteroduplex analyses or aberrations that were not identifiable by our screening methods, such as regulatory abnormalities. Although we cannot exclude genetic heterogeneity as the cause of Sotos syndrome in a small proportion of patients, our analyses demonstrate that Sotos syndrome is attributable to *NSD1* in the great majority of affected individuals.

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Web Resources

The URLs for data presented herein are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for Sotos syndrome, Weaver syndrome, and BWS)

R Project for Statistical Computing, http://www.R-project.org (for statistical analyses)

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