Characteristics of Sotos syndrome

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CHAPTER 3

Plasma Insulin-Like Growth Factors (IGFs), IGF-Binding Proteins (IGFBPs), Acid-Labile Subunit (ALS) and IGFBP-3 Proteolysis in individuals with clinical charcteristics of Sotos Syndrome

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Summary

Objective
Sotos syndrome is an overgrowth syndrome of poorly understood aetiology. We investigated whether this syndrome is related to alterations in plasma insulin-like growth factors (IGFs), IGF-binding proteins (IGFBPs), acid-labile subunit (ALS) and serum IGFBP-3 proteolysis.
Design
Based on clinical criteria, 32 patients diagnosed as having Sotos syndrome (median age 8.4 years, range 1.8-48.4) were categorised into three groups: typical (n=10, group 1), dubious (n=12, group 2) and atypical (n=10, group 3). Blood samples were obtained from 29 patients.
Measurements
Plasma IGF-I, IGF-II, E-II (pro-IGF-II and E-domain fragments), IGFBP-2, IGFBP-3, IGFBP-4, IGFBP-6 and ALS were measured by specific radioimmunoassays (RIAs). Except for E-II immunoreactivity, the concentrations were compared with those of age reference controls, and expressed as standard deviation scores (SDS). IGFBP-3 proteolysis was assessed by incubation of serum with [125I]-IGFBP-3, followed by gel electrophoresis and was then compared with that in normal serum and third trimester pregnancy serum.
Results
Patients in group 1 showed significantly reduced plasma levels of IGF-II (median -0.9 SDS; p=0.01), IGFBP-4 (-0.5 SDS; p=0.02) and IGFBP-3 (-1.0 SDS; p=0.01). Mean IGFBP-3 proteolysis was higher than in normal standard serum (61% vs 37%; p<0.01) but lower than in third trimester pregnancy serum (94%; p<0.01). Plasma IGF-I showed a tendency towards low values (median -0.9 SDS; p=0.09), IGFBP-6 and ALS a tendency towards elevated levels (median values +0.8 SDS; p=0.07 and +2.3 SDS; p=0.09), and IGFBP-2 was normal. The mean value of E-II immunoreactivity was 8.7 nmol/L, similar to that in pooled normal plasma (8.6 nmol/L). Plasma and serum parameters in group 2 and 3 were similar to references with the exception of plasma IGFBP-3 (in group 2 and 3 median < -1.1 SDS; p<0.02) and ALS (in group 3 median +1.3 SDS; p<0.01).
Conclusions
Patients with typical Sotos syndrome show low plasma IGF-II, IGFBP-3, IGFBP-4, and increased proteolysis of IGFBP-3 in serum. The extent to which these findings are associated with the pathophysiology of Sotos syndrome remains uncertain.

Introduction

Sotos syndrome (cerebral gigantism,OMIM117550) was first described in 1964 (1). The syndrome is characterised by the following features: 1) facial characteristics, which

include: frontal bossing, high hairline, dolichocephaly, prominent chin, high arched palate and antimongoloid slant of palpebral fissures; 2) overgrowth: large size at birth, rapid growth in the first four years and tall stature through childhood; 3) advanced bone age; 4) macrocephaly; 5) mental retardation and delayed motor development. Diagnosis is difficult because the features listed above are seldom present all together in one patient (2). The aetiology of the syndrome is poorly understood. Most cases are sporadic, but a few familial cases have been reported, with an apparently autosomal dominant inheritance. Cytogenetic aberrations have been reported in isolated cases (2-10). Recently, it was shown that in 77% of Japanese cases NSD1 haploinsufficiency is present (11).
Since intra-uterine and postnatal overgrowth is present in Sotos syndrome, biochemical growth parameters have been measured in earlier studies. In several studies GH levels were measured (12-18) and in the majority of cases normal levels were found. Although the biological activity of the insulin-like growth factors (IGFs), previously called "somatomedin activity" was normal in most cases (17, 19-22), elevated or lowered levels were also described (16, 18, 23-25). Plasma IGF-I, as determined by radioimmunoassay (RIA) was reported in three patients, of which one showed elevated levels (12).

Data on other important growth regulators, such as IGF-II, IGF-binding proteins (IGFBPs), acid-labile subunit (ALS) and specific IGFBP proteases, which are known to be involved in the bio-availability of IGFs, are lacking in Sotos syndrome. In Beckwith Wiedemann syndrome, and possibly in other overgrowth syndromes, IGF-II plays a role (26). Many tumours show an increased expression of (pro-)IGF-II.

In this study we investigated whether patients diagnosed as having Sotos syndrome show altered plasma levels of IGF-I, IGF-II, E-II (representing pro-IGF-II and E-domain fragments), IGFBP-2, IGFBP-3, IGFBP-4, IGFBP-6 and ALS. In addition, IGFBP-3 proteolysis was determined.

Methods

Study group

The study was conducted with the prior consent of the Medical Ethical Committee of the Leiden University Medical Center and all subjects and/or their parents included in the study gave informed consent to participate. Clinical geneticists, paediatric endocrinologists and the parent support group in The Netherlands received a letter requesting them to send an invitation to all families with a member diagnosed as having Sotos syndrome. Thirty-six families responded. In total 41 family members were previously diagnosed as having Sotos syndrome. Eight patients decided not to participate and one was excluded because of suspicion of Marfan syndrome. Finally 32

patients were included in the study, of whom 29 gave permission for blood samples to be taken.

Clinical data, including birth weight, birth length, parental height, radiographs for calculating bone age and photographs were obtained from the participants. Patients were examined and blood samples were taken. In the same session, standard photographs were made and, if possible, an intelligence test was performed by the patients. Chromosomal analysis was performed and Fragile-X was ruled out.

All data were studied and compared to the classical criteria (2) by an expert panel of three clinical geneticists and a paediatric endocrinologist. Patients were categorised into three groups: typical (group 1), dubious (group 2) and atypical (group 3) Sotos syndrome. To gain further insight into the relative weight of the criteria according to this panel, we developed a clinical scoring system, which best reflected the panel's decision on the categorisation of the patients. The total score was calculated by the sum of marks given for each of the following criteria: facial characteristics, growth in childhood, bone age, head circumference and development. A total score of 0-4 marks resulted in the category atypical, 5-8 marks in the category dubious, and 9-11 marks in the category typical for Sotos syndrome.

The method of scoring is shown in Table 1. The facial characteristics used in the score were: frontal bossing, high hairline, dolichocephaly, prominent chin, high arched palate and antimongoloid slant of palpebral fissures. Height, target height (TH) and head circumference standard deviation scores (SDS) were calculated using the Dutch standards of 1997(27). Target height (cm) was calculated as:

$$\frac{(\text{height father} + \text{height mother} \pm 13)}{2} + 4.5 \text{ cm}$$

where +13 applies to boys, –13 applies to girls, and where 4.5 cm is the mean secular trend per generation of 30 years. Bone ages of all available hand radiographs were assessed by the same investigator using the TW2 20 bones method (28). Dutch adaptations of the Wechsler Preschool and Primary Scale Intelligence-Revised(29), Wechsler Intelligence Scale for Children-Revised (30) and the Wechsler Adult Intelligence Scale (31) were used to assess IQ scores. If the patient was too young or not co-operative (10 patients), information was retrieved from medical records on motor and speech milestones. Delay was considered if the results were above the P90 for Dutch standards as described by Cools (32).

Other anthropometric measures used in the study were body mass index (BMI) (33), birth weight and birth length(34), which were all expressed as SDS.

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

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

Measurement of IGFs, IGFBPs and ALS

Plasma IGF-I, IGF-II, IGFBP-3, IGFBP-4 and IGFBP-6 were determined by specific RIAs. For each parameter as well as for the IGF-I/IGFBP-3 ratio extensive normative range values were available(35-39). ALS was determined in plasma samples of both patients and healthy controls of various ages (i.e. 258 females and 260 males) by a commercially available enzyme-linked immunosorbent assay (Diagnostics Systems Laboratories, Inc., Webster, TX). Smoothed references for the plasma parameters were constructed using the LMS method (40) as previously described for IGF-I, IGF-II,

IGFBP-2,IGFBP-3, IGFBP-4 (37) and IGFBP-6 (38, 39). Plasma levels were expressed as SDS according to the constructed references.

Plasma IGFBP-2 levels were determined by a newly developed RIA, using an antiserum that was raised in a New Zealand rabbit against recombinant bovine IGFBP-2 (rbIGFBP-2; GroPep, Adelaide, Australia). The antiserum was obtained after immunisation with 150 μg of protein in incomplete Freund's adjuvant. The assay buffer was composed of 50 mM sodium phosphate (pH 7.4), 10 mM ethyleen-diamino-tetra-acetaat (EDTA), 0.05% (w/v) Tween-20, 0.2% BSA and 0.02% NaN3. Recombinant hIGFBP-2 (GroPep, Adelaide, Australia) was used as a standard (range 0.02-15 ng/tube) and [125I]-rhIGFBP-2 as tracer. Iodination of rhIGFBP-2 was achieved following the procedure described for IGFBP-4 (39). The RIA incubation mixture consisted of 100 μL standard or diluted sample, 100 μL antiserum (final dilution in assay buffer: 1:60,000), and 100 μL tracer (≈12,000 cpm). After equilibrium incubation for 40-47 hrs at 4 C in polystyrene tubes, 100 μL Sac-Cel solid phase anti-rabbit IgG-coated cellulose suspension (Immunodiagnostic Systems, Boldon, UK) was added. The formation of complexes was complete after 30 min at room temperature, 0.5 mL distilled water was added to the samples, which were subsequently centrifuged at 10,000 x g for 4 min. Pellets were counted in a γ-counter (Packard Instrument Co.,Inc., Downers Grove, IL). Intra-assay variations (10 replicates) were 8.3, 4.9 and 4.5 % at mean plasma levels of 4.6 nmol/L (144 μg/L), 16.1 nmol/L (504 μg/L) and 76.0 nmol/L (2374 μg/L) respectively. Inter-assay variations (8 replicates) at mean levels of 7.8 nmol/L (243 μg/L) and 39.2 nmol/L (1224 μg/L) were 12.4 and 7.5 % respectively. The sensitivity of the assay was 0.003 nmol/L, i.e. 0.10 μg/L (absolute concentration). The antiserum showed no cross-reactivity with other IGFBPs and IGFs. The levels were compared to references based on 831 healthy individuals.

For the E-II [68-88] RIA, a synthetic peptide consisting of the first 21 amino acids of the predicted hpro-IGF-II E-domain region (E-II[68-88]) (MW:2355 D) was prepared by Sigma Genosys Biotechnologies, Inc. (Cambridgeshire, UK). E-II [68-88] was radioiodinated using the chloramine-T method, by reacting 1 μg of peptide with 0.5 mCi Na125I (Amersham International plc, Aylesbury, UK). Unbound radioactivity was removed using a Sep-Pak C18 cartridge (Waters, Millipore Corporation, Milford, MA). Following a wash with 20 mL of 0.1% trifluoroacetic acid (TFA), the radiolabelled peptide was eluted in 2 mL of 50% (v/v) acetonitrile, 0.1% (v/v) TFA, and stored at 4 C. Specific activities varied between 50-70 μCi/μg protein.
New Zealand White rabbits were immunised with 120 μg KLH-EII [68-88] in complete Freund's adjuvant, administered by multiple subcutaneous injections along the back and proximal limbs. Subsequent boosts (100 μg of KLH-coupled peptide in Freund's incomplete adjuvant) were given subcutaneously every 2 weeks. After 5 boosts plasmaphoresis was performed and an antiserum directed specifically against E-II [68-88] (WKZ6279) was obtained.

The assay buffer was the same as used in the RIA for IGFBP2, IGFBP-3 and IGFBP-6. The incubation mixture consisted of 200 μL standard or diluted sample, 50 μL WKZ6279 E-II [68-88] antiserum (final dilution in assay buffer: 1: 5400), and 50 μL [125I]-E-II [68-88] tracer (≈10,000 cpm). After equilibrium incubation for 17 hrs at 4 C in polystyrene tubes, separation of bound and free radioactivity was accomplished as described above for the RIA of IGFBP-2. Intra-assay variations (8 replicates) were 7.0 and 12.5 % at mean plasma levels of 15.7 nmol/L and 63.1 nmol/L E-II [68-88] immunoreactivity, respectively. Addition of an excess (i.e. 10 pmol/tube) of IGFBP-3 had no influence. Neither hIGF-II nor IGF-I at concentrations up to 17 pmol/tube cross-reacted in the EII [68-88] RIA. All samples were tested in the same RIA. RIAs with either exogenous EII [68-88] peptide or pro-IGF-IIE [68-87] (ranging from 91 to 127 nmol/L) added to plasma revealed that > 90 % of these proteins were recovered. Sensitivity of the assay was 0.13 nmol/L (absolute concentration). Levels were compared with those obtained for pooled normal plasma.

Proteolysis of IGFBP-3

IGFBP-3 proteolysis was assessed for several serum samples from each patient group, as described previously by Koedam et al (41). In brief, 2μL serum, in absence and presence of protease inhibitor EDTA (10 mM), was incubated for 16 hrs at 37 C with 40,000 cpm [125I]-IGFBP-3. Recombinant human IGFBP-3 (E.coli derived, non-glycosylated) was used. This was followed by Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE). Autoradiography was used to visualise intact 35 kDa IGFBP-3 and its lower molecular weight proteolysis fragments. After densitometry the amount of proteolysis was calculated by dividing the sum of all [125I]-IGFBP-3 by fragmented [125I]-IGFBP-3 and expressed as percentage. Results were compared with the amount of proteolysis in normal serum (pooled serum of 20 healthy individuals), and in third trimester pregnancy serum, which contains strong IGFBP-3 proteolytic activity. In an earlier study, 88% was found (41). For normal and pregnancy serum the mean of 2 respectively 4 experiments was calculated.

Statistical analysis

Data were analysed with SPSS for Windows version 10.0. To compare plasma levels and anthropometric data with the reference population, the Wilcoxon signed rank test was used. Differences between the three groups were studied with the Kruskal-Wallis test, followed by a Mann-Whitney test if a p-value < 0.05 was found. Percentages of proteolysis were compared with normal standard and pregnancy serum using the t-test. A difference with a p-value < 0.05 was considered statistically significant.

Results

Clinical data

The individual clinical scores are shown in Table 2. The expert panel considered facial features to be the most important characteristic for the diagnosis of Sotos syndrome. No

TABLE 2. Clinical scores for 32 patients diagnosed as having Sotos syndrome

	Sex M/F	Age (years)	*Fac	*Gr	*BA	*HC	*Dev	sum
Group 1 (Typical Sotos)								
1**	F	4.3	5	2	2	1	1	11
2	M	7.4	5	2	2	1	1	11
3	F	5.7	5	1	2	1	1	10
4	M	17.8	5	1	2	1	1	10
5	F	18.9	5	1	2	1	1	10
6	F	4.5	5	1	2	0	1	9
7**	M	3.5	5	1	1	1	1	9
8	M	4.6	5	1	2	0	1	9
9	M	15.2	5	1	1	1	1	9
10	M	33.5	5	1	1	1	1	9
Group 2 (Dubious Sotos)								
11	F	14.0	5	0	1	1	1	8
12	M	5.6	3	0	2	1	1	7
13	F	7.2	3	0	2	1	1	7
14**	M	1.8	3	0	2	0	1	6
15	M	3.0	3	0	1	1	1	6
16	M	6.2	3	1	1	0	1	6
17	M	7.6	3	0	2	1	0	6
18	M	36.3	3	0	1	1	1	6
19	M	2.1	3	0	0	1	1	5
20	M	10.2	0	1	2	1	1	5
21	M	12.8	0	2	2	1	0	5
22	F	36.3	3	1	1	0	0	5
Group 3 (Atypical Sotos)								
23	M	21.4	0	0	2	1	1	4
24	F	42.8	3	0	1	0	0	4
25	F	5.7	0	2	1	0	0	3
26	M	7.3	0	0	2	0	1	3
27	M	8.2	0	0	2	1	0	3
28	M	8.6	0	0	1	1	1	3
29	M	10.7	0	0	2	0	1	3
30	M	26.7	0	0	1	1	0	2
31	M	48.4	0	0	1	0	1	2
32	M	9.6	0	0	0	0	1	1

* Fac= facial characteristics, Gr=growth, BA=bone-age, HC=head circumference, Dev=development , if the score is underlined an IQ test was performed, for patient nr 24 and 30 normal IQ was assumed as there was no history of delayed developmental milestones and both received college education

** No bloodsample available

cases of facial flushing were noted. Median age, age range and anthropometric data of the three groups are shown in Table 3. Birth length was incorporated into the criteria with regard to growth. The anthropometric data not used in the clinical score were birth weight and BMI. In group 1 these parameters were not significantly different from the reference population. Median birth length SDS was 0.8, and showed a trend towards increase (p=0.07). An explanation for our finding of moderately increased birth length may be that in three cases mothers had pre-eclampsia. These children also showed a low birth weight. After exclusion of these cases, a median birth length of + 1.9 SDS was found, which is significantly higher than the reference population (p=0.03). In group 2 all parameters were significantly increased and group 3 showed values in the normal range. The only significant difference between the 3 groups was found for birth length SDS between groups 2 and 3 (p<0.01).

All patients in group 1 showed a normal karyotype. In group 2 one patient (case number 12) showed a small additional marker chromosome in 10 of the 30 analysed meta-phases. Fluorescence in situ hybridisation (FISH) analysis showed that the marker consisted of the centromere and a small part of the long arm of chromosome 8: 47,XY,+der(8)del(8)(p11.1)del(8)(q1?)[10]/46,XY[20]. Both parents showed a normal karyotype. This boy, who showed advanced bone age, a large head circumference (+2 SDS) and developmental delay, was categorised as dubious Sotos syndrome because his facial appearance (only frontal bossing and a high arched palate) and growth pattern (height SDS – TH SDS= +1.3 SDS) were not typical. Additionally, he had a lumbar lordosis, pes planus, genu valgus, 3 café-au-lait spots on his trunk, brittle nails and an everted lower lip. In group 3, one patient (case number 30) had a de novo balanced translocation, 46,XY,t(5;15)(q35;q22). Bone age and growth data were not available,

TABLE 3. Antropometric characteristics of patients diagnosed as having Sotos syndrome

	Group 1 (n=10) Typical Sotos		**Group 2 (n=12) Dubious Sotos**		**Group 3 (n=10) Atypical Sotos**	
	median	**range**	**median**	**range**	**median**	**range**
Age in years	6.6	3.5-33.5	7.4	1.8-36.3	10.2	5.7-42.8
Birth length SDS	0.8	-0.3-4.4	*1.5	0.5-3.3	-0.1	-1.0-1.0
Birth weight SDS	-0.2	-2.3-3.0	*0.8	-0.3-2.2	-0.3	-2.6-0.8
Height SDS -TH SDS**	*1.8	0.9-3.7	*1.4	-0.4-3.1	-0.2	-1.1-2.5
Head circumference SDS	*2.6	0.8-4.8	*2.5	0.4-5.3	*1.5	-0.1-3.3
BMI* SDS**	0.5	-1.0-3.1	*1.6	-1.6-3.2	1.3	-1.2-2.8

* significantly different from zero (p<0.05)

** TH = target height
TH SDS = target height SDS compared to Dutch references (Fredriks *et al.*,(27))

*** BMI = body mass index = weight (kg)/ height $(m)^2$ (Fredriks *et al.*,(33))

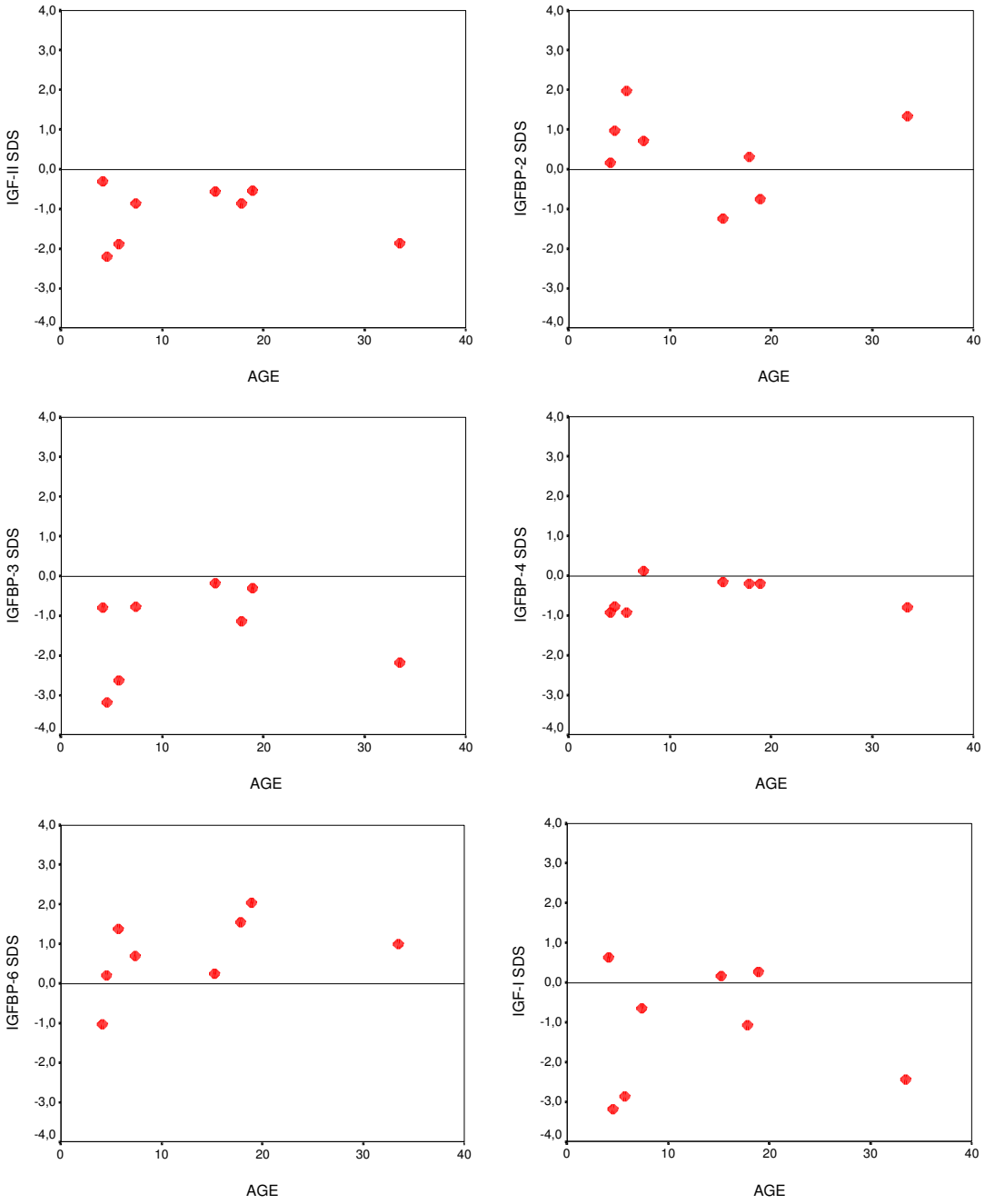

Figure 1. Scatterplots of the individual SD scores of plasma IGF-I, IGF-II, IGFBP-2, IGFBP-3, IGFBP-4 and IGFBP-6 levels of patients with typical Sotos syndrome.

the only feature was an enlarged head circumference. He had before been diagnosed as Sotos syndrome because his enlarged head circumference and he was said to be tall as a child, but no information of height measurements were available. There was no history

of delayed developmental milestones and he had received high school and college education.

Determination of IGFs, IGFBPs, ALS and IGFBP-3 protease activity

SDS values of the IGFs and various IGFBPs found in the circulation of patients designated to the typical Sotos group are shown in Figure 1. The median SDS values of the various laboratory parameters encountered for the three groups of patients are listed in Table 4. In group 1 SDS values of IGF-II, IGFBP-3 and IGFBP-4 in plasma were statistically significantly reduced ($p=0.01$, $p=0.01$ and $p=0.02$). In groups 2 and 3, plasma IGFBP-3 SDS were also low ($p=0.01$ and $p<0.01$). Circulating levels of ALS in the patient population studied showed a marked variation. The median plasma ALS SDS was elevated ($p<0.01$) for subjects in group 3. In group 1 a trend towards a low median value of IGF-I ($p=0.09$) and a slightly elevated IGFBP-6 ($p=0.07$) was found. The median IGF-I/IGFBP-3 ratios in group 1, 2, and 3 amounted –0.2 SDS, +0.6 SDS and +0.4 SDS, respectively. In group 3 this represented a significant difference with the reference population ($p=0.02$). When the 3 groups were compared a significant difference was noted only for IGF-II SDS between group 1 and 3 ($p<0.01$).

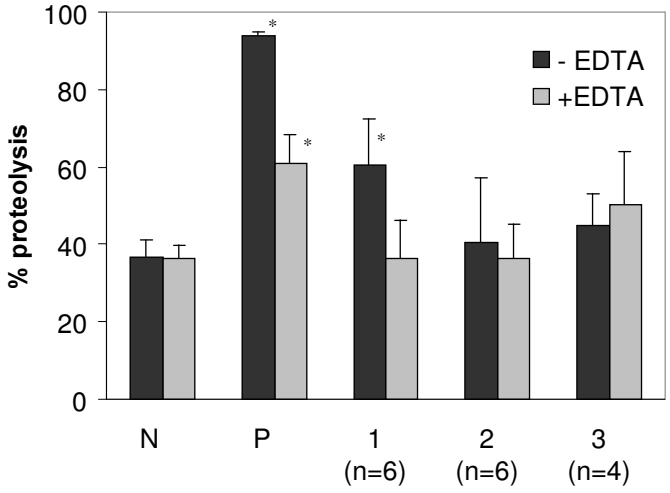

Figure 2. Quantitative graph of IGFBP-3 proteolysis. Proteolysis is expressed as the percentage (mean ± SD) of intact 35 kDa [^{125}I]-IGFBP-3 that migrated as lower molecular weight fragments. Proteolytic activity in normal and pregnancy serum were measured 2, respectively 4 times of which the mean was calculated. In patient serum it was measured 1 to 2 times, the mean of the different cases within one group was calculated. Significant difference between a group and normal standard serum is indicated with an asterisk.
normal serum: N, pregnancy serum: P, serum from patients of group 1(typical Sotos syndrome): 1, of group 2 (dubious Sotos syndrome): 2 and of group 3 (atypical Sotos syndrome): 3.

TABLE 4. plasma IGF-I, IGF-II, IGFBP-2, IGFBP-3, IGFBP-4, IGFBP-6 and ALS expressed as SDS

Test	**Group 1, 2 and 3 (n=29)**			**Group 1 (typical) n=8**			**Group 2 (dubious) n=11**			**Group 3 (atypical) n=10**		
	median	**range**	**p-value**	**median**	**range**	**p- value**	**median**	**range**	**p- value**	**median**	**range**	**p- value**
IGF-I	-0.5	-3.2- 2.1	0.09	-0.9	-3.2- 0.6	0.09	-0.3	-2.6– 1.7	0.60	-0.4	-1.0- 2.1	0.60
IGF-II	-0.5	-3.9- 1.3	0.03	-0.9	-2.2- -0.3	0.01	-0.5	-3.0-1.3	0.13	+0.3	-1.3- 0.8	0.40
IGFBP-2	+0.3	-2.5- 2.1	0.64	+0.5	-1.3- 2.0	0.26	+0.3	-2.5-1.6	0.88	0	-2.3- 2.1	0.52
IGFBP-3	-1.2	-5.7- 0.9	<0.01	-1.0	-3.2- -0.2	0.01	-1.5	-5.7-0.9	0.01	-1.2	-1.7- -0.1	<0.01
IGFBP-4	-0.3	-1.3- 6.7	0.09	-0.5	-0.9- 0.1	0.02	-0.4	-1.1-3.2	0.45	-0.1	-1.3- 6.7	0.88
IGFBP-6	+0.3	-1.0- 3.0	0.06	+0.8	-1.0- 2.0	0.07	+0.3	-1.0-3.0	0.25	0	-0.6- 1.5	0.88
ALS	+1.3	-3.9-10.6	<0.01	+2.3	-2.0-10.6	0.09	+0.4	-3.9-7.1	0.45	+1.3	0.3- 6.7	<0.01

Mean levels of E-II[68-88] immunoreactivity in groups 1, 2 and 3 were 8.7, 8.3 and 8.3 nmol/L, respectively. This was not different from pooled control plasma (8.6 nmol/L), suggesting normal pro-IGF-II processing.

Representative [125I]-IGFBP-3 proteolysis autoradiographs and a quantitative graph are shown in Figure 2. Mean percentages of proteolysed IGFBP-3 in serum of patients in group 1 (n=6) was higher ($p<0.01$) than those found for normal serum and lower ($p<0.01$) than third trimester pregnancy serum. Serum of patients from groups 2 (n=6) and 3 (n=4) showed no significantly different percentages when compared with normal serum. When comparing percentages of the three Sotos groups together with those of normal serum, proteolysis of IGFBP-3 was significantly higher in serum of the Sotos groups (49% in Sotos vs. 37% in controls).The addition of EDTA partially inhibited IGFBP-3 proteolysis in pregnancy serum and serum obtained from patients in group 1.

Discussion

The diagnosis of Sotos syndrome is difficult as there is no pathognomic clinical or biochemical feature. We developed a clinical score which best described the clinical assessment. Facial characteristics were considered the most important criteria for diagnosis. This is in conformity with the study of Cole et al. (2) in which categorisation of patients on facial characteristics alone showed much similarity with categorisation on facial characteristics in combination with other features: bone age, overgrowth and developmental delay. In our study 10 of 32 patients diagnosed as having Sotos syndrome were categorised by us as typical. Cole et al. (2) in their study designated 41 out of 79 patients as definitely Sotos syndrome. Other investigators have also proposed the existence of a ‘Sotos-like’ syndrome{161}.

In contrast to previous reports (2, 12) on this subject, our typical Sotos patients exhibited a normal median birth weight and BMI during childhood. Several studies have indicated that birth length would be a more important diagnostic indicator in Sotos syndrome than birth weight(1, 2). Birth length in our typical Sotos group showed a trend towards increase but the difference was not statistically significant. After exclusion of 3 cases of pre-eclampsia causing short length at birth, birth length in typical Sotos was significantly increased.

Marker chromosomes of a small pericentric region of chromosome 8, as found in one patient of the dubious group, have been previously reported (42-45). It is probable that the abnormalities found in the patient in our study are caused by the presence of a marker chromosome leading to a partial trisomy of chromosome 8. The extent to which this cytogenetic abnormality is involved in Sotos syndrome is not yet known.

One of our patients had a de novo balanced translocation 46,XY,t(5;15)(q35;q22). A patient with a similar chromosomal translocation was described by Maroun et al (6). Applying our clinical score to that patient, she would be categorised by us as typical Sotos syndrome. The patient in our study, however, was assigned to the atypical Sotos syndrome group since the only relevant feature was an enlarged head circumference. In a recent study, in which 77% of Japanese Sotos cases were shown to have NSD1 haploinsufficiency (11), a patient with another translocation of chromosome 5 was described, 46, XX, t(5;8)(q35;q24.1). The 5q35 breakpoint was located within NSD1, shown with FISH. With recent FISH studies we could not find haploinsufficiency for NSD1 in this patient, nor in all others. Further studies are required to elucidate the role of NSD1 in the aetiology of Sotos syndrome.

Despite apparent initial overgrowth it can be concluded that Sotos syndrome is not associated with elevated levels of IGF-I in plasma, as is usually found in constitutionally tall children (46). Moreover, patients with typical Sotos syndrome showed reduced levels of total IGF-II in their circulation. As all plasma samples investigated were taken when patients were older than 2.1 years of age, the reduced plasma IGF levels may be associated with the observed deceleration of growth in Sotos subjects after approximately 1 year of age (25). In that study, a high somatomedin activity was observed in the first year, which is the period of most excessive growth. Low levels were measured in samples of subjects between one and five years old. On the other hand, it is uncertain whether steady state plasma IGF levels reflect the bioavailability of these growth factors. Under normal circumstances, most of the IGFs circulate as a 150 kDa complex with IGFBP-3 and ALS, prolonging their half-life time in the circulation. Proteolysis of IGFBP-3 is believed to increase IGF bioavailability due to a diminished affinity of the IGFBP-3 fragments for IGFs (47, 48). Typical Sotos patients showed a wide range of plasma ALS concentrations but the median value for the whole group did not significantly differ from normal controls. Interestingly, an elevated IGFBP-3 protease activity in serum was found in this category of patients, in the face of reduced levels of IGFBP-3. This may point to alterations in plasma kinetics of the IGFs and their bioavailability to the tissue compartment. Blood specimens from the various patients were randomly obtained during the day. We therefore considered the measurement of free IGF-I (and IGF-II) to be irrelevant in these non-fasting samples (49). Instead, the IGF-I/IGFBP-3 ratio was determined as a surrogate indicator of free IGF-I, showing normal values. If the recent findings of NSD1 haploinsufficiency are confirmed in our and other patients with Sotos syndrome, further studies should focus on possible relations between NSD1 and IGFs and their binding proteins.

In conclusion, patients with typical Sotos syndrome exhibit significantly reduced plasma levels of IGF-II, IGFBP-3, IGFBP-4 and increased proteolysis of IGFBP-3 in serum samples. The extent to which these findings are associated with the pathophysiology of Sotos syndrome remains uncertain.

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