**DOI: 10.4274/jcrpe.galenos.2025.2025-6-8**

**Case Report**

# The Opposite Phenotype of Sotos Syndrome: 5q35.2q35.3 Microduplication Syndrome



Kübra Şen Küçük[1], Aydan Mengübaş Erbaş[2], Zehra Manav Yiğit[2], Ahmet Anık[1]
[1]Aydın Adnan Menderes University Faculty of Medicine, Department of Pediatrics, Division of Pediatric Endocrinology, Aydın, Türkiye
[2]Aydın Adnan Menderes University Faculty of Medicine, Department of Medical Genetics, Aydın, Türkiye

**What is already known on this topic?**
Duplications involving the *NSD1* gene are known to cause a phenotype opposite to Sotos syndrome, typically including microcephaly, short stature, and developmental delay. However, the number of reported cases remains limited, and detailed clinical characterization, especially from pediatric endocrinology perspectives, is still scarce in the literature.

**What this study adds?**
This case provides a detailed auxological and neurodevelopmental profile of a child with 5q35.2q35.3 microduplication involving *NSD1*. It contributes to the understanding of reverse Sotos phenotype and underscores the value of CMA in diagnosing rare genomic disorders with subtle but distinct clinical features.

**Abstract**
Sotos syndrome is a genetic disorder resulting from heterozygous pathogenic variants or deletions in the nuclear receptor-binding SET domain protein 1 (*NSD1*) gene. It is characterized by prenatal and postnatal overgrowth, macrocephaly, distinctive craniofacial features, learning disability, and advanced bone maturation. In contrast, Reverse Sotos syndrome, arises from duplications within the *NSD1* gene, presenting with an opposite clinical phenotype, including microcephaly, developmental delay, short stature and delayed bone maturation. To date, the reverse clinical phenotype associated with the 5q35.2q35.3 microduplication encompassing the *NSD1* gene has been reported in 43 cases. We present a novel case of a 4-year-11-month-old patient with a 5q35.2q35.3 duplication involving the *NSD1* gene. The patient, exhibited clinical features of microcephaly, short stature, low-normal weight, delayed bone age, developmental delay, attention deficit hyperactivity disorder, alongside normal routine biochemical tests, nutritional parameters, and insulin-like growth factor-1 levels. Chromosomal microarray analysis (CMA) identified a 714.1 kb duplication in the 5q35.2q35.3 region, including *NSD1*. This case underscores the significance of *NSD1* gene dosage alterations in manifesting a reverse clinical phenotype typified by microcephaly and short stature. Furthermore, it highlights the utility of CMA as a robust diagnostic tool for detecting microrearrangements and guiding clinical evaluation.
**Keywords:** Sotos syndrome, Reverse Sotos syndrome, NSD1, microduplication 5q35

Prof. Dr. Ahmet Anık, Aydın Adnan Menderes University Faculty of Medicine, Department of Pediatrics, Division of Pediatric Endocrinology, Aydın, Türkiye
ahmet.anik@yahoo.com
0000-0002-7729-7872

20.06.2025
12.09.2025

**Epub:** 25.09.2025

## Introduction
Over the past 19 years, several cases of 5q35 microduplication have been reported, demonstrating a broad spectrum of phenotypic variability. The condition is characterized by growth restriction, microcephaly, learning disabilities, behavioral changes, congenital anomalies, and distinctive craniofacial features, sometimes accompanied by cardiac, renal, or skeletal anomalies (1). To date, 43 patients, including both probands and their parents, have been identified with 5q35.2q35.3 duplications encompassing the nuclear receptor-binding SET domain protein 1 (NSD1) gene (1–4).
The *NSD* protein family comprises three histone methyltransferases: *NSD1*, *NSD2,* and *NSD3*, which are pivotal in maintaining chromatin architecture and integrity (5). Among these, *NSD1* is ubiquitously expressed across tissues, with predominant levels observed in the brain, endocrine glands, and reproductive organs. It plays a crucial role in modulating gene expression involved in cellular growth, keratinocyte differentiation, and bone morphogenesis (6). Haploinsufficiency of *NSD1* underlies Sotos syndrome, characterized by overgrowth, macrocephaly, and advanced bone age (7). In contrast, *NSD1* duplications result in a clinical presentation commonly described as "Reverse Sotos syndrome," marked by short stature, microcephaly, delayed bone age, and neurodevelopmental delay (1, 8–13).
In this case report, we present a rare instance of 5q35.2q35.3 duplication, identified through chromosomal microarray analysis (CMA), in a patient presenting with short stature, microcephaly, and syndromic features, thereby contributing to the expanding spectrum of clinical variability associated with this genomic alteration.

## Case report
### Clinical and Laboratory Features at Baseline
A 4-year-11-month-old male presented to the Pediatric Endocrinology Clinic with concerns about growth retardation and developmental delay. Since birth, the patient exhibited microcephaly, short stature, and poor weight gain. He was born at 38 weeks' gestation via cesarean section with a birth weight of 2560 g (-1.78 SD), a length of 47 cm (-1.28 SD), and a head circumference of 32 cm (-2.06 SD). Despite nutritional support, including enteral nutrition products provided under the supervision of the Pediatric Gastroenterology Division, his weight gain remained suboptimal. Additionally, he was followed by the Child and Adolescent Psychiatry Department for developmental delay, fine motor deficits, and Attention-Deficit/ Hyperactivity Disorder (ADHD), for which he was receiving methylphenidate therapy. The newborn hearing screening was passed successfully. Although no formal IQ testing had been performed, he demonstrated mild intellectual disability alongside a strong-willed personality. He was enrolled in special education programs. The family history revealed that the mother, father, and siblings were alive and healthy, with no notable health concerns. The parents were consanguineous (first-degree cousins). On physical examination, the patient's anthropometric measurements were as follows: weight of 13.5 kg (-2.5 SD; 0.66th percentile), height of 99.8 cm (-2.2 SD; 1.39th percentile), head circumference of 45.5 cm (-4.1 SD; <0.02nd percentile) and body mass index (BMI) of 13.5 kg/m² (-1.6 SD;

5.1st percentile). The target height, based on parental heights, was calculated at 168.4 cm (-1.26 SD; 10.38th percentile). Craniofacial features included a long face, a prominent nasal bridge with a broad nasal tip, a low-hanging columella, prominent ears with under folded helices, a broad inferior crus of antihelix, a smooth philtrum, thin upper and lower vermilion borders, retrognathia, and micrognathia (Figure 1A). Limb anomalies were noted, including bilateral clinodactyly of the fifth fingers (Figure 1B), and metatarsus adductus with slender toes (Figure 1C). He was prepubertal, with bilateral testicular volumes of 2 cc. The phenotypes of the mother, father, and siblings were similar. Complete blood count, routine biochemical tests, thyroid function tests, and nutritional parameters were within normal range. Celiac antibody screening was negative. Serum levels of Insulin-like Growth Factor-1 (IGF-1) and Insulin-like Growth Factor Binding Protein-3 (IGFBP-3) were within normal ranges. Bone age assessment revealed skeletal maturation consistent with that of a 4-year-old. Renal ultrasonography and echocardiography were both performed and revealed normal findings. Cranial Magnetic Resonance Imaging (MRI) also showed no structural abnormalities. A genetic panel for short stature was sent. During the three-year follow-up, the patient's weight consistently remained below -2 SD. His height standard deviation (SD) score fluctuated between -2.2 and -1.6 SD, while the BMI SD ranged from -3.2 to -1.6 SD. Notably, his bone age consistently lagged approximately one year behind his chronological age. Despite these findings, the patient demonstrated a height velocity exceeding 4 cm/year, with annual growth rates ranging from 4.0 to 7.3 cm/year. Given the adequate height velocity (>4 cm/year), normal IGF-1 and IGFBP-3 levels, and the absence of severe short stature, growth hormone (GH) stimulation test was not performed. Longitudinal follow-up was planned with height velocity monitoring. If there is further deterioration in height SD in subsequent visits, a GH stimulation test will be reconsidered.

**Genetic Diagnostics:**

To investigate the underlying genetic etiology of the patient's short stature and associated phenotypic features, exome sequencing was performed on genomic DNA extracted from peripheral blood leukocytes. Sequencing was carried out using the Sophia Genetics Clinical Exome Solution V3 Kit, which targets 5500 genes. The sequencing process utilized the Illumina NextSeq platform, and data analysis was conducted with the Sophia DDM V4 analysis platform. Exome analysis did not identify any pathogenic, likely pathogenic, or variants of uncertain significance (VUS) in single nucleotide variants (SNVs) or short insertions/deletions that were considered potentially relevant to the phenotype. However, copy number variation (CNV) analysis revealed a duplication encompassing all exons of *NSD1, UIMC1, FGFR4*, *SLC34A1, F12,* and *B4GALT7* within the 5q35.2q35.3 region of chromosome 5. This genomic alteration is characteristic of the duplication syndrome associated with Reverse Sotos syndrome. For confirmation, chromosomal microarray analysis (CMA) was performed using the Illumina Infinium Global Screening Array-24 v1.0 (GSA-Cyto) (Illumina, Inc., San Diego, CA) on genomic DNA derived from peripheral blood lymphocytes. Data analysis was facilitated by the NxClinical Interpret software (BioDiscovery, El Segundo, CA). Genome build hg19 was used for probe locations and data interpretation. Publicly accessible databases, including the Database of Genomic Variants (DGV), DECIPHER, OMIM, ClinVar, and ClinGen, were referenced for variant interpretation by established guidelines and literature recommendations. CMA analysis identified a 714.1 kb duplication in the 5q35.2q35.3 region (arr[hg19] 5q35.2q35.3(176,334,601-177,048,708)x3) encompassing 21 OMIM genes, namely *UIMC1* (OMIM *609433), *ZNF346* (OMIM *605308), *FGFR4* (OMIM *134935), *NSD1* (OMIM *606681), *RAB24* (OMIM *612415), *PRELID1* (OMIM *605733), *MXD3* (OMIM *609450), *LMAN2* (OMIM *609551), *RGS14* (OMIM *602513), *SLC34A1* (OMIM *182309), *PFN3* (OMIM *612812), *F12* (OMIM *610619), *GRK6* (OMIM *600869), *PRR7* (OMIM *618306), *DBN1* (OMIM *126660), *PDLIM7* (OMIM *605903), *DOK3* (OMIM *611435), *DDX41* (OMIM *608170), *FAM193B* (OMIM *615813), *TMED9* (OMIM *620436), *B4GALT7* (OMIM *604327) (Figure 2). Among these, *NSD1* has been well-documented as a causative gene for Reverse Sotos syndrome due to its dosage-sensitive role in growth and developmental processes. To determine the origin of the duplication, segregation analysis was performed on both parents. Neither parent carried the identified variant, confirming that the 5q35.2q35.3 duplication occurred *de novo* in the patient.

**Discussion**

Chromosomal microarray analysis identified a 714.1 kb microduplication in the 5q35.2q35.3 region encompassing the *NSD1* gene in our patient, who presented with mild intellectual disability, developmental delay, growth retardation, microcephaly and distinct dysmorphic features. The clinical phenotype of our patient is consistent with previously reported cases (1-4).

Although the phenotypic features observed in our patient align with those reported in prior studies, the variability in clinical expression suggests potential modifying influences, either genetic or environmental. Short stature and microcephaly were assessed based on standard growth curves, while dysmorphic features were evaluated through expert clinical dysmorphology assessment. The observed variability in neurodevelopmental and dysmorphic manifestations among patients raises questions about the degree of penetrance and the presence of potential genotype-phenotype correlations.

The hallmark clinical features of 5q35.2q35.3 duplication include short stature /height at the lower limit of normal, microcephaly/head circumference at the lower limit of normal, characteristic facial features, delayed bone age, mild intellectual disability, learning disabilities, and possible behavioral concerns. While birth length and weight may vary, less common anomalies include dental malformations, ear abnormalities, urogenital anomaly, cardiac defects, hypotonia, constipation, and tremors (2,8). In Sotos syndrome, cranial MRI has occasionally revealed nonspecific findings such as dilated ventricles, prominence of the trigone and occipital horns, and/or hypoplasia of the corpus callosum (14). *NSD1* protein is known to play a critical role in brain development (6). However, in patients with 5q35.2q35.3 duplication, cranial MRI findings have generally been reported as normal (8). Our patient presented with short stature, microcephaly, mild learning difficulties, aggressive behaviors, and ADHD. The newborn hearing test was normal, and no genital anomalies were observed. Renal ultrasonography and echocardiography were also normal. Importantly, cranial MRI revealed neither ventriculomegaly nor corpus callosum hypoplasia and was entirely unremarkable, thus ruling out the structural anomalies reported in some cases.

The term "Reverse Sotos syndrome" is frequently used, some studies argue against this nomenclature, noting that certain chromosomal imbalances similarly present with short stature and microcephaly without the distinctive inverted facial morphology seen in 5q35.2q35.3 duplications (8). Furthermore, while growth and neurodevelopmental trajectories seem to be contrasting, the underlying molecular mechanisms driving these phenotypic divergences remain to be fully elucidated.

Short stature associated with 5q35.2q35.3 duplications is generally not attributable to GH deficiency, as GH levels in affected individuals are typically within the normal range. Moreover, several trials of recombinant GH therapy in such patients have not demonstrated consistent or significant growth acceleration (2), suggesting that short stature in this context primarily results from intrinsic growth impairment rather than GH deficiency, further supporting the distinct genetic basis of this syndrome. However, a case report published in 2020 described a patient with Reverse Sotos syndrome who, despite having a normal GH stimulation test, received recombinant GH therapy due to severe short stature and declining height velocity. Treatment resulted in a marked improvement in height velocity and increased IGF-1 levels, ultimately enabling the patient to nearly reach normal stature (4). This finding underscores the heterogeneity of treatment responses and suggests that GH therapy may be considered on an individual basis in selected patients with significant growth failure. In our patient, severe short stature was not present; annual height velocity remained adequate, and both IGF-1 and IGFBP-3 levels were within the normal range. Therefore, a GH stimulation test was not performed. Nonetheless, we planned continued monitoring with height velocity follow-up, and in the event of a decline in growth velocity or further loss of height SDS, a GH stimulation test will be reconsidered.

Emerging evidence implicates *NSD1* overexpression in the pathogenesis of the undergrowth phenotype associated with 5q35.2q35.3 duplication (2,15). Experimental studies indicate that *NSD1* overexpression may modulate cell proliferation and growth via the *PI3K/AKT/mTOR* pathway, which is pivotal inregulating cell survival, protein synthesis, and metabolic control. Dysregulation of this pathway is implicated in various overgrowth and undergrowth syndromes. Notably, *mTOR* activation may occur independently of *PI3K*

signaling through the addition of branched-chain amino acids, such as leucine. However, further research is needed to elucidate these molecular interactions and their therapeutic implications (2).

The phenotypic variability observed among patients with 5q35.2q35.3 duplication suggests the involvement of additional genetic or epigenetic factors influencing disease expression. Although CMA remains the gold standard for identifying large CNVs, clinical exome sequencing (CES) is increasingly recognized for detecting smaller variants that may modulate clinical presentation. In our patient, CES was prioritized due to the complexity of the phenotype, including dysmorphic features, microcephaly, mild intellectual disability, ADHD and developmental delay. Although a more cost-effective method like CMA could have been sufficient for diagnosis, the complexity of the clinical phenotype prompted the use of CES. This case emphasizes the diagnostic complexity associated with phenotypic heterogeneity and underscores the importance of selecting appropriate genetic testing modalities. Given the heterogeneity of this syndrome, an integrated approach employing both CMA and next-generation sequencing (NGS) technologies may enhance diagnostic accuracy and refine genotype-phenotype correlations.

**Conclusion**

Sotos syndrome, classically defined by *NSD1* haploinsufficiency and overgrowth, contrasts sharply with the phenotype observed in 5q35.2q35.3 duplications, where gene dosage elevation results in short stature and microcephaly. Our findings reinforce the notion that 5q35.2q35.3 microduplication should be considered in the differential diagnosis of children presenting with short stature, microcephaly, dysmorphic features, delayed bone age, and neurodevelopmental delays. Furthermore, this study highlights the potential of NGS in detecting CNV, while also emphasizing the role of CMA in confirming structural variations. Further research is warranted to unravel the molecular mechanisms linking *NSD1* overexpression with growth impairment, potentially paving the way for targeted therapeutic strategies.

**Ethics**

**Informed Consent:** Written informed consent was obtained from the patient's parents for publication of this study.

**Authorship Contributions**

Surgical and Medical Practices: Kübra Şen Küçük, Ahmet Anık, Concept: Kübra Şen Küçük, Ahmet Anık, Design: Ahmet Anık, Data Collection or Processing: Kübra Şen Küçük, Aydan Mengübaş Erbaş, Zehra Manav Yiğit, Ahmet Anık, Analysis or Interpretation: Aydan Mengübaş Erbaş, Zehra Manav Yiğit, Literature Search: Kübra Şen Küçük, Aydan Mengübaş Erbaş, Zehra Manav Yiğit, Ahmet Anık , Writing: Kübra Şen Küçük, Aydan Mengübaş Erbaş, Zehra Manav Yiğit, Ahmet Anık.

**Conflict of interest:** None declared.

**Financial Disclosure:** The authors declared that this study received no financial support.

**References**

1.) van der Lugt NM, Weerts MJA, Veenma DCM, Lincke CR, Gischler SJ, Alders M, van Ierland Y. 5q35 duplication syndrome: Narrowing the critical region on the distal side and further evidence of intrafamilial variability and expression. Am J Med Genet A. 2023;191:835-841.
2.) Quintero-Rivera F, Eno CC, Sutanto C, Jones KL, Nowaczyk MJM, Wong D, Earl D, Mirzaa G, Beck A, Martinez-Agosto JA. 5q35 duplication presents with psychiatric and undergrowth phenotypes mediated by *NSD1* overexpression and mTOR signaling downregulation. Hum Genet. 2021;140:681-690.
3.) Takkar A, Arora V, Saxena R, Kumar P, Verma IC. Reversal of Clinical Phenotype of Sotos Syndrome Due to Microduplication of *NSD1* Gene. Indian J Pediatr. 2022;89:1137-1139.
4.) Bernhardt IT, Gunn AJ, Carter PJ. Growth hormone treatment for short stature associated with duplication of the *NSD1* Sotos syndrome gene. Endocrinol Diabetes Metab Case Rep. 2021;2021:20-0033.
5.) Morishita M, di Luccio E. Cancers and the NSD family of histone lysine methyltransferases. Biochim Biophys Acta. 2011;1816:158-163.
6.) Lucio-Eterovic AK, Singh MM, Gardner JE, Veerappan CS, Rice JC, Carpenter PB. Role for the nuclear receptor-binding SET domain protein 1 (*NSD1*) methyltransferase in coordinating lysine 36 methylation at histone 3 with RNA polymerase II function. Proc Natl Acad Sci U S A. 2010;107:16952-16957.
7.) Tatton-Brown K, Cole TRP, Rahman N. Sotos Syndrome. In: Adam MP, Feldman J, Mirzaa GM, Pagon RA, Wallace SE, Amemiya A (eds). GeneReviews®. University of Washington, Seattle; 2004.
8.) Dikow N, Maas B, Gaspar H, Kreiss-Nachtsheim M, Engels H, Kuechler A, Garbes L, Netzer C, Neuhann TM, Koehler U, Casteels K, Devriendt K, Janssen JW, Jauch A, Hinderhofer K, Moog U. The phenotypic spectrum of duplication 5q35.2-q35.3 encompassing *NSD1*: is it really a reversed Sotos syndrome? Am J Med Genet A. 2013;161A:2158-2166.
9.) Franco LM, de Ravel T, Graham BH, Frenkel SM, Van Driessche J, Stankiewicz P, Lupski JR, Vermeesch JR, Cheung SW. A syndrome of short stature, microcephaly and speech delay is associated with duplications reciprocal to the common Sotos syndrome deletion. Eur J Hum Genet. 2010;18:258-261.
10.) Novara F, Stanzial F, Rossi E, Benedicenti F, Inzana F, Di Gregorio E, Brusco A, Graakjaer J, Fagerberg C, Belligni E, Silengo M, Zuffardi O, Ciccone R. Defining the phenotype associated with microduplication reciprocal to Sotos syndrome microdeletion. Am J Med Genet A. 2014;164A:2084-2090.
11.) Rosenfeld JA, Kim KH, Angle B, Troxell R, Gorski JL, Westemeyer M, Frydman M, Senturias Y, Earl D, Torchia B, Schultz RA, Ellison JW, Tsuchiya K, Zimmerman S, Smolarek TA, Ballif BC, Shaffer LG. Further Evidence of Contrasting Phenotypes Caused by Reciprocal Deletions and Duplications: Duplication of *NSD1* Causes Growth Retardation and Microcephaly. Mol Syndromol. 2013;3:247-254.
12.) Chen CP, Lin SP, Lin CC, Chen YJ, Chern SR, Li YC, Hsieh LJ, Lee CC, Pan CW, Wang W. Molecular cytogenetic analysis of de novo dup(5)(q35.2q35.3) and review of the literature of pure partial trisomy 5q. Am J Med Genet A. 2006;140:1594-1600.
13.) Zhang H, Lu X, Beasley J, Mulvihill JJ, Liu R, Li S, Lee JY. Reversed clinical phenotype due to a microduplication of Sotos syndrome region detected by array CGH: microcephaly, developmental delay and delayed bone age. Am J Med Genet A. 2011;155A:1374-1378.
14.) Tatton-Brown K, Douglas J, Coleman K, Baujat G, Cole TR, Das S, Horn D, Hughes HE, Temple IK, Faravelli F, Waggoner D, Turkmen S, Cormier-Daire V, Irrthum A, Rahman N; Childhood Overgrowth Collaboration. Genotype-phenotype associations in Sotos syndrome: an analysis of 266 individuals with NSD1 aberrations. Am J Hum Genet. 2005;77:193-204.
15.) Jeong Y, Kim T, Kim S, Hong YK, Cho KS, Lee IS. Overexpression of histone methyltransferase NSD in Drosophila induces apoptotic cell death via the Jun-N-terminal kinase pathway. Biochem Biophys Res Commun. 2018;496:1134-1140.


**Figure 1.** Craniofacial dysmorphism and distal limb anomalies in the patient. A) Frontal view showing a long face, a prominent nasal bridge with a broad nasal tip, a low-hanging columella, prominent ears with underfolded helices and a broad inferior crus of the antihelix, a smooth philtrum, thin upper and lower vermilion borders, retrognathia, and micrognathia. B) Bilateral clinodactyly of the fifth fingers. C) Metatarsus adductus and slender toes.

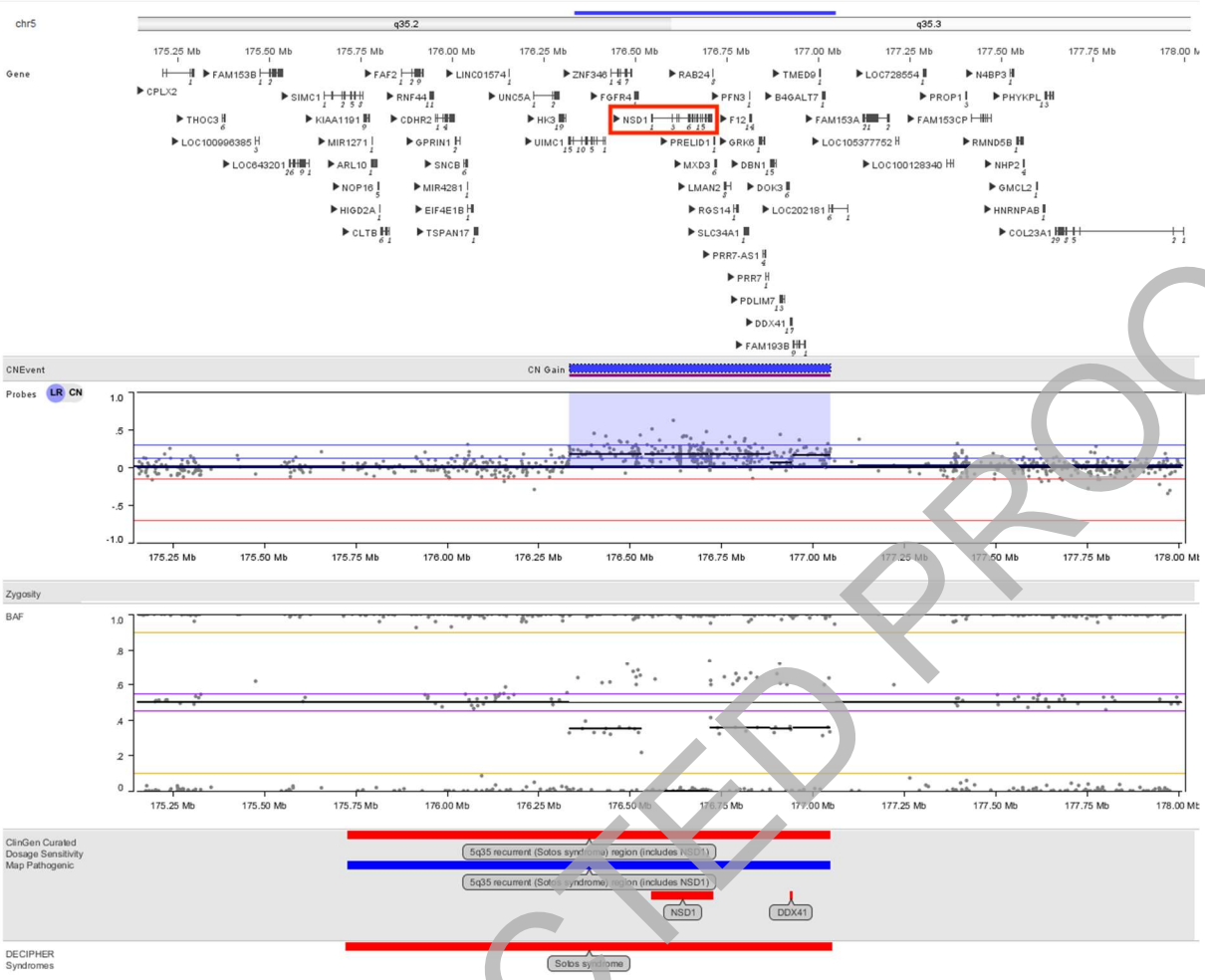


**Figure 2.** Chromosomal microarray analysis showed a 714.1 kb duplication at 5q35.2q35.3 involving the *NSD1* gene. Although this region overlaps the typical Sotos syndrome locus, the duplication is associated with a reverse Sotos phenotype. The copy number gain is indicated by an upward shift in the log2 ratio and a corresponding change in the B-allele frequency pattern.
*kb: kilobase, NSD1: Nuclear receptor-binding SET Domain protein 1*