Characteristics of Sotos syndrome

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CHAPTER 2

Genotype-phenotype correlation in patients suspected of having Sotos syndrome.

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Abstract

Background
Deletions and mutations in the NSD1 gene are the major cause of Sotos syndrome. We wanted to evaluate genotype-phenotype correlation in patients suspected of Sotos syndrome and determine the best discriminating parameters for the presence of a NSD1 gene alteration.
Methods
Mutation and FISH (Fluorescence In Situ Hybridisation) analysis was performed on blood samples of 59 patients, who were clinically scored into three groups. Clinical data were compared between patients with and without NSD1 alterations. With logistic regression analysis the best combination of predictive variables was obtained.
Results
In the group of typical, dubious and atypical Sotos syndrome 81%, 36% and 0% of the patients showed NSD1 gene alteration. Four deletions were detected. In 23 patients (two families) 19 mutations were detected (1 splicing defect, 3 non-sense, 7 frameshift and 8 missense mutations). The best predictive parameters for a NSD1 gene alteration were frontal bossing, downslant palpebral fissures, pointed chin and overgrowth. A higher incidence of feeding problems and cardiac anomalies was found. The parameters delayed development and advanced bone age did not differ between the two subgroups.
Conclusions
In our patients suspected of Sotos syndrome, facial features and overgrowth were highly predictive for a NSD1 gene aberration, whereas developmental delay and advanced bone age were not.

Introduction

Sotos syndrome, first described in 1964 (1), is an overgrowth syndrome, characterized by pre- and postnatal accelerated growth with advanced bone age, mental retardation and distinctive facial features including macrocephaly, dolichocephaly, frontal bossing with a high hairline, a high palate and a prominent jaw (2). In 2002 Kurotaki et al. (3) identified the NSD1 (Nuclear receptor Su-var, 3-9, Enhancer of zeste, Trithorax domain protein) gene, and showed that haploinsufficiency of this gene is the major cause of the syndrome. In a group of Japanese patients mainly deletions involving the NSD1 gene were found (3) and in studies in Europe (4-6) heterozygous intragenic mutations accounted for most of the Sotos syndrome phenotype.
In a British study (4), the majority of the mutations and deletions in the NSD1 gene were found in a group of patients with classical Sotos syndrome (76% of 37 patients).

In a group of Sotos-like patients (n=13) with atypical characteristics, especially concerning facial features, 30% showed a gene alteration of NSD1. Information about relations between genotype and clinical characteristics is limited (5-8). Rio et al. (5) observed that macrocephaly and facial gestalt were consistent findings in a group with gene alteration of NSD1, whereas overgrowth and advanced bone age were not. Comparison between patients with deletions and patients with mutations showed more severe mental retardation in the six patients with deletions. In another study (8) more central nervous system, cardiovascular and renal anomalies were found in patients with deletions. In a German study (6), no deletions were found but in 90% of the patients suspected of Sotos syndrome a mutation was detected. Facial characteristics, overgrowth, macrocephaly and developmental delay correlated best with their molecular results.
In this study we aimed at a detailed comparison of clinical data, especially height, head circumference and bone age data at different ages, between patients with and without NSD1 gene mutation or deletion. As in other studies (4-6) we categorised our patients. We used a clinical score to divide 59 patients, including three families, diagnosed as or suspected of Sotos syndrome in three categories ranging from typical to atypical Sotos syndrome. All were investigated for the presence of NSD1 mutations or deletions and we compared their growth patterns and other clinical characteristics. We addressed the following questions 1) What are the differences in clinical and growth characteristics between patients with and those without NSD1 gene aberrations? 2) Which parameters have the highest predictive values for detecting NSD1 gene aberration?

Patients and Methods

Clinical Analysis

This study was conducted with the prior consent of the Medical Ethical Committee of the Leiden University Medical Center and consent was given by the patients and/or their parents.
Blood samples were obtained from 59 patients who were diagnosed as or suspected of having Sotos syndrome. Clinical and biochemical data of 31 of them were described in a previous study (9) and 28 were included later. Clinical data of the first 31 patients were studied and compared with the classical criteria (2) by an expert panel of 4 clinical geneticists (SGK, AvH, JJvdS, EB - see acknowledgements) and a paediatric endocrinologist (JMW). Patients were categorised into three groups: typical (group 1) Sotos syndrome, dubious (group 2) and atypical (group 3) Sotos syndrome. A clinical scoring system was developed which best reflected the panel's decision on categorisation of the patients (see table 1) (9).
In short, in this clinical scoring system, marks were given for facial characteristics, growth, head circumference, bone age and development. The following facial characteristics were scored: antimongoloid slant of palpebral fissures, high palate,

TABLE 1. Clinical score for categorization of patients diagnosed as having Sotos Syndrome

Criteria		Marks*
facial characteristics**	5 or 6 present	5
	2, 3 or 4 present	3
	0 or 1 present	0
growth	Height SDS – TH***SDS > 2 (all measurements before final height)	2
	Height SDS – TH SDS ≤ 2 (before final height is reached), but in past measurements > 2	1
	Height SDS – TH SDS ≤ 2 (all measurements before final height)	0
bone age	> P90 (all available X-rays)	2
	too old to measure bone age or = P90	1
	< P90	0
head circumference	≥ 2 SDS	1
	< 2 SDS	0
development	IQ < 90 or delayed developmental milestones	1
	IQ ≥ 90	0

* sum 0-4=atypical Sotos syndrome, sum 5-8=dubious Sotos syndrome, sum 9-11=typical Sotos syndrome

** frontal bossing, high hairline, dolichocephaly, prominent chin, high arched palate, antimongoloid slant of palpebral fissures

*** Target Height

prominent jaw, dolichocephaly, frontal bossing and high hairline. The maximum score on growth was given when height standard deviation score (SDS) (10, 11) was above 2 for all measurements when corrected for target height (TH) SDS (gender corrected midparental height). In familial cases no correction for TH was made. A head circumference measurement above 2 SDS (11) gave a maximal score. A maximal score on bone age (2 marks) was given if at least one measurement was above 1.3 SDS (>p90) using the Greulich and Pyle method (12). A maximal score on development was given when a measured IQ (Intelligence Quotient) score was below 90 or developmental milestones were delayed. An IQ score of 90 coressponds with an SDS score of approximately –0.7. Patients with 9-11 marks were categorised as having typical Sotos syndrome, patients with 5-8 marks as dubious and 1-4 marks as atypical Sotos syndrome. No patients with Weaver syndrome were included in this study.

The 28 patients, included later were categorised by the panel using the scoring system. Besides the clinical information used for scoring, additional information such as birth size parameters (13), Body Mass Index (BMI) (Quetelet index, weight(kg)/ height(m)^2) (14), age of first teeth eruption, occurrence of neonatal jaundice, feeding problems in the first year, hypotonia, strabismus, epilepsy, febrile convulsions, pes planus, scoliosis, cardiac anomalies and brain anomalies seen on MRI (Magnetic Resonance Imaging) or CT (Computer Tomography) scan was collected.
One patient had shown an additional marker chromosome, consisting of the centromere and a small part of the long arm of chromosome 8 (patient #30)(9).

Families

Three families were included with more than one member showing characteristics of Sotos syndrome. The pedigrees are shown in Figure 1.

Family A:
The proband (patient number (#) 9, Figure 2A) was referred to the hospital at the age of four years with obesity and developmental delay. At birth and at the age of 4 years height, weight and head circumference were above the 97th percentile. Apart from slight facial features at physical examination muscular hypotonia with poor coordination and clumsiness was found. Her sister (#38, Figure 2B) was 22 months old at the first visit with a height, weight and head circumference above the 97th percentile. She showed typical facial features of Sotos syndrome, more than her sister. Physical examination showed muscle hypotonia and clumsiness. Father's (#44) height was 180 cm, his birth length was 57 cm (3.3 SDS). No intelligence test was performed, but he seemed slow and worked as a unskilled labourer, whereas his brother and sister have completed high school and work as professionals.

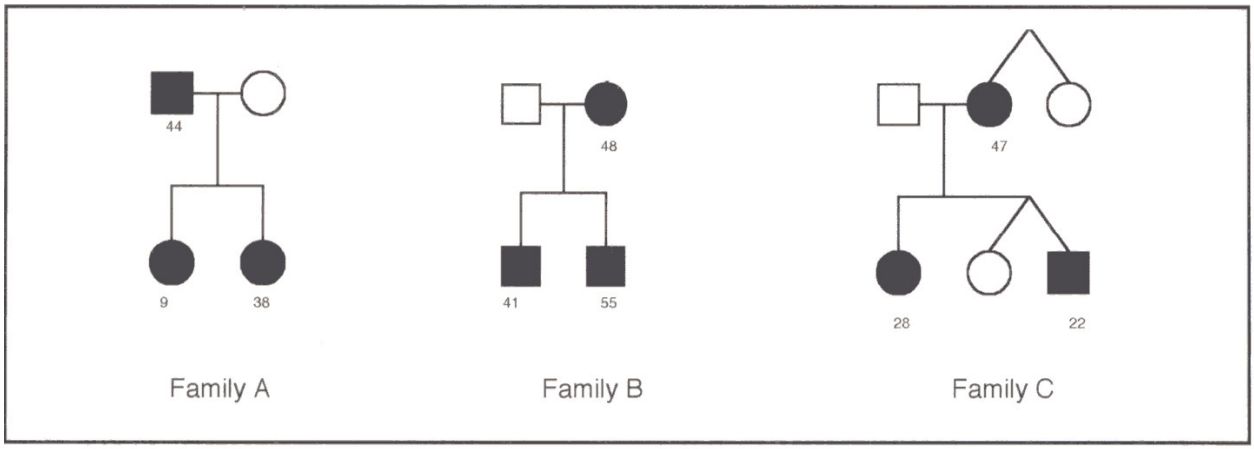

Figure 1. Pedegrees of the 3 familial cases.

Family B:
The proband (#41, Figure 2C), the eldest son of this family, was suspected of Sotos syndrome, because of advanced bone age and developmental delay. He did not have all the typical facial characteristics of Sotos syndrome. Later his brother (#55) was also suspected because he had a large head circumference and behaviour problems. His IQ was in the normal range. The mother (#48, Figure 2D) was very tall (+2.6 SDS), and showed some facial characteristics of Sotos syndrome. She had never had learning difficulties.

Family C
(Figure 2E): The proband, a boy (#22) who is part of a dizygotic twin, born after in vitro fertilisation (IVF), was suspected of Sotos syndrome at the age of 1.5 years, his head circumference was + 3.5 SD and he showed delayed developmental milestones. His twin sister appeared normal. His elder sister, also born after IVF, at that time 4 years old (#28), had no large head circumference but she showed some facial characteristics of Sotos syndrome. The mother (#47) was part of a dizygotic twin and childhood pictures showed facial characteristics of Sotos syndrome. She was very tall in childhood, but final height was in the normal range, and she had had some speech problems in childhood. Her twin sister showed no characteristics of Sotos syndrome.

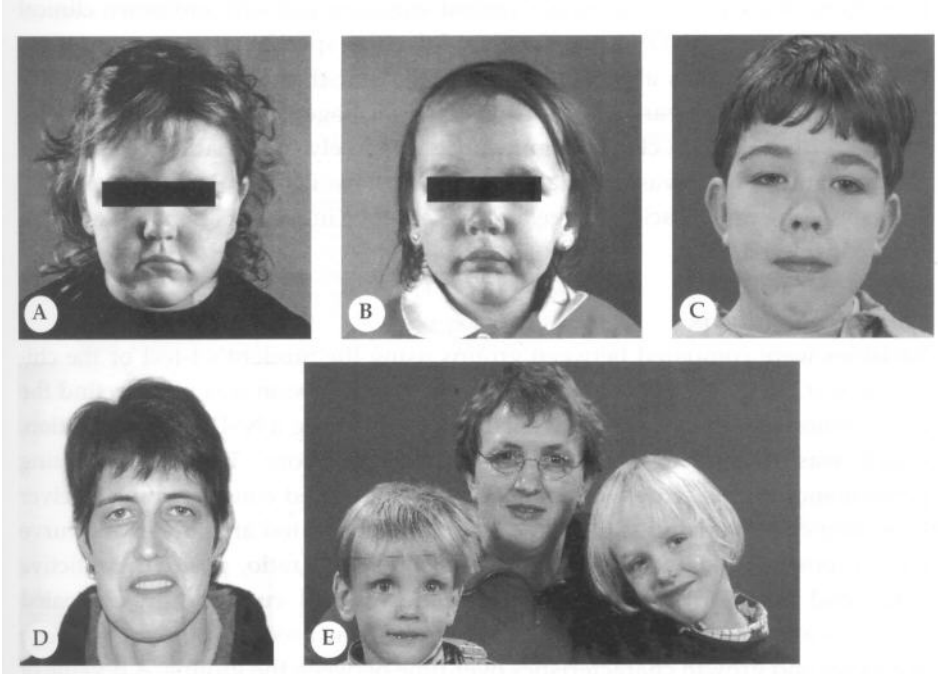

Figure 2. Photographs of familial cases. **A** Patient 9 (family A). **B** Patient 38 (family A). **C** Patient 41 (family B). **D** Patient 48 (family B). **E** Patient 22, 28 and 47 (family C).

Deletion analysis
Fluorescence in situ hybridisation (FISH) was performed as described previously (15). Deletion detection was performed by hybridising PAC clone RP1-251c21 (3), visualized in red, simultaneously with the control probe RP1-179p12 (5q23.2) visualized in green. To trace the parental origin of the deletion, a PCR-based microsatellite analysis using three markers located within the common deleted region (BV005165, BV005168, D5S2111) was carried out.

Mutation analysis
Blood samples from probands and their parents were obtained and genomic DNA was isolated from EDTA anticoagulated blood by a salting out procedure. The 22 coding exons of the NSD1 gene (exons 2-23) of the index patients were amplified by PCR using 34 primer pairs (a list available on request). PCR products were purified using the MultiScreen Assay System (Millipore, Billeria, Massachusetts, U.S.A.) or the Qiagen Qiamp PCR Purification kit (Westburg, Leusden, the Netherlands) and directly sequenced on both strands on an ABI PRISM 3730 DNA Analyzer (Applied Biosystems, Foster City, U.S.A.) using the BigDye Terminator chemistry according to the manufacturer's instructions (Applied Biosystems).
Mutations were considered pathogenic if they were likely to result in premature truncation of the protein. In case a variant was detected with unknown clinical significance, parental DNA was analysed for the specific sequence variation. When the variant was absent in both parents and the parents had no sign of Sotos syndrome, the variant was considered pathogenic. To decide whether a variant with unknown clinical significance was likely to be pathogenic, in case no parental DNA was available, its position in the NSD1 gene, the corresponding amino acid change and the absence in other patients or controls were considered.

Statistical analysis
Variables were compared between groups using the Student's t-test or the chi-square test, whatever was appropriate. Logistic regression was used to find the combination of the most predictive variables for having a NSD1 gene alteration, which was translated into an adjusted clinical score. The discriminating performance of this score was computed. This included constructing a receiver operating characteristics (ROC) curve with the computed area under the curve (AUC), positive likelihood ratio, negative likelihood ratio, positive predictive value and negative predictive value at the optimal cut-off limit. Repeated measures analysis, using a linear mixed model, was used to compare bone age SD scores and growth characteristics over time between the groups. A p-value of <0.05 was considered significant.

Results

In table 2 clinical scoring and the presence of a NSD1 gene mutation or deletion are listed for each patient. Percentages of NSD1 gene aberrations in group 1, 2 and 3 were 81%, 42% and 0%, respectively.

Deletions

With FISH analysis, we found a deletion of one of the copies of the NSD1 gene in four patients. Two deletions were of paternal origin (# 16, DNA of both parents available and #35, only maternal DNA available) and one deletion of maternal origin (#19, only maternal DNA available), of one patient (#4) no DNA of the parents was available. Two patients were categorised in group 1 (#4 and # 16) and two in group 2 (#19 and #35).

Mutations

DNA of 55 patients was screened for NSD1 mutations by sequencing. In 23 patients, including three families, 19 different mutations were found. The mutations are listed in table 3 and included a splicing defect, missense (n=8), nonsense (n=3) and frameshift (n=7) mutations. In two families missense mutations were found, which are discussed in the paragraph below. For the other six missense variants, parental DNA of both parents could be tested and the mutation occurred de novo, therefore these were considered pathogenic.

Table 3. NSD1 mutations found in 23 patients

exon	mutation		name	domain	patient and comment
5	1427T>A	L476X	nonsense	-	1
5	1969insA	E675fsX	frameshift	-	12
5	2809delCGinsT	R937X	frameshift	-	3
5	3531delT	F1177fsX1218	frameshift	-	8
5	3550insT	E1184fsX	frameshift	-	13 (also found by Douglas)
5	3680T>G	L1227X	nonsense	-	5
7	4108C>T	Q1370X	nonsense	-	34
11	4548_4549delGGinsC	E1516fsX	frameshift	-	2
13	4912delCACA	H1638fsX	frameshift	-	27
14	5129G>A	C1710Y	missense	PHDII	23
16	5435T>A	V1812D	missense	PWWPI	14
18	5737A>G	N1913D	missense	SAC	family A
18	5740C>T	R1914C	missense	SAC	20
18	5885T>C	I1962T	missense	SET	26
19	5950C>G	R1984G	missense	SET	11 (5951G>A, R1984Q, found by Rio)
21	6241T>G	L2081V	missense (p?)	-	family C
23	6604T>C	C2202R	missense	-	36
23	7576C>T	P2526S	missense (p?)	-	family C
23	7618delT	S2540fsX	frameshift	-	18
intron	IVS5+33A>T		splicingdefect		10

*p? means pathogenic?, both mutations were detected in one family, it is not known which one is pathogenic

Table 2. Clinical scores high to low for 59 patients diagnosed as having Sotos syndrome

	patient	sex	age	Fac*	1			4	5	6	Gr*	Ba*	Hc*	Dev*	sum	NSD1**
Group 1	1	F	4.3	5	+	+	+	+	+	+	2	2	1	1	11	mut
	2	M	7.4	5	-	+	+	+	+	+	2	2	1	1	11	mut
	3	M	17.8	5	+	+	+	+	+	+	2	2	1	1	11	mut
	4	M	20.3	5	+	+	+	-	+	+	2	2	1	1	11	del
	5	F	1.5	5	-	+	+	+	+	+	1	2	1	1	10	mut
	6	M	4.6	5	+	+	-	+	+	+	1	2	1	1	10	-
	7	M	5.8	5	+	+	+	+	+	+	1	2	1	1	10	-
	8	F	18.9	5	+	+	+	+	+	+	1	2	1	1	10	mut
	9[x]	F	4.0	3	-		+	-	+	-	2	2	1	1	9	mut
	10	M	4.6	3		+		+	+	+	2	2	1	1	9	mut
	11	F	5.7	5	+	+	+	+	+	+	1	1	1	1	9	mut
	12	M	7.8	5	+	-	+	+	+	+	2	0	1	1	9	mut
	13	M	8.5	5	+	+	+	+	-	+	0	2	1	1	9	mut
	14	M	15.2	5	+	+	+	-	+	+	1	1	1	1	9	mut
	15	M	15.3	3	-		+	+	-	-	2	2	1	1	9	-
	16	M	25.6	5	+	+	+	+	+	+	0	2	1	1	9	del
Group 2	17	F	3.3	3	-	+	+	+	2	2	0	1	8	-		
	18	M	3.5	5	+	-	+	+	+	+	1	0	1	1	8	mut
	19	M	4.2	5	+	+	+	+	+	+	1	0	1	1	8	del
	20	M	7.5	3	+		+	-	+	+	1	2	1	1	8	mut
	21	F	33.4	3	-	+	-	+	+	+	1	2	1	1	8	-
	22[x]	M	2.1	5	+	+	-	+	+	+	0	0	1	1	7	mut
	23	M	3.0	3	-	+	-	+	+	+	0	2	1	1	7	mut
	24	M	3.8	3	+	-	+	-	+	+	0	2	1	1	7	-
	25	M	4.1	3	+	-	+	-	+	+	1	2	0	1	7	-
	26	F	4.3	3	+	-	-	-	+		2	2	0	0	7	mut
	27	F	4.4	5	+		+	+	+	+	0	0	1	1	7	mut
	28[x]	F	4.5	3	+	-	+	-	+	+	1	2	0	1	7	mut
	29	M	4.9	5	-	+	+	+	+	+	0	0	1	1	7	-
	30	M	5.6	3	-	+	-	+	+	-	0	2	1	1	7	-
	31	F	7.2	3	-	+	+	-	+	+	0	2	1	1	7	-
	32	F	9.2	3	-	+	-	+	-	-	0	2	1	1	7	-
	33	M	20.6	3	+	+	-	-	+	+	0	2	1	1	7	-
	34	M	33.5	3	-	+	+	-	+	+	1	1	1	1	7	mut
	35	M	33.8	3	+		+	-	+	+	0	2	1	1	7	del
	36	F	0.8	3	-	-	-	+	+	+	0	2	1	0	6	mut
	37	M	1.8	3	-	-	-	+	+	+	0	2	0	1	6	-
	38[x]	F	1.8	3	+		+	-	+	+	2	0	1	0	6	mut
	39	F	4.8	3	-	+	-	+	+	+	1	0	1	1	6	-
	40	F	6.3	0	-		-	-	-	-	2	2	1	1	6	-
	41[x]	M	10.7	3	-	+	-	+	-	-	0	2	0	1	6	-
	42	F	14.0	3	-	+	+	-	+	+	0	1	1	1	6	-
	43	M	36.3	3	-	+	-	-	+	+	0	1	1	1	6	-
	44[x]	M	38.0	3	-		+	-	+	-	0	1	1	1	6	mut
	45	M	6.2	3	-	+	-	-	+	+	1	0	0	1	5	-
	46	F	10.2	0	-	-	-	-	+	-	1	2	1	1	5	-
	47[x]	F	36.3	3	-	-	+	-	+	-	1	1	0	0	5	mut
	48[x]	F	42.8	3	+	+	+									
	49	M	48.4	3	-	+	+									
Group 3	**50**	**M**	**7.6**	**3**	**-**	**-**	**+**	**-**	**+**	**-**	**0**	**0**	**1**	**0**	**4**	
	51	**M**	**10.1**	**0**	**-**	**-**	**-**	**-**	**-**	**-**	**2**	**1**	**0**	**1**	**4**	
	52	**F**	**11.5**	**3**	**-**	**+**	**+**	**-**	**-**	**-**	**0**	**1**	**0**	**0**	**4**	
	53	**M**	**12.8**	**0**	**-**	**+**	**-**	**-**	**-**	**-**	**2**	**0**	**1**	**0**	**3**	
	54	**F**	**5.7**	**0**	**-**	**+**	**-**	**-**	**-**	**-**	**2**	**0**	**0**	**0**	**2**	
	55[x]	**M**	**8.2**	**0**	**-**	**+**	**-**	**-**	**-**	**-**	**1**	**0**	**1**	**0**	**2**	
	56	**M**	**8.6**	**0**	**-**	**-**	**-**	**-**	**-**	**-**	**0**	**0**	**1**	**1**	**2**	
	57	**M**	**8.8**	**0**	**-**	**-**	**-**	**-**	**-**	**-**	**0**	**0**	**1**	**1**	**2**	
	58	**M**	**7.3**	**0**	**-**	**-**	**-**	**-**	**-**	**-**	**0**	**0**	**0**	**1**	**1**	
	59	**M**	**9.6**	**0**	**-**	**-**	**-**	**-**	**-**	**-**	**0**	**0**	**0**	**1**	**1**	

* Fac=facial characteristics 1=down slant palpebral fissures, 2=high arched palate, 3=prominent jaw, 4=dolichocephaly, 5=frontal bossing, 6=high hairline, Gr=growth, Ba=bone age, Hc=head circumference, Dev=development ** NSD1 mut=mutation, del=deletion

Families

Family A. Father and both daughters were carrier of a missense mutation 5737A>G, N1913D. This mutation is located in a conserved residue of the SAC (SET associated Cys-rich) domain and was therefore considered pathogenic. The proband was categorised into group 1, her sister and father into group 2.

Family B. In DNA of the mother and eldest son the missense variant 607G>A,V203I, was detected. The other son did not carry this variant. It was not located in a functional domain, the amino acid change did not lead to a change in polarity and in exon 2 no previous pathogenic mutations have been reported. Therefore this missense variant was considered non-pathogenic. DNA of the grandparents was not available. The proband and the mother were categorised into group 2 and the brother into group 3.

Family C. In DNA of the mother, daughter and son two missense variants were found, 6241T>G, L2081V and 7576C>T, P2526S, which were both not detected in DNA of the mother's twin sister, who showed no clinical characteristics of the syndrome. Neither was one of these detected in one of the other patients. The first variant is located between the SET (su-var 3-9, enhanced-zeta, trithorax) domain and the PHDIII (plant homeodomain) domain and this variant was not detected in 58 controls. In contrast with the second variant the amino acid change does not lead to change in polarity. We assume one of these mutations is pathogenic, but it is unclear which variant is the pathogenic one. DNA of the grandparents was not available. All three family members were categorised into group 2.

Genotype- phenotype correlation

For all clinical and growth characteristics mentioned below a comparison was made between patients with deletions and patients with mutations and no significant difference was found. For subsequent genotype-phenotype correlations we divided all patients in two groups, one having a NSD1 gene aberration and one that has not.

Clinical score

In the group of patients in which NSD1 deletions or mutations were found the total score and the subscores for facial characteristics and head circumference were significantly higher than in the group in which no NSD1 gene abnormalities were found (see table 4). The subscores for growth and bone age were also higher, but the difference was not significant. The subscore for development was not different between groups. Percentages of patients with developmental delay were similar in both groups. Mean IQ scores for the ones who were tested were 75 in the group with mutations or deletions (n=11) and 78 for the other group (n=17). Bone age SD scores were compared in a repeated measures analysis and no overall statistical difference between the groups was detected (p=0.46). Also the patterns over time were similar (test for interaction between age and group yields p=0.14). In figure 3 the difference between bone age and calendar age is shown as a function of calendar age.

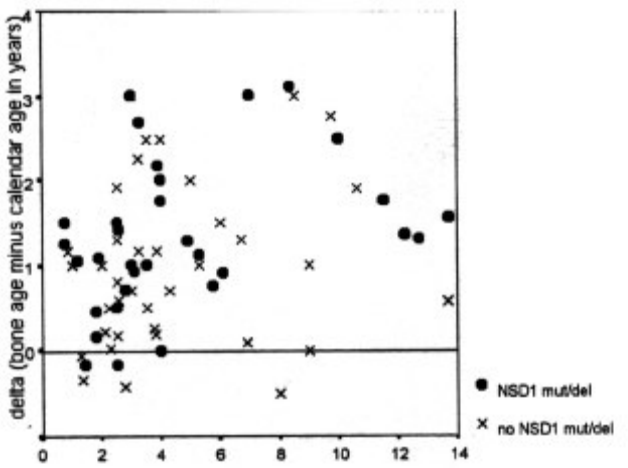

Figure 3. Bone age (Greutlich and Pyle method) - calender age in years

Predictive variables for the presence of a NSD1 gene aberration

With an adjusted score, computed by logistic regression analysis, we determined the parameters with the highest predictive value for NSD1 gene alteration. Variables, which showed univariate significancy with a p-value of 0.20 or lower, were included in a logistic regression model with a backward selection procedure. The resulted prognostic score was: -4.71(1.37) + 1.26(0.74) x (downslant palpebral fissures) + 1.3(0.75) x (pointed chin) + 3.1 (1.18) x (frontal bossing) + 0.82(0.49) x (growth score). The numbers between brackets are the standard errors. For simplification, numbers were rounded resulting in the following scoring system: Total score = 1x (downslant palpebral fissures) + 1x (pointed chin) + 2x (frontal bossing) + 0.5 x (growth score). The score for growth as mentioned in table 1 would be replaced by 0, 0.5 or 1 mark. This means frontal bossing is the most important characteristic, followed by the two other facial characteristics and overgrowth. The total score ranges from 0 till 5. The AUC of the score from a constructed ROC curve was 0.88, in comparison with 0.82 for the initial score. The optimal cut-off limit was at a score $\geq$ 3.5. At this cut-off limit the positive likelihood ratio was 3.16 (confidence interval (CI) 1.79-5.59) and the positive predictive value 0.73 (CI 0.60-0.83). The negative likelihood ratio was 0.15 (CI 0.05-0.46) and the negative predictive value 0.89 (CI 0.72-0.96).

Growth characteristics

Until the age of 10 years height SD scores are higher in the group with NSD1 gene alteration (see table 5). A significant difference between mean SD scores of length was found at the age of 1 year. Repeated measures analysis showed that the group with NSD1 gene alteration did have a significantly larger length SDS (estimated difference : 0.75: 95% confidence interval 0.22, 1.29). The pattern over time did not differ significantly between the two groups (test for interaction between time and group p=0.86). When a correction was made for target height SDS, no change in significance

was found, height SDS was higher in the patients with NSD1 gene alteration at all ages. Head circumference SD scores tended also to be higher, but the difference was not significant (estimated difference 0.45: 95% CI –0.025, 0.92, test for interaction between time and group p=0.06). No significant difference was found in birth weight SDS, 0.54 (n=22) in the group with a mutation or deletion and 0.32 (n=27) in the other group (p=0.62). BMI did not differ significantly between groups.

Table 4. Comparison of the clinical scores and clinical problems between patients with mutations or deletions in the NSD1 gene and those without.

	Group with NSD1 mutation or deletion (n=27)			Group without NSD1 mutation or deletion (n=32)			p-value
Total clinical score (mean)		8.3			5.5		<0.001
Score facial characteristics	0	3	5	0	3	5	
	0 %	44 %	56 %	31 %	59 %	9 %	<0.001
Down-slanted palpebral fissures		67 %			19 %		<0.001
high arched palate		52 %			56 %		0.74
prominent jaw		82 %			38 %		0.001
dolichocephaly		56 %			34 %		0.11
frontal bossing		96 %			50 %		<0.001
high hair line		85 %			44 %		0.001
sum 6 items (mean)		4.4			2.4		<0.001
Score growth	0	1	2	0	1	2	
	30 %	37 %	33 %	53 %	28 %	19 %	0.07
Score bone age	0	1	2	0	1	2	
	22 %	19 %	59 %	34 %	19 %	47 %	0.29
Score head circumference		89 %			63 %		<0.05
Score development		85 %			81 %		0.69
neonatal jaundice		58 % n=7			36 % n=8		0.22
feeding problems		86 % n=12			35 % n=7		<0.01
hypotonia		68 % n=13			59 % n=17		0.50
strabismus		40 % n=8			42 % n=11		0.88
cardiac anomaly		21 % n=5			0 % n=0		<0.01
epilepsia		0 % n=0			7 % n=2		0.22
febrile convulsions		29 % n=5			13 % n=3		0.18
scoliosis		17 % n=3			21 % n=5		0.74
pes planus		40 % n=6			29 % n=6		0.48
brain anomaly on CT scan		63 % n=5			47 % n=8		0.48
otitis		71.5 % n=10			70.5 % n=12		0.96

Table 5. Growth characteristics in mean Standard Deviation Scores (SDS) of patients suspected of Sotos syndrome.

NSD1	birth	0.5 years	1 year	2 years	4 years	10 years	adult
Height SDS							
mut/del	1.90 n=23	2.03 n=15	2.47* n=16	2.07 n=19	2.58 n=18	2.19 n=5	0.94 n=8
no mut/del	1.00 n=25	1.49 n=20	1.69 n=23	1.81 n=23	1.96 n=26	1.96 n=13	1.16 n=8
Head circumference SDS							
mut/del	1.52 n=17	2.16 n=15	2.61 n=17	2.80 n=19	2.72 n=19	3.25 n=6	2.77 n=7
no mut/del	1.19 n=24	1.80 n=21	2.04 n=22	2.24 n=21	2.35 n=23	2.88 n=12	2.64 n=7

* $p<0.05$

Clinical problems

Information on several clinical problems was compared between both groups (Table 4). A significantly higher incidence of feeding problems in the first year and of cardiac anomalies was found in the group with NSD1 gene alteration. Cardiac anomalies consisted of persistent ductus arteriosus, atrial septum defect, mitral insufficiency and coarctation of the aorta. One of these patients (#19) had a NSD1 deletion, the others a mutation. Brain anomalies seen on CT scan were large ventricles. Age of eruption of first teeth in months was 6.1 (n=11) for the group with NSD1 mutations or deletions and 6.2 (n=14) for the group with no gene abnormalities (p=0.88).

Discussion

Our study clearly showed that, using the clinical scoring system, high scores predicted the detection of a NSD1 mutation or deletion in patients suspected of Sotos syndrome. Significant differences between patients with gene alteration of NSD1 and patients without, were higher length SDS in the former and higher incidence of the following facial characteristics: antimongoloid slant of palpebral fissures, prominent chin, frontal bossing and high hairline. Also a higher incidence of feeding problems and cardiac anomalies was found. The most important parameters for predicting NSD1 mutations or deletions in this study were frontal bossing, followed by antimongoloid slant of palpebral fissures, prominent jaw and overgrowth.

There is quite some variation between the ways of categorisation of the patients in our study and three previously published studies (4-6). Although four similar criteria were used in all studies: bone age (>1.3 SDS), growth (>2 SDS), head circumference (>2 SDS) and dysmorphic facial features, development and congenital anomalies were not consistent criteria. Furthermore, the number of fulfilled criteria for inclusion in the different studies varied. We included patients if diagnosis of Sotos syndrome was considered, which resulted in inclusion of patients with only one feature, whereas in other studies patients had to have more features. Both ways can result in a bias in the collection of patients. In our study height SDS was corrected for target height SDS, which was not done in other studies. NSD1 gene alterations were detected in 81% of our patients in the typical Sotos syndrome group. This percentage is comparable to the

British study, which reported a percentage of 76% (4). In the French study (5) the percentage was 70% and in a German study 90% (6). This high percentage of 90% could be due to the small size of their study group. Differences among the studies could also be due to a different number of fulfilled criteria necessary for inclusion in the typical Sotos groups.

In the European studies the amount of deletions was small (n=3, n=6 and n=0) in 75, 39 and 37 patients (4%, 15% and 0 %) (4-6). Deletions were also found in Sotos-like patients (n=1, n=4) (4, 5). We found 4 deletions (6%), of which two in patients with typical and two in patients with dubious Sotos syndrome. This low percentage in European countries is in contrast to the high rate of deletions (67%) observed in a study in Japan (3). The difference is thought either to be attributable to patient selection bias or a population specific genomic structure (16).

Locations of two mutations in our study have been described in previous studies. The frame shift mutation 3550insT, E1184fsX was detected earlier (4). In our study a patient showed a missense mutation located in the SET domain: 5950C>G, R1984G. In another study (5) a patient with a missense mutation in the same amino acid was described 5951G>A, R1984Q.

In accordance with others (17) the majority of the detected mutations are de novo and apparently there are no clear hot spots for mutations. Most missense mutations detected in our study are located in one of the functional domains. Three are not located in a functional domain. For one case, parental DNA did not show the mutation, therefore this mutation in exon 23 was considered pathogenic. The other two were found in one family (family C) and located in exon 21 and 23, respectively. It is not yet clear which of these mutations (6241T>G or 7576C>T) is the pathogenic one. DNA of the grandparents was not available.

Our observation in families A and C indicate that phenotypes can differ between patients with the same genotype. For example the two sisters in family A showed different facial characteristics. This suggests that interactions between NSD1 and as yet undefined other genetic or environmental factors may influence the expression of typical features of Sotos syndrome. We have one observation (#47) of less typical facial features in adulthood than in childhood.

Facial characteristics were also identified by others (5, 6) as consistent findings in patients with NSD1 gene alterations. In our study frontal bossing, followed by antimongoloid slant of palpebral fissures, prominent jaw and a high hairline were most predictive. A high hairline was highly associated with frontal bossing and therefore not found in the score developed by logistic regression analysis. Dolichocephaly and a high arched palate were less predictive, although for the last feature data were missing for 9 patients. In our study overgrowth was more extreme in the group with gene alteration of NSD1. Although cross-sectional data were used in our study, the

overgrowth is seen especially in the first years of life as described before for a group of patients with suspicion of Sotos syndrome (18). Mean SD scores were above 2 for ages between 0.5 and 10 years. The number of patients who have already reached final height is small in our study. Overgrowth was not a consistent finding in the French study (5). Other studies (6, 8) pointed to overgrowth as an important predictor.

Advanced bone age in patients with gene alteration of NSD1 was not a consistent finding in other studies (5). We found mean bone age of all patients suspected of Sotos syndrome advanced with no difference between patients with gene alteration of NSD1 and those without. In our study also developmental delay was not discriminating because both groups showed the same percentage of patients with developmental delay. Both advanced bone age and delayed development are important findings for considering Sotos syndrome, but in the selected group of patients suspected of Sotos syndrome they were not more extreme in patients with mutations or deletions in the NSD1 gene.

Muscular hypotonia (68%) or the developmental delay could play a role in the higher incidence of feeding problems in the first year. In our study, a higher incidence of cardiac anomalies was found in the group with NSD1 gene alterations, although it should be noted that the number of patients was very small. Height SD scores of these patients where between +0.4 and +3.2 SDS. In contrast with a previous study (8), in which cardiac anomalies were exclusively found in patients with deletions, in our study anomalies were also found in patients with mutations. Other studies have compared the patients carrying mutations with the patients carrying deletions and found patients with deletions to have more severe mental retardation (4, 8) and less overgrowth. We compared all studied parameters between these two groups, but could not find significant differences. However, one should note that our group of patients with deletions was very small.

Nowadays most patients suspected of Sotos syndrome will be screened for mutations or deletions in the NSD1 gene. The combination of the parameters with the highest predictive value for NSD1 gene alteration translated into a score could assist in deciding for mutation or deletion screening. No deletions or mutations were detected in our patients with a score below 2 and all patients with the maximum score of 5 showed gene alteration of NSD1. However, this score should be evaluated in a new group of patients. Furthermore it should be realised that the study group is a pre-selected group of patients with one or more of the clinical characteristics. In some typical Sotos patients, we did not detect alterations in NSD1. It is possible that they are carriers of genomic rearrangements in the NSD1 gene like small deletions and duplications that will not be detected by direct sequencing and FISH analysis as performed in our study.

In conclusion, in this study on genotype-phenotype correlation in patients suspected of Sotos syndrome, frontal bossing, pointed chin, downslant palpebral fissures and overgrowth were the most important predictive variables for having a gene alteration

in one of the copies of the NSD1 gene. Besides detecting gene alteration in typical Sotos patients, mutations were also detected in some patients showing only one or a few characteristics.

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