Sotos syndrome associated with Hirschsprung's disease: a new case and exome-sequencing analysis

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BACKGROUND: Sotos syndrome (SoS) is an overgrowth disorder with various congenital anomalies and is usually accompanied by other clinical problems. However, anorectal malformations have not been documented as part of the SoS entity. Our objective is to report on a case of SoS associated with Hirschsprung's disease (HSCR) and subsequent genetic analysis.

METHODS: A 2-year-old boy with SoS experienced constipation since infancy and ultimately showed an aganglionic segment in the histopathologic examination, which was followed by exome-sequencing analysis.

RESULTS: In the genetic test for SoS diagnosis, two novel mutations of *NDS1*, c.2465C > A (p.Ser822Tyr) and c.4347T > A (p.Cys1449*), were observed and verified by resequencing in the patient and his parents. In further whole-exome-sequencing analysis using the patient's blood DNA, which was followed by a comparison analysis with the results of our previously reported genome-wide association study (GWAS) of HSCR, three genes (*ZNF827*, *FGD2*, and *KCNJ12*) with significance for HSCR from our previous GWAS were overlapped among the genes showing variants in the exome sequencing.

CONCLUSION: This is the first reported patient with SoS and HSCR. Further studies are required to determine whether there is a genetic relationship between SoS and HSCR.

Sotos syndrome (SoS, OMIM 117550) is an overgrowth disorder characterized by distinctive facial features, craniomegaly, advanced bone age, early and excessive growth with accompanying neurodevelopmental and cognitive delay, and intellectual disability (1,2). Characteristic facial features include a prominent forehead; sparse frontal hairline; a sharp, pointed mandible; and downward-slanting eyes. Learning disability can range from mild to severe, with difficulty in speech and communication. Growth is usually premature, with the birth length, head circumference, and bone age ranging above the 90th percentile (3,4).

This syndrome is usually accompanied by other clinical problems such as spine deformities, and cardiac and genitourinary disorders (3). Cardiac anomalies occur in ~20% of patients, and these may include patent ductus arteriosus, atrial septal defect, or ventricular septal defect. Ventricular dilatation and vesicoureteral reflux are the most common cranial and renal abnormalities, respectively (4). Other symptoms or features associated with SoS range from constipation to hearing or vision loss, cryptorchidism, hernias, hydrocele, gastroesophageal reflux, and many others (4).

Mutations of *NSD1* are the known etiologic factors for this disease entity, which may include frameshift and nonsense mutations, partial gene deletions, and microdeletions. *NSD1* molecular genetic screening is used to confirm any suspected case of SoS (4). However, additional confounders, such as *APC2* and the *MAPK/ERK* signaling pathway, have recently been identified as risk factors for SoS development based on genome-wide levels discovered through whole-exome-sequencing (WES) and genome-wide expression studies (5,6).

To the best of our knowledge, Hirschsprung's disease (HSCR) has not been documented as part of SoS. Whether the combined phenotype of SoS with HSCR is sporadic or associated with the syndrome is yet to be discovered. Our objective is to report on a case of SoS associated with HSCR, perform a genetic analysis, and compare the results with genetic variations in our previous genome-wide association study (GWAS) of HSCR (7).

RESULTS

Case

A 2-year-old boy, who was known to have SoS, visited our Seoul National University Bundang Hospital as he was suffering from constipation since birth. Bowel movements were painful and frequency varied from once a day to once in 2 days. According to the birth record, he was born at

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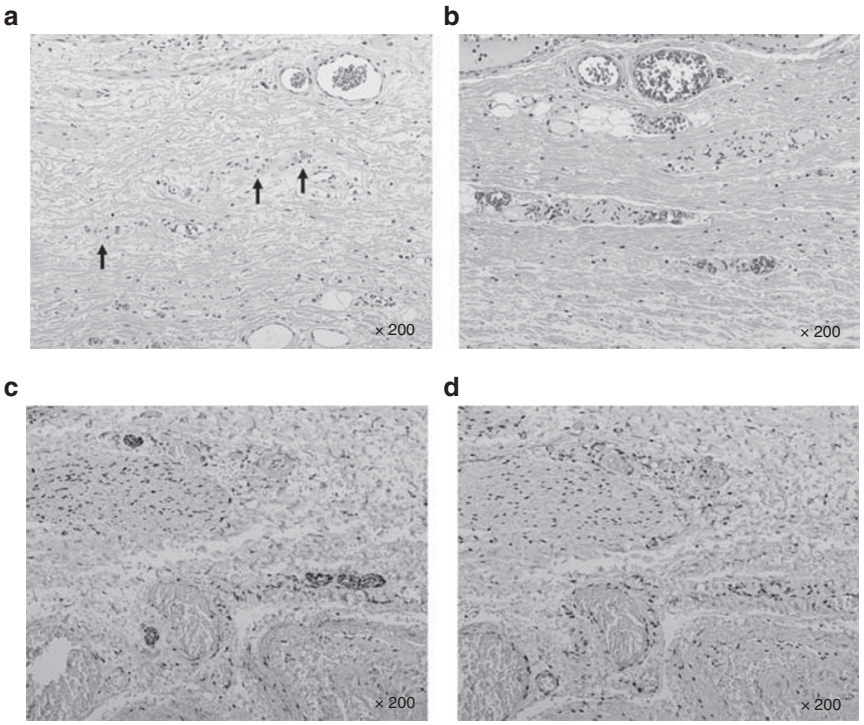

Figure 1. Histological features of HSCR diagnosis. (**a**) The presence of ganglion cells on hematoxylin and eosin (H&E) staining. Ganglion cells of proximal segment are indicated by arrow. (**b**) No ganglion cell on H&E staining of the distal segment. (**c**) CD56 positive and (**d**) MAP2 negative on immunohistochemistry staining of the distal segment.

37 ± 2 weeks age of gestation via normal spontaneous vaginal delivery weighing4.07 kg, and he had a history of polyhydramnios and neonatal jaundice. There was passage of meconium within 24 h of birth, with irregular bowel movements.

In addition, the boy had a history of feeding problems (such as abdominal distension aggravated by feeding, frequent small volume of loose stool-like incontinence, and occasional projectile vomiting) and developmental milestones were delayed. He also experienced poor head control since infancy. Speech and walking were observed at 2 years of age. Past medical history included cryptorchidism on the right side, for which he underwent orchidopexy. An echocardiogram was not completed because of patient restlessness and noncooperation. However, brain magnetic resonance imaging showed prominent extra-axial fluid collection in the frontal convexities bilaterally with focal hemosiderin deposit in the left cerebellar hemisphere.

Physical examination showed typical facial features, with prominent forehead; macrocephaly; receding hairline; downslanting palpebral fissure; long, narrow face; and long chin. The height was 0.98 m (>95th percentile) and weight was 17.0 kg (>95th percentile). The abdomen was soft and markedly distended, with palpable soft to firm masses and abundant fecal material on colonic irrigation.

The patient underwent a laparoscopic-assisted trans-anal pull-through Soave procedure with rectal biopsy on frozen sections. There was an adequate number of normal ganglion cells on histopathologic frozen biopsy of the upper rectum. The patient started a liquid diet on the first postoperative day. The diet was progressed, and the rest of the patient's hospital stay was unremarkable. Final histopathologic examination of the resected bowel showed an aganglionic segment 13 mm distally and a ganglionic segment 87 mm proximally (**Figure 1**), with no identified immature ganglion cells, submucosal and myenteric plexus hyperplasia, or giant ganglia.

Genetic Analysis

In the previous genetic test for SoS diagnosis of the patient, two mutations of *NDS1*, c.2465C>A (p.Ser822Tyr) and c.4347T>A (p.Cys1449*), were observed. The c.4347T>A (p.Cys1449*) mutation, leading a novel nonsense mutation in *NSD1*, was assumed to be a cause for this clinical syndrome, whereas both parents of the patient tested negative for the mutation (**Figure 2a**). In the case of c.2465C>A (p.Ser8225Tyr), this variant was also identified as novel, as it was not found in the dbSNP database of NCBI (http://www.ncbi.nlm.nih.gov/snp/), and there was a negative genotype in the father and a positive genotype in the mother (**Figure 2b**).

In this study, WES was performed at a mean coverage of 87× for the patient with SoS associated with HSCR. Among a total of 12,219 missense and nonsense variants identified, filtering (see detailed description in the Methods section) ultimately revealed 58 variants in 56 genes (**Table 1**). In order to find a relationship between SoS and HSCR, further

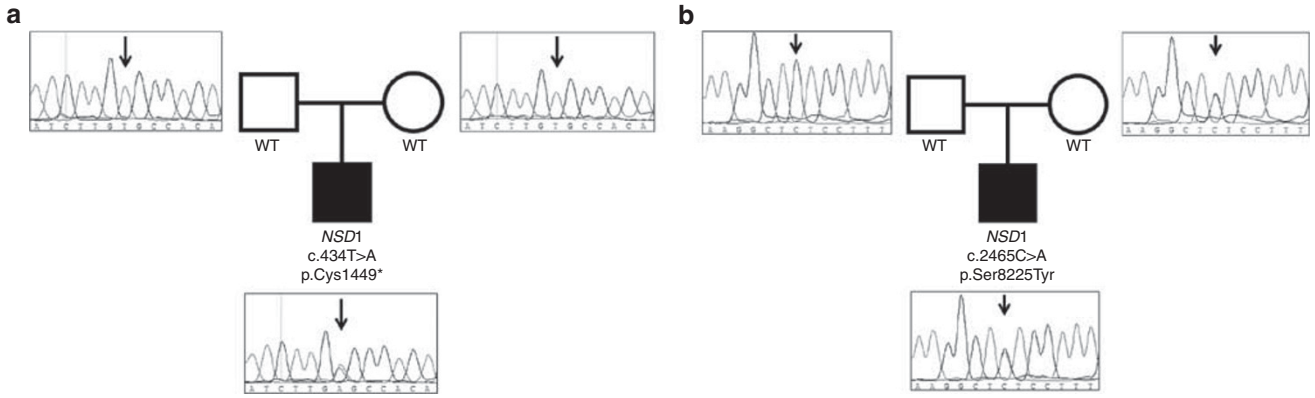

Figure 2. Resequencing in the patient and his parents. (**a**) *NSD1* c.4347T > A (p.Cys1449*) and (**b**) c.2465C > A (p.Ser8225Tyr). Primer pairs (5′-ccctgtctgttggagcattt-3′ and 5′-CAGATTTTGGGCAAACCAGT-3′ for c.4347T > A; 5′-TGTTCACAAACCCCAGTCAG-3′ and 5′-TTGATGGCTTTGATGTTCCA-3′ for c.2465C > A) are used. WT, wild type.

comparison between WES in this study and our recently meta-analyzed GWAS of HSCR carried out across Chinese, Korean, and European populations was performed. Our results showed that mutations in several well-known HSCR-related genes (*RET*, *NRG1*, *NRG3*, *EDNRB*, *GDNF*, *NRTN*, *SOX10*, and *SEMA3*) and variations with additional strong signals observed in our previous GWAS (*SLC6A20*, *IL-11*, *SLC6A20*, and *VAMP5*) (7–10) were not found in the patient with SoS associated with HSCR. However, among the genes showing variants in WES, three (*ZNF827*, *FGD2*, and *KCNJ12*) that had shown significance for HSCR in our previous GWAS were overlapped.

DISCUSSION

This report is the new occurrence of HSCR that has not previously been reported in SoS. As shown in this case, the occurrence of HSCR could be purely co-incidental and may not be a feature of SoS. The incidence of HSCR (1.4–2.8 in 5,000 live births) varies across ethnic groups, with the highest incidence in Asians (11). In the patients with SoS, gastrointestinal symptoms, including constipation (> 15% of cases) as a common complaint, have also been reported (1,12,13). This clinical feature could hypothesize that sometimes the constipation observed in SoS could have a component of poor ganglion formation.

The diagnosis of SoS is made on the basis of history and physical examination, which includes the cardinal features of typical facial dysmorphism; increased velocity of growth and weight with a relative development rate of > 2 or the 75th percentile; advanced bone age; and some form of developmental delay such as in talking, walking, or other developmental milestones (1–3,14). These cardinal features are present in > 90% of patients with SoS. Other symptoms associated with SoS encompass cognitive problems, mild-to-severe intellectual disability, behavioral problems, and cardiac problems. These problems may include enlarged ventricles and other congenital heart diseases; neurologic abnormalities such as seizures; increased subarachnoid spaces, or even pituitary and hypothalamic tumors; and genitourinary problems such as vesicoureteric reflux disease, cryptorchidism, or dysplastic kidneys (1–3,5). Although all these disease entities have been associated with SoS, anorectal malformations have not been documented. Research and retrospective data have found that HSCR with associated anomalies or syndromes has poor outcomes such as long hospital stays and long time for recovery (15).

Genetic analyses of SoS have revealed mutations of *NSD1* in 80% of affected patients, and it is believed to be an autosomal dominant disorder (14,16). Various mutations, including nonsense, frameshift, and microdeletion in chromosome 5q35, have been detected in SoS patients (12,14,16–18). In the case of HSCR, many confounding factors, including the major *RET* mutations, are known to be associated with HSCR susceptibility. In addition, HSCR has been shown to be associated with other syndromes involving genetic mutations or chromosomal anomalies (11). Among the identified variations in this study, p.R261H and p.I262S of *KCNJ12* are common variants with a population frequency of 0.163 and 0.178, respectively, suggesting that they may have little possibility to affect this rare disease. In the case of other overlapping genes (*ZNF827* and *FGD2*), no literature clues related to SoS could be found. Although the functions of these genes are little understood, they have been reported to have roles in hepatic traits and leukocyte signaling (19,20). On the other hand, considering that constipation is commonly seen in SoS, future studies for the possibility that the overlapping genes could relate to constipation or HSCR are required.

In additional gene ontology (GO) analysis of the detected genes with variants in the patient using the WEB-based GEne SeT AnaLysis Toolkit (http://bioinfo.vanderbilt.edu/webgestalt/), two genes (*BRCA1* and *TUBGCP6*) involved in gamma-tubulin-related categories (GO:0008274, GO:0000931 and GO:0000930) were predicted to have a significant value in SoS associated with HSCR (minimum $P = 0.0002$; $_{adj}P = 0.01$ for multiple test adjustment). Considering that tubulins function as a component of the enteric nervous system and show

Table 1. Mutations detected by whole-exome sequencing

Gene	Mutation type	Chr.:position	Ref Seq accession	Nucleotide change	Amino acid change	Population frequency[a]
H6PD	Missense	Chr1:9324725	NM_004285	c.2173C>A	p.P725T	
TNFRSF8	Missense	Chr1:12202406	NM_001243	c.1606G>A	p.E536K	
SZT2	Missense	Chr1:43898455	NM_015284	c.5416G>T	p.D1806Y	
CELSR2	Missense	Chr1:109810618	NM_001408	c.6254T>G	p.L2085R	
AMPD2	Missense	Chr1:110169028	NM_001257360	c.672C>G	p.I224M	
CCDC181	Missense	Chr1:169364443	NM_021179	c.1369T>C	p.W457R	
NLRP3	Missense	Chr1:247599379	NM_001127462	c.2435A>T	p.D812V	
ATG7	Missense	Chr3:11383746	NM_006395	c.1118C>T	p.T373M	0.0000912
EOMES	Missense	Chr3:27761734	NM_001278182	c.964G>C	p.A322P	
PTPN23	Missense	Chr3:47453783	NM_015466	c.4189G>T	p.G1397C	
PCGF3	Missense	Chr4:731308	NM_006315	c.260A>G	p.E87G	
SEC24D	Missense	Chr4:119754838	NM_014822	c.14G>A	p.G5D	0.000008255
ZNF827[c]	Missense	Chr4:146823752	NM_178835	c.659A>G	p.N220S	0.000008238
SDHA	Missense	Chr5:236649	NM_004168	c.1367C>T	p.S456L	0.02354792
ARL15	Missense	Chr5:53409080	NM_019087	c.414A>T	p.L138F	
PPIC	Missense	Chr5:122359593	NM_000943	c.616G>A	p.V206M	0.00006593
MZB1	Missense	Chr5:138723511	NM_016459	c.513T>G	p.C171W	
NSD1[b]	Missense	Chr5:176637865	NM_022455	c.1658C>A	p.S822Y	
NSD1[b]	Nonsense	Chr5:176671240	NM_022455	c.3540T>A	p.C1449Stop	
GRM6	Missense	Chr5:178413934	NM_000843	c.1405C>T	p.R469W	0.0001998
MAK	Missense	Chr6:10804051	NM_001242957	c.565A>G	p.I189V	0.00003295
GNL1	Missense	Chr6:30515169	NM_005275	c.1238C>A	p.P413Q	
HLA-DQA2	Missense	Chr6:32713619	NM_020056	c.383C>A	p.P128H	0.00022007
FGD2[c]	Missense	Chr6:36982708	NM_173558	c.923G>T	p.G308V	
LRIT2	Missense	Chr10:85982062	NM_001284223	c.1297C>T	p.L433F	
TOLLIP	Missense	Chr11:1298403	NM_019009	c.691G>A	p.E231K	0.00000834
STK33	Missense	Chr11:8486301	NM_001289058	c.285G>C	p.K95N	
CAT	Missense	Chr11:34478336	NM_001752	c.1028T>C	p.I343T	
ESRRA	Missense	Chr11:64083269	NM_004451	c.1103G>A	p.G368D	0.02258727
SPTBN2	Missense	Chr11:66483314	NM_006946	c.296C>T	p.S99L	
MMP3	Missense	Chr11:102714228	NM_002422	c.50C>A	p.A17D	
POU2F3	Missense	Chr11:120188063	NM_001244682	c.1267A>T	p.N423Y	
MCRS1	Missense	Chr12:49952449	NM_001278341	c.793G>A	p.A265T	
PRIM1	Missense	Chr12:57135290	NM_000946	c.911G>A	p.R304Q	0.00001663
DZIP1	Missense	Chr13:96293958	NM_198968	c.188G>A	p.R63Q	
ERCC5	Missense	Chr13:103518688	NM_000123	c.2276G>A	p.R759Q	0.00000824
CCDC88C	Missense	Chr14:91739363	NM_001080414	c.5693C>G	p.P1898R	
MAPK6	Missense	Chr15:52356168	NM_002748	c.1137G>C	p.Q379H	
CCDC78	Missense	Chr16:774760	NM_001031737	c.686A>G	p.N229S	
ALG1	Nonsense	Chr16:5122035	NM_019109	c.185C>A	p.S62Stop	
KIF22	Nonsense	Chr16:29811296	NM_007317	c.1207C>T	p.R403Stop	
NFAT5	Missense	Chr16:69660389	NM_001113178	c.211G>A	p.G71S	0.000008299
PER1	Missense	Chr17:8052027	NM_002616	c.983C>T	p.S328L	
KCNJ12[c]	Missense	Chr17:21319436	NM_021012	c.782G>A	p.R261H	0.16303167
KCNJ12[c]	Missense	Chr17:21319439	NM_021012	c.785T>G	p.I262S	0.1776513

Table 1 continued on next page

Table 1 Continued

Gene	Mutation type	Chr.:position	Ref Seq accession	Nucleotide change	Amino acid change	Population frequency[a]
BRCA1	Missense	Chr17:41222964	NM_007300	c.5030G>C	p.G1677A	
CBX4	Missense	Chr17:77808942	NM_003655	c.499A>C	p.K167Q	
SH2D3A	Missense	Chr19:6755199	NM_005490	c.624G>C	p.K208N	0.00001763
ZNF799	Missense	Chr19:12502265	NM_001080821	c.947A>C	p.K316T	
ZNF626	Missense	Chr19:20807298	NM_001076675	c.1385T>C	p.F462S	
ZNF792	Missense	Chr19:35451885	NM_175872	c.47T>C	p.F16S	
SPTBN4	Missense	Chr19:41066234	NM_020971	c.5840T>C	p.F1947S	
ZNF587	Missense	Chr19:58370895	NM_001204817	c.1112G>T	p.R371L	
KREMEN1	Missense	Chr22:29490344	NM_001039570	c.196C>A	p.H66N	0.00004972
PATZ1	Missense	Chr22:31740987	NM_014323	c.602G>A	p.G201D	
TUBGCP6	Missense	Chr22:50662615	NM_020461	c.2225A>G	p.E742G	
ARSA	Missense	Chr22:51065432	NM_001085426	c.514G>T	p.G172C	0.00001739
RENBP	Missense	ChrX:153209587	NM_002910	c.158G>T	p.G53V	

GWAS, genome-wide association study; HSCR, Hirschsprung's disease.
[a]Allele frequency obtained from ExAC (Exome Aggregation Consortium) or dbSNP database (https://www.ncbi.nlm.nih.gov/snp/).
[b]Mutations used for the diagnosis of Sotos syndrome.
[c]Bolded mutations are overlapped with significant variants for HSCR in our previous GWAS (7).

differential expressions along the entire colonic tract related to HSCR (21,22), further studies of gamma-tubulin may be valuable in understanding SoS-associated gastroenterological diseases.

Recognizing HSCR early, especially in an SoS patient, is a significant part of management of the syndrome. Moreover, constipation in SoS patients may point to the possibility of an associated HSCR. To our knowledge, this is the first case report regarding the association of the two disease entities. Treatment of these individual diseases requires a multidisciplinary approach and collaborative treatment plan. The recognition of the genetic etiology may hopefully lead to the possibility of screening, as well as raising awareness that SoS and HSCR may coexist. In conclusion, HSCR should be considered as part of the clinical and diagnostic workup in SoS patients with constipation, as untreated or undiagnosed HSCR may contribute to severe morbidity. Further studies are also required to determine whether there is a genetic relationship between SoS and HSCR.

METHODS

This study was approved by the Institutional Review Board (IRB number B-1504-294-702) of Seoul National University Bundang Hospital. After the patient's guardian provided written informed consent, the patient's medical records were reviewed.

In this study, we performed a genetic analysis using WES to investigate genetic variations and compared them with our previous GWAS of HSCR. Isolated, genomic, blood DNA from the SoS patient was used for exome library construction using the Ion AmpliSeqTM Library Kit 2.0 (Life Technologies, Waltham, MA). Library templates were then sequenced using the Ion PI Chip (Life Technologies) base on the Ion Proton Sequencer System (Life Technologies) according to the manufacturer's instructions.

After processing sequencing reads using the Ion Torrent Suite software v 4.0.2 (Life Technologies), high-quality sequencing reads were mapped to the complete hg19 human genome (UCSC version, February 2009). On the basis of the variant discovery using Torrent Variant Caller v 4.2 (Life Technologies), significant variants were identified as follows: (i) mutations predicted to be positioned at frameshift, nonsense, or essential splice sites or (ii) missense variants predicted to affect the function with SIFT score (http://sift.bii.a-star.edu.sg) <0.05 and Polyphen2 score (http://genetics.bwh.harvard.edu/pph2) >0.85. Then, known common variants with a frequency over 0.01 in 1000 Genomes Project data were excluded.

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Disclosure: The authors declare no conflict of interest.

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