

## NSD1 Gene Analysis in Sotos Syndrome

### Clinical Features:

The three cardinal features of Sotos syndrome include a characteristic facial gestalt, learning difficulties, and macrocephaly, and greater than 90% of patients are noted to have all three features.<sup>1</sup> The characteristic facial gestalt is most recognizable between the ages of one and six and consists of a long, thin face with down-slanting palpebral fissures, a broad forehead, malar flushing, and fronto-temporal hair sparsity. Learning disabilities are present in 97% of individuals with Sotos syndrome, but the extent of impairment is largely variable.<sup>2</sup>

Approximately 90% of individuals with Sotos syndrome have height and/or head circumference at least 2 standard deviations above the mean in childhood. Other major features present in more than 15% of patients include advanced bone age, cardiac anomalies, cranial magnetic resonance imaging or CT abnormalities, hyperlaxity, maternal pre-eclampsia, neonatal hypotonia, neonatal jaundice, neonatal poor feeding, renal anomalies, scoliosis, and seizures. As with other overgrowth syndromes, there is an increased risk for tumors in Sotos syndrome. The risk is estimated to be 2-3% and includes embryonal tumors such as Wilms tumor, neuroblastoma, and hepatocellular carcinoma, as well as other benign and malignant tumors including leukemia, lymphoma, and sacrococcygeal teratoma.<sup>3</sup> In previous studies, Sotos syndrome has demonstrated complete penetrance, as all individuals with Sotos syndrome exhibit at least some characteristics of the disorder.

### Genetics:

Autosomal dominant. Greater than 95% of cases result from de novo variants.<sup>2</sup> No cases of germline mosaicism have been reported to date.<sup>4</sup> Sotos syndrome is caused by heterozygous variants or deletions of the NSD1 (nuclear receptor SET domain-containing protein) gene, located at chromosome 5q35.<sup>4,5</sup> The NSD1 gene consists of 23 exons and encodes a 2696 amino acid histone methyltransferase. Although the function of the protein is not well characterized, it is believed to play a role in chromatin regulation. The protein contains multiple functional domains, including the SET domain, a SET-associated (SAC) domain, a C5HCH domain, five plant homeodomains (PHDs), two proline-tryptophan-tryptophan-proline (PWWP) domains and two nuclear-receptor interaction domains, NID-L and NID+L.<sup>4</sup>

Some genotype-phenotype correlations have been established, although additional research is necessary to better characterize the spectrum of phenotypes associated with specific types of NSD1 variants. Patients with 5q35 microdeletions have been noted to have more severe learning disabilities and are more likely to have cardiac anomalies than patients with NSD1 intragenic variants.<sup>8,2</sup> However, it is also possible for individuals with the same variant in the NSD1 gene to present with strikingly different phenotypes.<sup>2</sup>

## Test Methods:

Using genomic DNA from a submitted blood specimen, bi-directional sequence analysis of exons 1-23 and the splice sites of the NSD1 gene is performed. Concurrently, targeted array CGH analysis with exon-level resolution (ExonArrayDx) is performed to evaluate for a deletion or duplication of one or more exons of the NSD1 gene. The presence of a variant or deletion is confirmed by repeat analysis using sequencing, restriction fragment analysis, qPCR or another appropriate method.

## Test Sensitivity:

The analysis performed by GeneDx is expected to identify a pathogenic variant or deletion/duplication in the NSD1 gene in approximately 90%-93% of non-Japanese patients and 63% of Japanese patients with a clinical diagnosis of Sotos syndrome.<sup>5,8,2</sup> Specifically, in non-Japanese patients, 71%-77% will have an intragenic variant identifiable by sequencing, while 10%-13% will have a 5q35 microdeletion and 5%-6% will have a partial gene deletion or duplication identifiable by ExonArrayDx deletion/duplication analysis.<sup>9,8,2</sup> In Japanese patients, approximately 12% will have an intragenic variant identifiable by sequencing, while 52% are expected to have a 5q35 microdeletion.<sup>5</sup>

Variants in the NSD1 gene appear to be specific to Sotos syndrome. Although one study reported variants in NSD1 in 3/6 (50%) of patients with atypical Weaver syndrome,<sup>10</sup> on further analysis two of these patients were determined to have classic Sotos syndrome and the other patient was diagnosed with "possible Sotos syndrome."<sup>2</sup> To date, NSD1 variants have not been reported in patients with classic Weaver syndrome or nonspecific overgrowth in the absence of other features of Sotos syndrome.<sup>2,11,12</sup>

## Variant Spectrum:

The spectrum of variants differs among Japanese and non-Japanese patients. In Japanese patients, the predominant cause of Sotos syndrome is a recurrent 1.9-Mb microdeletion on chromosome 5q35 that includes the NSD1 gene.<sup>5</sup> The microdeletion occurs due to non-allelic homologous recombination between low-copy repeats flanking the NSD1 gene, and it is observed more frequently in Japanese patients due to the presence of a common inversion polymorphism.<sup>2,6</sup>

Although 5q35 microdeletions also occur in non-Japanese patients with Sotos syndrome, the size of the deletion varies greatly. While some non-Japanese patients have the 1.9Mb microdeletion, other reported deletions range in size from a 482 kb deletion of NSD1 only to a 5Mb deletion of 54 genes including NSD1.<sup>2</sup> The primary cause of Sotos syndrome outside of the Japanese population is intragenic variants in the NSD1 gene. In a previous report of 266 patients primarily of European ancestry with an NSD1 variant, 88% were found to have an intragenic variant, while ~12% had a chromosome 5q35 microdeletion and 3% had a partial

deletion of the NSD1 gene. Intragenic variants are scattered throughout the gene and include frameshift (34%), missense (24%), nonsense (22%), and splice site (4%) variants. Pathogenic missense variants cluster in the SET, SAC, C5HCH, and PHD functional domains, whereas missense substitutions in the central region of the protein, which does not contain any known functional domains, are typically not pathogenic.<sup>9,2,8</sup> Although most patients have novel variants, several variants have been reported in multiple unrelated patients with Sotos syndrome.<sup>7,2</sup>

## References:

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