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## Abstract

*NSD2* dimethylates histone H3 at lysine 36 (H3K36me2) and is located in the Wolf-Hirschhorn syndrome (WHS) critical region. Recent descriptions have delineated loss-of-function (LoF) variants in *NSD2* with a distinct disorder. The oncogenic missense variant p.Glu1099Lys occurs somatically in leukemia and has a gain-of-function (GoF) effect. We describe two individuals carrying p.Glu1099Lys as heterozygous de novo germline variant identified by exome sequencing (ES) of blood DNA and subsequently confirmed in two ectodermal tissues. Clinically, these individuals are characterized by intellectual disability, coarse/ square facial gestalt, abnormalities of the hands, and organomegaly. Public cell lines with *NSD2* GoF variants had increased K36me2, DNA promoter methylation, and dysregulated RNA expression. *NSD2* GoF caused by p.Glu1099Lys is associated with a novel phenotype different from WHS and Rauch–Steindl syndrome (RAUST).

Bernt Popp, Melanie Brugger, Vincent Strehlow, and Theresa Brunet contributed equally to this work

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**KEYWORDS**

gain-of-function, Glu1099Lys, neurodevelopmental disorder, NSD2, Rauch–Steindl syndrome, Wolf–Hirschhorn syndrome

## 1 | INTRODUCTION

Variants affecting genes encoding epigenetic proteins cause neurodevelopmental disorders (NDDs) and have been dubbed “Mendelian Disorders of the Epigenetic Machinery” (MDEM).<sup>1</sup> Clinically, these entities often comprise intellectual disability (ID) and growth abnormalities.

Epigenetic modifications of histone N-terminal lysine (K) residues affect DNA accessibility and transcriptional activity. Depending on the modified amino acid, they can serve as chromatin repressive (H3K27 methylation) or activating (H3K36 methylation) markers.

The nuclear receptor binding SET domain protein 2 (NSD2) catalyzes H3K36 methylation writing.<sup>2</sup> The previous name, Wolf–Hirschhorn syndrome (WHS, OMIM #194190) candidate 1 (WHSC1) indicates the gene's location in the critical deletion region. WHS was cytogenetically characterized before the microarray era and refined as a contiguous gene deletion syndrome with two candidates (NSD2 and LETM1) on 4p16.3 (OMIM #194190). The core clinical features of WHS include NDD, a typical facial dysmorphism described to resemble a “Greek warrior helmet” and intrauterine/ postnatal growth retardation.<sup>3,4</sup> A recent study described truncating and missense variants with loss of methylation activity in 18 individuals with mild developmental phenotypes that only partially overlap with WHS (Rauch–Steindl syndrome, RAUST, OMIM #619695).<sup>5</sup>

The other name for NSD2, “multiple myeloma SET domain containing protein” (MMSET), highlights the gene's involvement in hematologic malignancies.<sup>6</sup> The somatic t(4;14) translocation, present in 15–25% of multiple myeloma, fuses NSD2 to the immunoglobulin heavy-chain (IGH) and causes overexpression. Sequencing studies identified a recurring somatic hotspot missense mutation in NSD2 (E1099K; c.3295G > A, p.Glu1099Lys) in pediatric acute lymphoblastic leukemia (AML).<sup>7,8</sup> Both IGH-NSD2 and p.Glu1099Lys cause an expansion of H3K36me2 methylation.<sup>7,9</sup> Cryo-electron microscopy showed that p.Glu1099Lys destabilizes autoinhibition, increases substrate recognition and H3K36 methyltransferase activity.<sup>10,11</sup>

Here, we describe the NSD2 GoF variant p.Glu1099Lys as a constitutional, germline heterozygous de novo variant in two individuals with a syndromic NDD.

## 2 | MATERIALS AND METHODS

### 2.1 | Ethics approval and consent

Leipzig University's Ethical Committee approved genetic testing for Ind\_1 (224/16-ek, 402/16-ek). For Ind\_2, the study was approved by

the Technical University of Munich (#5360/12S). Written consent from both parents to publish genetic, clinical, and image data was received and archived.

### 2.2 | Genetic analyses and comparison of phenotypes

NSD2 (NM\_001042424.2) variants in the index cases were identified by trio ES and confirmed by Sanger sequencing. For retrospective phenotyping and case reviews, we used an Excel questionnaire. Supplemental notes, Data S1, provide details and analyses of the variant spectrum, 3D structure, and omics data from cancer cell lines.

## 3 | RESULTS

### 3.1 | NSD2 variant spectrum

ES detected a heterozygous de novo missense variant in NSD2 (NM\_001042424.3: c.3295G > A, p.Glu1099Lys; chr4[hg19]: g.1962801G > A) in both individuals. The constitutional status of the c.3295G > A, p.Glu1099Lys variant was confirmed in different tissues (Figure S1).

This variant had not previously been described as disease-causing when mutated in the germline and was listed once (1/251470 alleles) in gnomAD (possible mosaicism; Figure S2). As the phenotype of the individuals did not match the LoF descriptions (RAUST/WHS) the variant was classified as uncertain significance (VUS) according to the ACMG (American College of Medical Genetics and Genomics). Based on the clinical overlap between the two individuals, we upgraded to PS2\_strong, added PM1\_strong and PP2, allowing classification as “pathogenic.”

NSD2 LoF variants are dispersed throughout the protein (Figure S3A). Missense variants with a presumed LoF effect are located in functional domains but show no clustering in the tertiary structure, while the two missense variants with a proven GoF effect are close to each other and near lysine position 36 of H3 when the two proteins interact (Figure S3B).

### 3.2 | Comparing NSD2 allelic disorders

Both individuals described here showed remarkable phenotypic overlap with complications in pregnancies and prenatal/postnatal periods as dominant features, prenatally detectable organomegaly, and postnatally evident dysmorphic features characterized by coarse/square facies (Figure 1A–D,K) and large hands with tapering fingers (Figure 1E–J, Q–S; case reports in Supplementary notes).



**FIGURE 1** Clinical images. (A, B) frontal and (C, D) lateral facial images of Ind\_1. (E, F) dorsal and (G, H) palmar sides of the right hand of Ind\_1. (I, J) dorsal side of the feet of Ind\_1. Paired images are at age of 2 years 8 months and 8 years 3 months. (K) Full frontal body view of Ind\_1. (L) Hip X-ray of Ind\_2 showing almost horizontal acetabulum, widened proximal femoral metaphysis and prominent incisura ischiadica indicative of skeletal dysplasia. (M, N) abdominal MRI images of Ind\_2 showing hepatomegaly and nephromegaly. (O) Abdominal ultrasound of Ind\_2 displays kidney hypertrophy, diminished corticomedullary differentiation, irregular parenchyma, multiple small renal cysts, (P) hepatomegaly with rounded caudal margin in Ind\_2, and (Q, R) dorsal and palmar sides of the right hand of Ind\_2 showing short/ tapered fingers and hypoplastic fingernails. (S) Dorsal feet of Ind\_2 illustrating hypoplastic toenails. See Figure S5 for cranial MRI results of Ind\_1. [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

Our review found 26 individuals with (likely) pathogenic and presumed LoF variants carrying 23 variants, mostly truncating ( $n = 18$ , 78.3%) and a few missense ( $n = 5$ , 21.7%). We found varied facial dysmorphologies. GoF cases have coarse and square facial features, while LoF cases have a triangular face. LoF cases had a broad forehead with a high anterior hairline, while GoF cases had a low anterior hairline and a smaller forehead area. In both GoF cases, anteverted nares and an exaggerated cupid's bow were reported. A long philtrum was described in both GoF and 1/15 LoF cases.

In other phenotype categories, both GoF individuals and 0/12 LoF had short necks. Tapered fingers were described in both GoF cases but in only 1/18 LoF cases. Four other hand phenotypes were described in both GoF cases but were not phenotyped in the LoF cases (Table S2; compare Figure 1E–H,Q–R). Both GoF (nonspecific patent ductus arteriosus in Ind\_1) and 3/18 LoF cases had abnormal heart morphology. Abnormalities of the genitourinary system were described in both GoF cases but also in 7/23 (30.4%) of LoF cases and abnormal liver morphology was present in both GoF cases but only one LoF; assuming the other LoF cases were healthy, this is an important difference (Figure 1M–P). Both GoF cases had moderate, while most LoF cases had mild intellectual disability (73.7%; 14/19). Behavioral anomalies (47.4%; 9/19) and autistic behavior (30.4%; 7/23) were reported in LoF but not in the GoF cases. The two NSD2 GoF carriers had low-normal height (Ind\_1) or short stature (Ind\_2) and discordant head circumferences; macrocephaly in Ind\_1 and microcephaly in Ind\_2. The description of constitutional measurements is hindered by two factors: only two described individuals and postnatal intracerebral hemorrhage and growth hormone deficiency in Ind\_2. Compare Tables S1 and S2.

Our analysis of cancer cell lines showed NSD2 GoF has higher mono- (me1) and dimethylation (me2) levels at H3 lysine 36 (K36). Differential DNA methylation and RNA expression analysis identified a large fraction of dysregulated genes that can be clustered into functional modules correlating with NSD2 function (Figure S4).

## 4 | DISCUSSION

A genetic disorder can be described by the affected gene, inheritance pattern, phenotypic description of the associated disease, and pathogenic variants' effect on protein function. This differentiation helps standardize genetic diagnostics for well-characterized rare disease cohorts and was advanced through the ClinGen group framework.<sup>12</sup>

NSD2 was a candidate in the WHS region, and LoF variants are associated with a milder NDD entity (Rauch–Steindl syndrome; RAUST). The NSD2 missense variant p.Glu1099Lys has been extensively studied because of its somatic recurrence in leukemia caused by a GoF effect. Here, we report two individuals with a syndromic NDD presentation harboring this GoF variant in the germline. When comparing the described individuals with NSD2 LoF individuals, some facial features seem inverted. While GoF carriers have a square face with a low anterior hairline and small forehead, LoF carriers have a triangular face with a high hairline and large forehead. The nasal

bridge is wide and depressed in the two GoF individuals, but thin and elevated in the LoF cohort. With organomegaly and skeleton/hand/foot abnormalities, NSD2 GoF appears syndromic compared to LoF. These phenotypic differences are expected given GoF and LoF's opposing cellular effects (compare Figure S4).

While the variant in Ind\_1 was identified by clinical ES about 6 years prior to this report, its highly suspected pathogenicity remained unclear until a second case was identified. This anecdotal diagnostic odyssey, combined with no other germline descriptions of this variant, argues for a rare disorder. No other GoF variant carriers have been identified despite posting the case to Clinvar, GeneMatcher, and genetic conferences. Still, ClinVar enabled the identification of the second individual and the description of this rare entity.

Due to the rarity of NSD2 GoF, it is hard to predict the clinical outcome and recommend treatment and surveillance. Both GoF individuals required intensive care postnatally. The course of Ind\_2 was complicated by brain hypoxia, which hampers assessing his development. On the other hand, Ind\_1 shows a mild to moderate intellectual disability, which seems surprising given the strong histone methylation changes induced by the GoF variant (compare Figure S4). Similarly, while both individuals had organ abnormalities and organomegaly (kidneys, liver), laboratory findings were within normal ranges, indicating no signs of beginning organ failure. Due to the strong leukemia association of p.Glu1099Lys, the most obvious concern in the two cases remains a possibly increased blood cancer risk, despite mostly unremarkable repeated blood counts (case report in Supplementary notes). An increased cancer risk has been described in other MDEM caused by variants in NSD1, DNMT3A and SETD2.<sup>13–15</sup> A murine conditional knock-in model of p.Glu1099Lys in the B-cell lineage did not lead to the spontaneous leukemia development in immunocompetent mice.<sup>16</sup> Enrichment in AML relapses and functional studies showing induction of glucocorticoid resistance indicate that p.Glu1099Lys NSD2 GoF may cause clonal advantage and not be a primary driver.<sup>17</sup> We still recommend at least yearly blood counts, coupled with developmental assessment and abdominal organ ultrasound.

We describe a syndromic NDD entity associated with increased NSD2 activity and histone methylation disturbance. The specific phenotype, along with coarse facial features and hand abnormalities, may allow clinically supported diagnosis of a MDEM in individuals with other NSD2 variants. GoF variation may cause a distinct NSD2-related disorder due to its clinical distinguishability from syndromes associated with NSD2 LoF and an antagonistic molecular consequence. As more individuals with NSD2 GoF variants are described, a distinct name could be chosen.

## AUTHOR CONTRIBUTIONS

Bernt Popp, Melanie Brugger, Theresa Brunet, Rami Abou Jamra, and Vincent Strehlow conceived the study concept. Bernt Popp, Melanie Brugger, Matias Wagner, Tobias Bartolomeaus, Rami Abou Jamra, Theresa Brunet and Vincent Strehlow analyzed genetic data. Vincent Strehlow, Melanie Brugger and Theresa Brunet coordinated collection of clinical/genetic data. Melanie Brugger, Theresa Brunet and Bernt Popp reviewed literature data and standardized the HPO terms.



Melanie Brugger, Sibylle Poschmann, Maximilian Radtke, Nataliya Di Donato, Janina Gburek-Augustat, Elisabeth Graf, Matias Wagner, Tobias Bartolomaeus, Julia Hentschel, Thomas Meitinger, Ina Sorge, Rami Abou Jamra, Theresa Brunet, and Vincent Strehlow provided clinical/genetic data and performed clinical assessments. Bernt Popp performed structural protein analysis, analyzed all data and created all main figures. Bernt Popp, Theresa Brunet, and Melanie Brugger created the Supplementary files. Bernt Popp, Melanie Brugger, Vincent Strehlow, and Theresa Brunet wrote and edited the manuscript. All authors reviewed, commented on, and agreed on the final draft manuscript.

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## CONFLICT OF INTEREST

All authors declare no competing interest for this study.

## PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1111/cge.14241>.

## DATA AVAILABILITY STATEMENT

All data generated or analyzed can be found either at the publisher's website or has been uploaded to Zenodo at <https://zenodo.org/> (File S2, S3, S4: DOI: <https://doi.org/10.5281/ZENODO.6206868>).

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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