Olgu Sunumu
Case Report

A Rare Pathogenic Frameshift Mutation in NSD1 Gene Related to Sotos Syndrome in a Turkish Patient

SOTOS SENDROMLU BİR TÜRK HASTADA NSD1 GENİNDE NADİR PATOJENİK ÇERÇEVE KAYMASI MUTASYONU

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ABSTRACT

Sotos syndrome which autosomal dominant inheritance has been observed, caused by the mutations and deletions in *NSD1* gene. *NSD1* gene consists 23 exons and localizes on chromosome 5q35.3. The prevalance of the Sotos syndrome is 1:14000 live births and the disease is characterized by excessive growth resulting in tall stature, a characteristical face appearance, advanced bone age, neurological disorder with intellectual disability and etc. Over 90% of the patients represent overgrowth, learning disability and macrocephaly. It has been shown that *NSD1* gene mutations and microdeletions in 5q35.3 were common cause of Sotos syndrome. In this study we describe a 4 years old boy with Sotos syndrome harbouring a pathogenic *NSD1* frameshift mutation. Clinical exome sequencing was performed using 2 ml of peripheral blood sample of the patient. The high-throughput data was analyzed using SOPHIA DDM database. The pathogenity of the mutations were evaluated based on in silico prediction tools (ClinVar, SIFT, Polyphen2, MutationTaster).We detected a pathogenic frameshift variant in *NSD1* gene, 2386_2389delGAAA by clinical exome sequencing. Although the diagnosis of Sotos syndrome can be made clinically, molecular analyzes are also important in diagnosis. Numerious NSD1 gene mutations and deletions have been identified to date. However, 2386_2389delGAAA pathogenic variant in the *NSD1* gene associated with Sotos syndrome will be reported for the first time in Turkey.

Keywords: Sotos syndrome, *NSD1*, clinical exome sequencing

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ÖZ

NSD1 genindeki mutasyonlar ve delesyonlar Otozomal dominant kalıtım görülen Sotos sendromuna neden olmaktadır. Kromozom 5q35.3 bölgesinde lokalize olan *NSD1* geni 23 eksondan oluşmaktadır. Sotos sendromunun prevalansı 14000 canlı doğumda bir olmakla birlikte, hastalık anormal büyüme, ileri kemik yaşı, entelektüel yeti yitiminin eşlik ettiği nörolojik bozukluklar, vb. ile karakterizedir. Sotos sendromlu bireylerin %90'nda karakteristik yüz görünümü, öğrenme güçlüğü, uzun boy ve makrosefali görülmektedir. Yapılan çalışmalarda *NSD1* gen mutasyonları ve 5q35.3 bölgesindeki delesyonlarının Sotos sendromuna neden olduğu gösterilmiştir. Bu çalışmada Sotos sendromlu 4 yaşındaki bir erkek çocukta yeni nesil dizileme yöntemi ile *NSD1* geninde patojenik çerçeve kayması mutasyonu saptanmıştır. Hastadan alınan 2 ml kan örneği kullanılarak klinik ekzom dizileme işlemi gerçekleştirilmiştir. SOPHIA DDM veritabanı kullanılarak data analizi yapılmıştır. Mutasyonların patojenitesi in siliko algoritmalar kullanılarak (ClinVar, SIFT, Polyphen2, MutationTaster) değerlendirilmiştir. *NSD1* geninde patojenik çerçeve kayması mutasyonu (2386_2389delGAAA) saptanmıştır. Sotos sendromunun tanısı klinik olarak konulabilmekle birlikte moleküler analizlerin de tanı da önemi büyüktür. Günümüze kadar pekçok *NSD1* gen mutasyonu ve delesyonu tanımlanmıştır. Ancak, Sotos sendromu ile ilişkili *NSD1* geninde saptadığımız 2386_2389delGAAA patojenik varyantı Türkiye'deki ilk kez rapor edilecektir.

Anahtar Kelimeler: Sotos sendromu, *NSD1*, klinik ekzom dizileme

Sotos syndrome is an autosomal dominant genetic condition which is characterized by excessive growth during childhood, macrocephaly, advanced bone age, typical facial features accompanied by non-progressive neurological disorder with intellectual disability. The prevalance is 1:14000 live births (1). Most cases are sporadic, but several familial cases have also been described exhibiting autosomal dominant inheritance patern (2). The *NSD1* gene was first characterized by Kurataki et al in 2001. The gene consists of 23 exons and the translated protein contains 2,696 amino acids (3). *NSD1* encodes a histone methyltransferase which is expressed in various tissues including muscle, brain, lung, thymus, kidney and spleen (4). Haploinsufficiency of the Nuclear receptor Set Domain containing protein 1 (*NSD1*) gene found to cause Sotos syndrome in 2002 (5). *NSD1* gene localizes on chromosome 5q35.3. To diagnose Sotos syndrome, identification of mutations and deletions are essential for revealing that *NSD1* gene mutations are responsible in 90% of affected patients (6-8). The range of clinical features associated with Sotos syndrome varies depending on underlying molecular mechanisms that are implicated in their generation. In particular, 5q35.3 microdeletions were shown to be the common cause of Sotos syndrome in Japanese population but in non-Japanese population 5q35.3 microdeletions accounts for only 10% of affected patients (9,10). Sotos syndrome is characterized by excessive growth resulting in tall stature, a characteristical face appearance, advanced bone age, neurological disorder with intellectual disability and etc. Over 90% of the patients represent overgrowth, learning disability and macrocephaly. (9).

Upto date there are limited studies identifying the mutations in *NSD1* gene related to Sotos syndrome. Kurataki et al (5) reported 4 different de novo mutations, 1 nonsense mutation, 2 frameshift mutations and 1 splice site mutation in NSD1 gene in 42 patients diagnosed with Sotos syndrome. Douglas et al (6) evaluated 75 individuals with

childhood overgrowth carrying intragenic mutations and large deletions in *NSD1* gene and reclassified them in 3 groups of which 2 of them as having 'typical Sotos syndrome' and the third one as 'possible Sotos syndrome' and they reported 28 gene alterations in 37 patients. Turkmen et al. (7) reported 19 mutations in 21 Sotos patients in turkish population and concluded that the great majority of the patients have mutations in *NSD1* gene, despite the fact that in patients with Weaver syndrome or other overgrowth phenotypes no mutations were detected in *NSD1* gene. On the other hand Nagai et al (11) reported 21 patients with approximately 2.2 Mb deletion and 5 patients with point mutations in *NSD1* gene.

Here we describe a 4 years old male patient with Sotos syndrome harbouring a pathogenic *NSD1* frameshift variant detected by next generation sequencing technology, 2386_2389delGAAA, as the first report in Turkey.

CASE

Four years old boy who admitted to our clinic was suffering from asthma, diagnosed as bilateral sensoryneural hearing lost, had a hydrocele in the left testicle, neurodevelopmental retardation. He was born at 38 weeks of gestation with 3730 gr weight and having secundum atrial septal defect in the first 5 months of his life. He has no siblings and none of relatives in the family were found to have Sotos syndrome. Writen consent was obtained from the parents of the participant. All procedures performed in our study involving human participants were in accordance with the ethical standards of the Pamukkale University Hospital ethics committee and with the 1964 Helsinki Declaration.

2-ml peripheral blood sample was collected using K_2EDTA vacutainers. DNA extraction was performed via QIAcube instrument with QIAamp DNA Blood Mini QIAcube Kit (Qiagen) according to the instructions of the manufacturer. Fluorometric quantitations of DNA samples and purified libraries were performed by Qubit instrument (Thermo Fisher, USA).

Clinical exome libraries were prepared with Clinical Exome Solution Kit (Sophia Genetics, USA) that is spanning 12Mb of target region covering more than 4495 genes with known hereditary mutations associated with various diseases including mental retardation, cardiomyopathies, hereditary cancer, autism, autoinflammatory diseases and etc. The sizes of the generated libraries were analyzed by using Agilent 2100 Bioanalyzer. Sequencing was done using NextSeq-500 sequencer (Illumina, Inc., San Diego, CA, USA) to produce 2 × 150 bp reads.

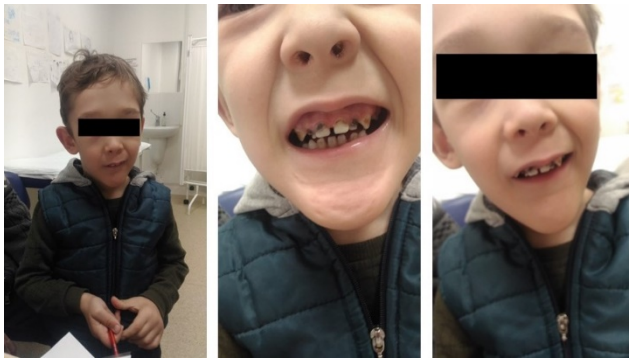
The amplified sequencing products are generated using Sequencing by synthesis (SBS) technology allowing four fluorescently labeled nucleotides to sequence millions of clusters on a flow cell surface at the same time. Illumina platform records each nucleotide via imaging techniques then converts into base calls. Sophia DDM® (v4.3.1)

algorithms were used for the bioinformatics analysis of data enabling to detection of SNV, Indels and CNV (Sophia Genetics SA, Switzerland). Generated FastQ files were uploaded and trimmed in order to remove primer sequences. Sequence reads were then aligned to the targeted regions and coverage was > 200x for Copy Number Variant (CNV) analyses which were also performed in silico on NextSeq dataset by means of commercial software Sophia DDM v4.3.1. The pathogenity of the mutations were evaluated based on in silico prediction tools (SIFT, Polyphen2, MutationTaster), the inheritance mode (OMIM), database entries (HGMD, ClinVar), and ACMG recommendations.

RESULTS

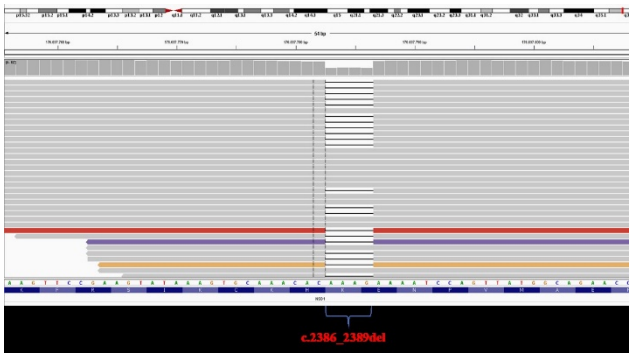
Physical examinations revealed that the patient has macrodolichocephaly, a receding hairline, long-thin face, frontotemporal thin hair, extremely slanted palpebral fistula, distinct mandibula and bilateral premolar forth and fifth teeth as well as their cavity are missing, all together 8 teeth (Figure 1).

Figure 1. Distinctive facial features of the proband.



Clinical exome sequencing (CES) analysis revealed a four base heterozygous deletion, 2386_2389delGAAA, in the exon 5 of *NSD1* gene (Figure 2).

Figure 2. Represantation of the pathogenic frameshift variant using Integrative Genomics Viewer (IGV).



Harbouring a deletion in *NSD1* gene and characteristical clinical findings (dysmorphic features, mental retardation, etc) have confirmed the diagnosis of the patient as Sotos syndrome.

DISCUSSION

The *NSD1* gene was found to be responsible for Sotos syndrome and until now, more than 400 mutations including 35% deletions, 25% missense variants, 20% nonsense variants, 15% insertions, 5% splice variants were identified in the *NSD1* gene (12). A familiar face with a disproportional extension of the forehead continuing with the pointed chin, macrodolichocephaly, a receding hairline, apparent hypertelorism with down slanted palpebral fissures are typical in the infancy of Sotos syndrome. Most patients found to have a non-progressive neurological dysfunction. As we described in results section, most of the symptoms of Sotos syndrome were detected in our patient.

The frameshift and duplication/deletions in *NSD1* gene could also be detected by FISH (fluorescence in situ hybridization) analysis to detect microdeletions, MLPA (multiplex ligation-dependent probe amplification) and also DNA analysis by genome sequencing to determine the specific *NSD1* gene mutations (13-16). Besides the importance of evaluating the characteristic clinical Picture of Sotos syndrome, molecular genetic testing is also extremely recommended to identify the disease. Therefore, in order to evaluate phenotype-genotype correlation of the patient we performed clinical exome sequencing which reveals that the patient was carrying a pathogenic heterozygous frameshift variant, 2386del_2389GAAA in *NSD1* gene.

It has been shown that *NSD1* gene alterations either point mutaitons or deletions are detected related to Sotos syndrome. There are numerous reports identifying point mutations and deletions in the patients with Sotos syndrome. Kurateki et al (5,17). identified 16 point mutations in 112 patients . Höglund et al (18) identified a novel 896delC mutation in a male infant and his father. Dougles et al (6) reported 9 pathogenic missense mutations (H1616L, L1637P, C1674W,C1792V, C1925R, R2005Q, R2017Q,H2143E, C2183S), 7 deletions (1171delC, 1727delA, 2576delAT, 3383delCT, 4883delT, 6001delC, 6302delA) and 6 insertions (2807_2808insA, 3549_3550insT, 5008_5009insG, 5744_5745insT, 6431_6432ins17, 6450_6451insC) in the molecular genetic analysis of *NSD1* gene in 75 patients with childhood overgrowth. Türkmen et al (7) identified 19 mutations (R498X, R1031X, 3141delC, 3160delA, 3541delGAAA, 3536delA, 4160insC, IVS8-2A>G, 4806delTGTTAAA, 4885C>T, 4895delG, 5194G>T, IVS15-1G>T, 5386G>T, 5398insT, 5611A>T, IVS19-2A>G, 6013C>T, 6532delTGCCCCAGC) in 37 patients. Baujat et al. (19) reported a 4 base deletion, 7968delGACA, in *NSD1* gene. F

Tatton-Brown et al (9) reported 180 NSD1 intragenic gene mutations in 233 NSD1-positive patients of which 80 of them were frameshift mutations including 2386_2389delGAAA and 45 missense mutations . Kotilainen et al (20) reported 11 point mutations (3383_3384delCT, R2017Q, 4827delC, Y1870C, 2954_2955delCT, Q546X, 3281dupA, R604X, R788X, 3004_3005delAA, c.4497+1G → A). Vieira et al (21) described a novel C1593W mutation in a cohort of Brazilian patients. Bae Sohn et al (22) identified 4 novel NSD1 mutations (c.4797_4798insTG, c.4591_4592insA, c.4778G>A and c.4967-1G>A) in 7 out of 15 Korean patients. Lacetta et al (2) identified V174A mutation in *NSD1* gene in a 3 generation family with gigantism.

Non-allelic homologous recombination occurs between DNA sequences that have been duplicated and thus have low copy repeats (LCRs). Visser et al reported a 3-kb major recombination hotspot within LCRs and mapped deletion breakpoints of the patients carrying 1.9 Mb microdeletion by array CGH analysis (10,23). Numerous reports have revealed that there were no correlation between deletion size and clinical phenotype (9, 13, 23). This study has the importance of being the first report for detecting a pathogenic frameshift variant, 2386_2389delGAAA, in a Turkish patient with Sotos syndrome. Although the diagnosis of Sotos syndrome can be made clinically, molecular analyzes are also important in diagnosis.

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