ONLINE MUTATION REPORT

Identification of eight novel *NSD1* mutations in Sotos syndrome

J Kamimura, Y Endo, N Kurotaki, A Kinoshita, N Miyake, O Shimokawa, N Harada, R Visser, H Ohashi, K Miyakawa, J Gerritsen, A M Innes, L Lagace, M Frydman, N Okamoto, R Puttinger, S Raskin, B Resic, V Culic, K Yoshiura, T Ohta, T Kishino, M Ishikawa, N Niikawa, N Matsumoto

J Med Genet 2003;40:126 (<http://www.jmedgenet.com/cgi/content/full/40/11/126>)

Sotos syndrome or cerebral gigantism (SoS, OMIM #117550) is a well-known disorder characterised by overgrowth with advanced bone age, craniofacial anomalies, developmental delay, and occasional seizures.^{1,2} A typical face has a large head circumference, frontal bossing with high anterior hairline, down slanting of palpebral fissures, flat nasal bridge, and prominent jaw.³ The hands and feet are usually large. Height and weight tends to normalise in adulthood.⁴ EEG abnormalities, hypotonia, strabismus, congenital heart defects, kyphoscoliosis, and cancer have also been noted.^{3,5-10} Since the original report in 1964,¹ more than 300 affected cases have been reported. Most cases are sporadic, while several familial cases have been described, suggesting that SoS is an autosomal dominant disorder.^{8,11-17}

We have previously isolated the nuclear receptor SET domain containing gene 1 (*NSD1*) from the 5q35 translocation breakpoint in a Japanese SoS patient with t(5;8)(q35;q24.1).^{18,19} *NSD1* encodes 2696 amino acids (GenBank accession no. AF395588), and the gene has several putative functional domains, such as NID^{-L}, NID^{+L}, SET, SAC, PWWP-I, PWWP-II, PHD-I, PHD-II, and PHD-III, suggesting that the *NSD1* protein may be associated with chromatin mediated transcriptional regulation.¹⁹⁻²⁴ In 42 Japanese sporadic cases of SoS, we identified 20 submicroscopic deletions including the entire *NSD1* gene and four point mutations, the data indicating that SoS is caused by haploinsufficiency of *NSD1*.²⁵ Recently, a UK group reported 29 novel *NSD1* point mutations and only three microdeletions in 37 typical SoS and 13 SoS-like patients, and suggested that *NSD1* intragenic mutations instead of microdeletions were the major cause of SoS.²⁶

In this study, we validated the spectrum of *NSD1* intragenic mutations among 30 newly collected SoS patients.

MATERIALS AND METHODS

The subjects studied included 13 Japanese and 17 non-Japanese patients with SoS. No patient with Weaver syndrome was included in this study. The 17 non-Japanese cases comprised four Canadians including two Hutterite, three each of Brazilians, Germans, and Italians, and one each of Israeli-Arab, Israeli, Austrian, and Croatian. Three main features were considered at the clinical diagnosis: (a) typical craniofacial dysmorphology including macrocephaly, high anterior hairline, down slanting palpebral fissures, and prominent jaw, (b) developmental delay (intelligence quotient or development quotient <80), and (c) history of overgrowth (height and weight >+2 SD). Advanced bone age was not evaluated, because sufficient data were not available. Adequate clinical information was available in 22 patients. In the other eight cases, only limited information was provided

Key points

- Sotos syndrome (SoS) (OMIM #117550) is an autosomal dominant overgrowth disorder with developmental delay, typical dysmorphic craniofacial features, and advanced bone age. The syndrome is caused by haploinsufficiency of *NSD1*.
- In the Japanese population, a common microdeletion including *NSD1* accounts for about a half of SoS patients. In contrast, only 6% of SoS or SoS-like cases in the UK were shown to have a deletion, but 58% had *NSD1* point mutations. Thus far, 38 intragenic mutations of *NSD1* have been reported.
- To investigate the spectrum of *NSD1* point mutations in SoS patients, we performed *NSD1* mutation analysis by direct sequencing in 30 subjects whose microdeletions were already ruled out by fluorescent in situ hybridisation analysis.
- We identified eight intragenic mutations: one insertion, three small deletions, and four nonsense mutations. All mutations were novel and were predicted to cause protein truncations. The data obtained thus far do not support the presence of hot spots for *NSD1* point mutations.
- Low frequency of *NSD1* mutations in this series may be explained in part by a significant portion of collected patients showing atypical SoS or Sotos-like syndrome, and possible genetic heterogeneity.

for this study. All patients were referred to us after microdeletions were ruled out by fluorescent in situ hybridisation analysis using a P1 derived artificial chromosome probe (RP11-118M12), as described previously.²⁰ Six patients (SoS123a (daughter) and SoS123b (mother) from a Japanese family, SoS153a (son) and SoS153b (father) from a Canadian-Hutterite family, and SoS152a (brother) and SoS152b (sister) from an Italian family) were originally suspected to be familial.

Peripheral blood samples were collected from the patients after obtaining informed consent, and genomic DNA was extracted according to a standard method. The study was approved by the ethics committee of Nagasaki University.

Abbreviations: SoS, Sotos syndrome

Table 1 Summary of *NSD1* mutations identified in 30 patients with Sotos syndrome

Mutation	Amino acid change	Exon	Patient	Origin	Type of mutation
Insertion 4769insT	Frameshift	13	SoS122	Japanese	De novo
Deletion 1807delT	Frameshift	5	SoS127	Japanese	NC
2053–2057del	Frameshift	5	SoS121	Japanese	NC
3273delT	Frameshift	5	SoS150	Israeli-Arab	NC
Nonsense 1130G→A	W377X	4	SoS154	Austrian	NC
3886A→T	K1296X	6	SoS153a	Canadian-Hutterite	De novo
3964C→T	R1322X	7	SoS147	Brazilian	De novo
5229G→A	W1743X	15	SoS155	Croatian	NC

NC, not confirmed because parental DNA was not available for analysis.

The 22 exons covering the *NSD1* coding region (exons 2–23) as well as intron–exon boundaries were amplified by PCR. The primer sequences designed at our previous study are available for a request.²⁵ PCR was cycled 35 times at 95°C for 30 s, 50°C for 30 s, and 72°C for 1 min in volume of 25 µl, containing 1× PPCR buffer with 2 mmol/l MgCl₂, 0.2 mmol/l each dNTP, 1 µmol/l each primer and 2.5 U *Taq* polymerase. Amplified PCR products were purified using the QIAquick PCR purification kit (Qiagen, Chatsworth, CA, USA), sequenced on both strands with the BigDye Terminator Cycle Sequencing Ready Reaction kit (version 3.0; PE Applied Biosystems, Foster City, CA, USA), according to the manufacturers’ protocols, and analysed on an ABI 3100 automated DNA sequencer with the sequence analysis software and the AutoAssembler software (version 2.1.1) (all PE Applied Biosystems).

RESULTS

We identified eight different intragenic mutations of *NSD1* in the 30 SoS patients analysed (table 1). All were heterozygous mutations. Bidirectional sequence analyses were carried to be sure that all heterozygous mutations are authentic. These mutations included one insertion (4769insT), three small deletions (1807delT, 2053–2057del, and 3273delT), and four nonsense mutations (1130G→A (W377X), 3886A→T (K1296X), 3964C→T (R1322X), 5229G→A (W1743X)). All mutations were novel and observed through exons 4 to 15 (fig 1). All mutations found are predicted to result in protein truncations. One insertion (4769insT) and two nonsense mutations (Lys1296X, Arg1322X) were judged after sequencing their parental DNA to have occurred de novo. Ages of patients with and without mutations were 3 months to 14 years (mean 6.4 years) and 9 months to 40 years (mean 13.2 years), respectively.

Figure 1 Schematic representation of the genomic structure of *NSD1*, and location of detected mutations. Open and grey/filled boxes depict exons and functional domains of *NSD1*, respectively. Arrows indicate mutations.

DISCUSSION

NSD1 intragenic mutations were found in eight (27%) of 30 newly collected SoS patients. Microdeletions had already been ruled out in this series of patients before the sequence analysis. The present data, combined with those from previous studies,^{25–28} give a total of 38 *NSD1* intragenic mutations distributed in exons 2, 4–7, 10, 13–16, 18–20 and 22–23. The data do not support the presence of hot spots for *NSD1* point mutations. Different types of mutations were found, insertion, deletion, and nonsense mutations, and all may lead to protein truncations.

Mutations were not found in the other 22 patients (73%). Among these, adequate clinical information was available in 15 patients. Nine patients out of the 15 showed only one (a typical craniofacial feature) or two of three main features of SoS, suggesting that 22 patients without any *NSD1* mutations might have included many atypical SoS or Sotos-like patients. Instead, seven patients with mutations whose clinical information was fully available showed all three features. Erroneous diagnosis might also be possible, as the patients in this study were referred to us by a number of physicians. The average age of patients with mutations was younger than that of patients without any mutations. We assume that correct diagnosis in infancy and childhood is easier than in adulthood because overgrowth and developmental delay are prominent. Other mutational events that cannot be detected by current methods may be observed in some of our patients, such as intronic mutations that affect transcription, silent mutations including exon skipping, or *NSD1* promoter mutations. Alternatively, another SoS locus might exist.

Among six patients suspected to be familial, only one patient (SoS153a) showed an *NSD1* mutation, 3886A→T (K1296X). His father (SoS153b) who showed a similar craniofacial feature, but no developmental delay or overgrowth, had no such abnormality. SoS123a (daughter) with all three features and SoS123b (mother) with a similar phenotype but no overgrowth in a Japanese family, and SoS152a (brother) and SoS152b (sister) both showing all three features in an Italian family, presented with normal *NSD1* sequences. Thus, we could not identify any transmitted *NSD1* mutations in the three families. To date, only one point mutation (896delC) in a father and son from a Finnish family has been reported.²⁷ Previous studies suggested reproductive reduction in SoS patients, as some cases of menstrual dysfunction or spontaneous abortion were known.^{3, 8} Familial cases with *NSD1* mutations may be rare.

In conclusion, we have provided additional data of *NSD1* mutations in SoS patients. These findings will contribute to further understanding of the function and structure of *NSD1* and facilitate accurate diagnosis of SoS from other overgrowth syndromes.

ACKNOWLEDGEMENTS

We thank the patients and their parents for their participation to this study. We also express our gratitude to M Kazumi Miyazaki, Naoko Takaki, Naoko Yanai, and Yasuko Noguchi for their excellent technical assistance; and Drs A Orrico, Medical Genetics, Azienda Ospedaliera Senes, Italy; F Mari, Medical Genetics Unit, Department of Molecular Biology, University of Siena, Italy; Hidefumi Tonoki, Chitose Municipal Hospital, Japan; J Seidel at Klinikum Der Friedrich Schiller University at Jena, Germany; Junji Nishimoto, Division of Pediatrics, Kinki Central Hospital, Japan; Kouji Inui, Department of Pediatrics, Osaka University Graduate School of Medicine, Japan; Mayumi Touyama, Okinawa Child Development Center, Japan; Reiko Kawamura, Hiroshima Jidou-Ryouiku-Shidou Center, Japan; Ryuji Fukuzawa, Cancer Genetics Laboratory Department of Biochemistry, University of Otago, New Zealand; Vazken M Der Kaloustian, Clinical Genetics Unit, Montreal Children's Hospital, Canada; Yan-Ling Fei, Hospital For Sick Children, Canada, for their cooperation in sampling. This study was supported in part by CREST, Japan Science and Technology Corporation (JST).

Authors' affiliations

J Kamimura, Y Endo, N Kurotaki, A Kinoshita, N Miyake, N Harada, R Visser, K Yoshiura, N Niikawa, N Matsumoto, Department of Human Genetics, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

J Kamimura, M Ishikawa, Department of Obstetrics and Gynecology, Asahikawa Medical College, Asahikawa, Japan

J Kamimura, A Kinoshita, N Miyake, N Harada, K Yoshiura, T Ohta, T Kishino, N Niikawa, N Matsumoto, CREST, Japan Science and Technology Corporation, Kawaguchi, Japan

O Shimokawa, N Harada, Kyushu Medical Science Nagasaki Laboratory, Nagasaki, Japan

T Ohta, T Kishino, Division of Functional Genomics, Research Center for Frontier Life Sciences, Nagasaki University, Nagasaki, Japan

R Visser, International Consortium for Medical Care of Hibausha and Radiation Life Science, The 21st Century COE (Center of Excellence), Nagasaki, Japan

R Visser, Department of Pediatrics, Leiden University Medical Center, Leiden, The Netherlands

H Ohashi, Division of Medical Genetics, Saitama Children's Medical Center, Saitama, Japan

K Miyakawa, Division of Pediatrics, Niigata City General Hospital, Niigata, Japan

J Gerritsen, A M Innes, Department of Medical Genetics, University of Calgary, Calgary, Canada

L Lagace, East Central Health, Alberta, Canada

M Frydman, Danek Gertner Institute of Human Genetics, Chaim Sheba Medical Center, Tel-Aviv University, Ramat Gan, Israel

N Okamoto, Department of Planning and Research, Osaka Medical Center and Research Institute for Maternal and Child Health, Izumi, Japan

R Puttinger, Klinische Genetik, St. Johanns-Spital, Salzburg, Austria

S Raskin, Laboratorio Genetica, Curitiba Parana, Brazil

B Resic, V Culic, Clinical Hospital Split, Pediatrics Clinic, Department of Developmental Neurology and Medical Genetics, Spinciceva, Croatia

Correspondence to: Dr N Matsumoto, Department of Human Genetics, Nagasaki University School of Medicine, Sakamoto 1-12-4, Nagasaki 852-8523, Japan; naomat@net.nagasaki-u.ac.jp

REFERENCES

- 1 **Sotos JF**, Dodge PR, Muirhead D, Crawford JD, Talbot NB. Cerebral gigantism in childhood: a syndrome of excessively rapid growth with acromegalic

features and non progressive neurologic disorder. *N Engl J Med* 1964;**271**:109-16.

- 2 **Cole TR**, Hughes HE. Sotos syndrome. *J Med Genet* 1990;**27**:571-6.
- 3 **Cole TR**, Hughes HE. Sotos syndrome: a study of the diagnostic criteria and natural history. *J Med Genet* 1994;**31**:20-32.
- 4 **Agwu JC**, Shaw NJ, Kirk J, Chapman S, Ravine D, Cole TR. Growth in Sotos syndrome. *Arch Dis Child* 1999;**80**:339-42.
- 5 **Kaneko H**, Tsukahara M, Tachibana H, Kurashige H, Kuwano A, Kajii T. Congenital heart defects in Sotos sequence. *Am J Med Genet* 1987;**26**:569-76.
- 6 **Hersh JH**, Cole TR, Bloom AS, Bertolone SJ, Hughes HE. Risk of malignancy in Sotos syndrome. *J Pediatr* 1992;**120**:572-4.
- 7 **Noreau DR**, Al-Ata J, Jutras L, Teebi AS. Congenital heart defects in Sotos syndrome. *Am J Med Genet* 1998;**79**:327-8.
- 8 **Opitz JM**, Weaver DW, Reynolds JF Jr. The syndromes of Sotos and Weaver: reports and review. *Am J Med Genet* 1998;**79**:294-304.
- 9 **Le Marec B**, Pasquier L, Dugast C, Gosselin M, Odent S. Gastric carcinoma in Sotos syndrome (cerebral gigantism). *Ann Genet* 1999;**42**:113-16.
- 10 **Sweeney E**, Fryer A, Donnai D. Sotos syndrome: two cases with severe scoliosis. *Clin Dysmorphol* 2002;**11**:121-4.
- 11 **Hansen FJ**, Friis B. Familial occurrence of cerebral gigantism, Sotos' syndrome. *Acta Paediatr Scand* 1976;**65**:387-9.
- 12 **Zonana J**, Sotos JF, Romshe CA, Fisher DA, Elders MJ, Rimoin DL. Dominant inheritance of cerebral gigantism. *J Pediatr* 1977;**91**:251-6.
- 13 **Smith A**, Farrar JR, Silink M, Judzewitsch R. Investigations in dominant Sotos syndrome. *Ann Genet* 1981;**24**:226-8.
- 14 **Winship IM**. Sotos syndrome—autosomal dominant inheritance substantiated. *Clin Genet* 1985;**28**:243-6.
- 15 **Bale AE**, Drum MA, Parry DM, Mulvihill JJ. Familial Sotos syndrome (cerebral gigantism): craniofacial and psychological characteristics. *Am J Med Genet* 1985;**20**:613-24.
- 16 **Scarpa P**, Faggioli R, Voghenzi A. Familial Sotos syndrome: longitudinal study of two additional cases. *Genet Couns* 1994;**5**:155-9.
- 17 **Goldstein DJ**, Ward RE, Moore E, Fremion AS, Wappner RS. Overgrowth, congenital hypotonia, nystagmus, strabismus, and mental retardation: variant of dominantly inherited Sotos sequence? *Am J Med Genet* 1988;**29**:783-92.
- 18 **Imaizumi K**, Kimura J, Matsuo M, Kurosawa K, Masuno M, Niikawa N, Kuroki Y. Sotos syndrome associated with a de novo balanced reciprocal translocation t(5;8)(q35;q24.1). *Am J Med Genet* 2002;**107**:58-60.
- 19 **Kurotaki N**, Harada N, Yoshiura K, Sugano S, Niikawa N, Matsumoto N. Molecular characterization of NSD1, a human homologue of the mouse Nsd1 gene. *Gene* 2001;**279**:197-204.
- 20 **Huang N**, vom Baur E, Garnier JM, Lerouge T, Vonesch JL, Lutz Y, Chambon P, Losson R. Two distinct nuclear receptor interaction domains in NSD1, a novel SET protein that exhibits characteristics of both corepressors and coactivators. *EMBO J* 1998;**17**:3398-412.
- 21 **Jenuwein T**, Laible G, Dorn R, Reuter G. SET domain proteins modulate chromatin domains in eu- and heterochromatin. *Cell Mol Life Sci* 1998;**54**:80-93.
- 22 **Jenuwein T**. Re-SET-ting heterochromatin by histone methyltransferases. *Trends Cell Biol* 2001;**11**:266-73.
- 23 **Aasland R**, Gibson TJ, Stewart AF. The PHD finger: implications for chromatin-mediated transcriptional regulation. *Trends Biochem Sci* 1995;**20**:56-9.
- 24 **Stec I**, Nagl SB, van Ommen GJ, den Dunnen JT. The PWWP domain: a potential protein-protein interaction domain in nuclear proteins influencing differentiation? *FEBS Lett* 2000;**473**:1-5.
- 25 **Kurotaki N**, Imaizumi K, Harada N, Masuno M, Kondoh T, Nagai T, Ohashi H, Naritomi K, Tsukahara M, Makita Y, Sugimoto T, Sonoda T, Hasegawa T, Chinen Y, Tomita HA, Kinoshita A, Mizuguchi T, Yoshiura K, Ohta T, Kishino T, Fukushima Y, Niikawa N, Matsumoto N. Haploinsufficiency of NSD1 causes Sotos syndrome. *Nat Genet* 2002;**30**:365-6.
- 26 **Douglas J**, Hanks S, Temple IK, Davies S, Murray A, Upadhyaya M, Tomkins S, Hughes HE, Cole TR, Rahman N. NSD1 mutations are the major cause of Sotos syndrome and occur in some cases of Weaver syndrome but are rare in other overgrowth phenotypes. *Am J Hum Genet* 2003;**72**:132-43.
- 27 **Hoglund P**, Kurotaki N, Kytola S, Miyake N, Somer M, Matsumoto N. Familial Sotos syndrome is caused by a novel 1 bp deletion of the NSD1 gene. *J Med Genet* 2003;**40**:51-4.
- 28 **Nagai T**, Matsumoto N, Kurotaki N, Harada N, Niikawa N, Ogata T, Imaizumi K, Kurosawa K, Kondoh T, Ohashi H, Tsukahara M, Makita Y, Sugimoto T, Sonoda T, Yokoyama T, Uetake K, Sakazume S, Fukushima Y, Naritomi K. Sotos syndrome and haploinsufficiency of NSD1: clinical features of intragenic mutations and submicroscopic deletions. *J Med Genet* 2003;**40**:285-289.