ONLINE MUTATION REPORT

Identification of eight novel *NSD1* mutations in Sotos syndrome

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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, PWWWP-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.

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, thre 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.

MATERIALS AND METHODS

The subjects studied included 13 Japanese and 17 non-Japanese patients with SoS. No patient with Weaver synrome 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 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℃ for 30 s, 50℃ for 30 s, and 72℃ for 1 min in volume of 25 µl, containing 1× PPCR buffer with 2 mmol/l $MgCl_2$, 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.

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 developmantal 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.

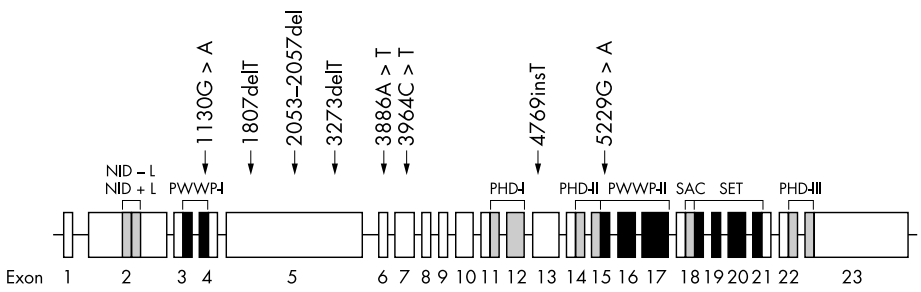

Figure 1 Schematic representation of the genomic structure of *NSD1*, and location of detected mutations. Open and grey/filled boxes depict exons and functional domains of *NSD1*, respectively. Arrows indicate mutations.

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