Mutations in *NSD1* and *NFIX* in Three Patients with Clinical Features of Sotos Syndrome and Malan Syndrome

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Abstract

Keywords

- ▶ next generation sequencing
- ▶ autistic features
- ▶ behavioral abnormality

Mutations in nuclear receptor SET domain-containing protein 1 gene (*NSD1*) are related to Sotos syndrome, which is characterized by overgrowth, macrocephaly, distinctive features, and neurodevelopmental disabilities. On the other hand, mutations in the nuclear factor I/X gene (*NFIX*) can lead to Malan syndrome, also known as Sotos-like syndrome, or to the Marshall–Smith syndrome. In this study, using next generation sequencing (NGS), we identified de novo mutations in *NSD1* and *NFIX* in three patients with developmental disabilities associated with overgrowth or macrocephaly. Overall, we confirmed that clinical entities of congenital malformation syndromes can be expanded by molecular diagnoses via NGS.

Introduction

Congenital malformation syndromes can be diagnosed through clinical evaluations. If the patients show specific key findings, a differential diagnosis can be easily made. However, the final diagnosis often requires a genetic analysis.

Certain congenital malformation syndromes, including Sotos syndrome and Sotos-like syndrome, are related to overgrowth and macrocephaly.^{1,2} Most patients with Sotos syndrome show distinctive facial features, i.e., an inverted triangular face and down-slanting palpebral fissures.¹ Such key features aid in the clinical diagnosis of these syndromes. Although the molecular diagnosis of these syndromes has been challenging, next-generation sequencing (NGS) provided an easy approach to identify disease-specific mutations. Using NGS, disease-specific mutations can be detected even if the patients show atypical findings, thus allowing for an expansion of disease entities.³

Here, we report three patients with developmental disabilities associated with overgrowth or macrocephaly. De novo disease-causing mutations were identified via NGS.

Patient Reports

Patient 1

A 5-year-old boy was born at 37 weeks of gestation, with birth weight of 3,376 g (75–90th centile); length of 52.7 cm (>97th centile); and occipito-frontal circumference (OFC) of 36.5 cm (>97th centile), indicating prenatal overgrowth. He was diagnosed with a mild intellectual disability (intellectual quotient of 58) associated with autistic features and hyperkinetic behaviors. When the patient was 5 years old, his height, weight, and OFC were 119.3 cm (>97th centile), 30.5 kg (>97th centile), and 56.2 cm (>97th centile), respectively, indicating overgrowth. He also showed distinctive facial features, including a flat nasal bridge and an inverted triangular face, which are typical of Sotos

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syndrome. Brain magnetic resonance imaging (MRI) showed no abnormalities, and fluorescence in situ hybridization analysis of the nuclear receptor SET domain-containing protein 1 gene (*NSD1*) region showed no deletions.

Patient 2

A 10-year-old girl was born at term, with birth weight of 3,564 g (90–97th centile); length of 55.0 cm (>97th centile); and OFC of 35.2 cm (90–97th centile), as the third child of healthy parents. At birth, a cleft lip and palate were noted. She showed a developmental delay with head control at 6 months of age. She suffered febrile seizures at 13 months of age. Since she showed autistic features, a psychological therapy was initiated. Her height, weight, and OFC were 146.9 cm (90–97th centile), 44.9 kg (90–97th centile), and 55.0 cm (75–90th centile), respectively, indicating a relative overgrowth. Her bone age was evaluated by Tanner-Whitehouse 2 (TW2) method and scored as 11 years and 3 months by 20 Bone, 11 years and 3 months by RUS11, and 10 years and 10 months by Carpal. These results indicated an advanced bone age of approximately 1 year. Her developmental quotient (DQ) was evaluated as 32 by the Kyoto development test. Brain MRI showed no abnormalities. A retrospective evaluation confirmed the characteristic facial findings of Sotos syndrome

Patient 3

A 4.6-year-old boy was born with birth weight of 3,490 g (75–90th centile); length, 50.5 cm (75–90th centile); and OFC of 35.8 cm (= 97th centile). He showed a developmental delay since early infancy, with the walking noticed at 33 months. There were no distinctive facial findings. He

did not show interests to the surrounding environment and the eye contact was poor. His behavior was over-familiar at interpersonal relations. At present, his height, weight, and OFC are 103 cm (mean), 19.0 kg (75–90th centile), and 55.2 cm (>97th centile), respectively, indicating macrocephaly. The Enjoji development test showed a moderate global developmental delay with a DQ of 30. Brain MRI showed no abnormalities.

Methods and Results of Genetic Testing

This study was performed in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Tokyo Women's Medical University. After receiving written informed consent from the patients' families, we obtained blood samples from them and their parents, and extracted genomic DNA. NGS was performed using the TruSight One v1.0 Sequencing Panel (Illumina, San Diego, California, United States), as previously described.⁴ The extracted data was mapped to the GRCh37/hg19 reference genome using the BWA Enrichment v1.0 software (Illumina), and annotated and filtered using the VariantStudio software (Illumina). The identified variants were reconfirmed by Sanger sequencing according to the standard methods. All the results are summarized in ►Table 1.

In patient 1, a 1-bp insertion was identified in exon 13 of *NSD1*—NM_022455.4(*NSD1*):c.4809_4810insA [NP_071900.2:p.Arg1605LysfsTer13], which leads to a frame shift at Arg1605, resulting in a new reading frame with a premature stop codon (►Fig. 1A–C). Patient 2 showed a missense mutation in exon 19 of *NSD1*—NM_022455.4(*NSD1*):c.5951G > A [NP_071900.2:p.Arg1984Gln] (►Fig. 1D–F).

Table 1 Summary of the study

	Patient 1	Patient 2	Patient 3
Gender	Male	Female	Male
Age (y)	5	9	4.5
Intellectual disability	Mild	Moderate	Moderate
Developmental disability	+	+	+
Prenatal overgrowth	+	+	±
Overgrowth (>97th centile)	+	±	–
Macrocephaly (>97th centile)	+	–	+
Distinctive features	+	+	–
Advanced bone age	ND	+	ND
Genes affected	<i>NSD1</i>	<i>NSD1</i>	<i>NFIX</i>
Affected exon	exon 13	exon 19	exon 2
Nucleotide variants	c.4809_4810insA	c.5951G > A	c.375G > T
Amino-acid alterations	p.Arg1605LysfsTer13	p.Arg1984Gln	p.Lys125Asn
Mechanism	Frameshift	Missense mutation	Missense mutation
Novel/Recurrent	Novel	Recurrent	Novel
Origin	ND	De novo	De novo

Abbreviations: ND, not determined; *NFIX*, nuclear factor I/X; *NSD1*, nuclear receptor SET domain-containing protein 1.

Fig. 1 Results of the molecular analysis. An insertion mutation in patient 1 (A–C), a missense mutation in patient 2 (D–F), and a missense mutation in patient 3 (G–I) are shown. (A, D, G) Desktop images of the identified mutations visualized by Integrative Genomics Viewer (IGV, <http://software.broadinstitute.org/software/igv/>). (B, E, H) Electropherograms of Sanger sequencing confirming the mutations in the patients. (C, F, I) Conservation of amino-acid sequences among various species. Red arrows indicate the locations of the identified mutations.

Both parents harbored no mutation in this site, indicating a de novo occurrence. Patient 3 showed a missense mutation in exon 2 of the nuclear factor I/X gene (*NFIX*)—NM_002501.2(*NFIX*: c.375G > T [NP_002492.2:p.Lys125Asn]) (►Fig. 1G–I). This mutation was not detected in both the parents, indicating a de novo occurrence.

The prediction scores for c.5951G > A in *NSD1* and c.375G > T in *NFIX* were calculated through WANNONAR (webANNOVAR, <http://wannovar.wglab.org/>) and listed in ►Table 2. Most of the scores indicated that these mutations were pathogenic.

Discussion

The clinical diagnosis of congenital malformation syndromes is sometimes challenging for pediatricians who are not familiar with such syndromes. For patient 1, but not patients 2 and 3, Sotos syndrome was initially suspected as a clinical diagnosis. Developmental disability was the main concern of families and pediatricians for these patients. NGS was performed for a more rapid and comprehensive analysis for these patients, and disease-causing mutations were identified.

Sotos syndrome can be clinically recognized by characteristic facial appearances, learning disabilities, and overgrowth (height and/or OFC >97th centile), with each finding presenting in at least 90% of the reported Sotos patients.¹ Besides the cardinal features, several major features, such as seizures, advanced bone age, cardiac anomalies, and others, were observed in more than 15% of the affected individuals.¹ Loss-of-function mutations and chromosomal interstitial deletions involving *NSD1* are the main causes of Sotos syndrome.^{5,6}

The insertion c.4809_4810insA (p.Arg1605LysfsTer13) was identified in patient 1. To our knowledge, 382 variations (mutations, deletions, and insertions) in *NSD1* have been reported previously.⁷ Since this mutation has not been reported previously, we determined this to be a novel

mutation. This mutation causes a frame shift and subsequent premature termination, resulting in a loss-of-function *NSD1* protein lacking the SET domain. Patient 2 showed a previously reported mutation, c.5951G > A (p.Arg1984Gln) in *NSD1*.⁷ There are two other types of known mutations, c.5950C > G (p.Arg1984Gly) and c.5950C > T (p.Arg1984Ter), reported at the same amino acid position.^{8,9}

On the other hand, patient 3 showed a de novo missense mutation, c.375G > T (p.Lys125Asn), in *NFIX*, which is a gene responsible for Malan syndrome, also known as Sotos-2. Although this patient showed no distinctive findings, macrocephaly was the only finding compatible with Malan syndrome. Because predicted scores of p.Arg1984Gln suggested it as pathogenic (►Table 2), we concluded that this mutation is disease-causing.

So far, there have been only seven missense and two nonsense mutations reported to cause Malan syndrome.^{2,10–13} The c.375G > T (p.Lys125Asn) mutation reported in this study had not been reported previously. Martinez et al (2015) suggested that mutations giving rise to Malan syndrome cluster at exons 2 and 3, while mutations causing the more severe Marshall-Smith syndrome are distributed along the exons 6 to 10.¹² This may be due to a different fate of the messenger RNA.² Although the mutation, c.1012C > T located in exon 7, was reported by Klaassens et al (2014) in a patient with Malan syndrome,¹¹ the novel mutation reported here, located in exon 2 causing Malan syndrome, supported that speculation.

Molecular diagnosis for patients with overgrowth is complicated owing to the existence of a complex genetic background. NGS analysis could present as an easy, yet comprehensive diagnostic tool. Patients with both Sotos syndrome and Malan syndrome often show autistic features and behavioral abnormalities, as observed in all patients in this study who presented initially with a developmental delay. Clinical entities of such congenital malformation syndromes will be expanded in the future.

Table 2 Predicted scores provided through wANNOVAR

	Patient 2	Patient 3
	<i>NSD1</i>	<i>NFIX</i>
	c.5951G > A	c.375G > T
	p.Arg1984Gln	p.Lys125Asn
SIFT_score	0	0
SIFT_pred	D	D
PolyPhen2_HDIV_score	1	0.997
PolyPhen2_HDIV_pred	D	D
PolyPhen-2_HVAR_score	0.991	0.996
PolyPhen-2_HVAR_pred	D	D
LRT_score	0	0
LRT_pred	D	N
MutationTaster_score	1	1
MutationTaster_pred	D	D
MutationAssessor_score	3.965	2.54
MutationAssessor_pred	H	M
FATHMM_score	−3.03	−1.38
FATHMM_pred	D	T
RadialSVM_score	1.059	0.479
RadialSVM_pred	D	D
LR_score	0.928	0.674
LR_pred	D	D
VEST3_score	0.979	0.679
CADD_raw	5.758	3.344
CADD_phred	36	17.26
GERP + +_RS	5.77	4.21
phyloP46way_placental	2.884	1.19
phyloP100way_vertibrate	8.021	7.925
SiPhy_29way_logOdds	19.958	14.746

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Conflict of Interest

None.

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