A NOVEL *NONO* NONSENSE VARIANT IN A FETUS WITH RENAL ABNORMALITIES

Short running title: Novel *NONO* variant in a fetus with renal abnormalities

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Key points

What is already known about this topic?

- Hemizygous pathogenic variants in the *NONO* gene are confirmed to cause a rare X-linked syndromic disorder (OMIM#300967)

- Developmental delay and intellectual disability, macrocephaly, structural abnormalities involving the corpus callosum and /or cerebellum, left ventricular

noncompaction and other congenital heart defects are commonly described among affected individuals

What does this study add?

- A novel hemizygous pathogenic variant c.355C>T, p.(Arg119Ter) [NM_007363.5]in the *NONO* gene was identified in a male fetus with renal anomalies.
- This case highlights the need to better delineate the prenatal clinical spectrum of loss-of-function variants in *NONO*.

Data availability statement: n/a

Ethics statement

Autopsy and exome sequencing were performed as a clinical service under clinical consent forms. Retrospective collection of data from patient records was granted by a waiver of informed consent. All clinical data in this report has been anonymized.

ABSTRACT

At 16+6-weeks a fetal scan performed in the second pregnancy of a 42 y.o. woman identified a right multicystic dysplastic kidney, left renal agenesis, absent urinary bladder, myocardial hypertrophy, increased nuchal fold, a single umbilical artery, and oligohydramnios. Trio exome sequencing analysis detected a novel pathogenic *NONO* variant. Postmortem examination after termination of pregnancy confirmed the ultrasound findings and also revealed pulmonary hypoplasia, retrognathia and low-set ears. The variant was a novel *de novo* hemizygous pathogenic loss-of-function variant in *NONO* [NM_007363.5], associated with a rare X-linked recessive neurodevelopmental disorder, named intellectual developmental disorder, X-linked syndromic 34 (OMIM#300967). The postnatal characteristic features of this disorder include intellectual disability, developmental delay, macrocephaly, structural abnormalities involving the corpus callosum and /or cerebellum, left ventricular noncompaction and other congenital heart defects. In the prenatal setting, the phenotype has been poorly described, with all described cases presenting with heart defects.

71 This case highlights the need of further clinical delineation to include renal abnormalities in
72 the prenatal phenotype spectrum.
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1. REPORT

FETAL PHENOTYPE

A 42-y.o. gravida 1 para 1, with no relevant personal or family medical history and no exposure to any known teratogen, had an ultrasound examination at 16+6 gestational weeks that revealed a male fetus with a right multicystic dysplastic kidney, left renal agenesis, absent urinary bladder, oligohydramnios, myocardial hypertrophy, increased nuchal fold (7.3 mm) and a single umbilical artery (Figure 1A and 1B, Table 1A). Amniocentesis was offered and chromosomal microarray analysis (CMA) was performed using a genome-wide oligonucleotide array (qChipPrenatal microarray (qGenomics, Spain) based on an Agilent 8×60 K format). The results revealed a normal male profile, arr(XY)x1,(1-22)x2.

DIAGOSTIC METHOD

Trio exome sequencing was performed using DNA Prep with Enrichment (Exome capture, Illumina, San Diego, CA, USA) on a NextSeq 500 sequencer (Illumina, San Diego, CA, USA), with a targeted mean coverage of 100× and a minimum of 90% of bases sequenced to at least 20×. Bioinformatic analysis consisted of alignment to the reference human genome (hg38) using BWA MEM (v0.7.17) and Bowtie2 (v2.4.1) short-read aligners, genotyping using Haplotype Caller from Genome Analysis Toolkit (v.4.2) and VarDict (v1.7.0) variant callers, and annotation using Ensembl Variant Effect Predictor (v104). Variant interpretation and classification were performed according to the ACMG recommendations (Richards et al., 2015).

DIAGNOSTIC RESULTS AND INTERPRETATION

A *de novo* hemizygous nonsense variant, c.355C>T, p.(Arg119Ter), in *NONO* [NM_007363.5] was identified in the proband (Table 1B). The variant is novel, absent in the population databases (gnomAD, 1000G) and is consistent with a loss-of-function mechanism.

PREGNANCY OUTCOME AND POSTNATAL FINDINGS

Based on ultrasound findings, the parents opted for an elective pregnancy termination at 17 weeks. Postmortem examination showed a male fetus with frontal bossing, low-set ears, micrognathia with retrognathia, and nuchal edema 7 mm in thickness (Figure 1C). Left renal fossa was empty with a discoid and verticalized left adrenal gland and single umbilical artery. Right kidney was large (4.9 g, expected weight of renal tissue at 17 weeks gestation < 2 g), with multicystic dysplasia, connected to an empty urinary bladder in the lesser pelvis through a thin ureter (Figure 1D). Lungs were hypoplastic (Potter sequence).

2. DISCUSSION

The *NONO* (non-POU domain containing octamer-binding; OMIM*300084) gene is located on chromosome Xq13.1 and encodes a protein that belongs to the highly conserved Drosphila behavior/human splicing (DBHS) protein family which are nuclear proteins involved in various aspects of RNA metabolism (Mircsof et al., 2015). Loss-of-function variants in *NONO* have been associated with an X-linked recessive neurodevelopmental disorder characterized by developmental delay and intellectual disability, dysmorphic features, and central nervous system abnormalities typically involving the corpus callosum. Left ventricular noncompaction and other congenital heart defects are commonly described among affected individuals (Carlston et al., 2019; reviewed in Roessler et al., 2023). The disorder is named intellectual developmental disorder, X-linked syndromic 34 (OMIM#300967).

Several prenatal cases have been reported, all of them characterized by cardiac defects (Sun et al., 2020; Sewani et al., 2020). However, due to the scarcity of the clinical reports and high risk of gestational loss, the prenatal clinical findings are still uncertain. Similarly to ours, the case reported by Sewani and colleagues (2020) presented with renal agenesis, retrognathia and low-set ears as additional clinical findings. Although it can be argued that the low position of the ears could be physiological and that some of the dysmorphic features might be secondary to the renal agenesis and associated with oligohydramnios sequence, the

involvement of renal abnormalities in the clinical core phenotype of this emerging disorder should be reconsidered.

The present case expands the prenatal phenotype associated with *NONO* loss-of-function variants and in addition to cardiac defects, renal anomalies should be considered to be included in its phenotypic spectrum.

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FIGURE LEGEND

Figure 1. Prenatal ultrasound images of the present case at 16+6 weeks showing: A) right multicystic dysplastic kidney and B) increased nuchal fold. C) Postmortem images showing frontal bossing, microretrognathia, low-set ears, and nuchal edema. D) Renal multicystic dysplasia: Anomalous renal differentiation with scarce glomeruli and normal looking renal tubules with dilated (cystic) structures surrounded by a variably cellular stroma that accumulates around immature tubular structures. (H&E, original magnification 2x).

Table 1 A. Clinical data

Case	Parental details	Gestation at diagnosis	Phenotypes (HPO terms)	Obstetric history	Family history	Outcome
1	Maternal Age 42 y-o. Ethnicity Caucasian Paternal Age 43y-o. Ethnicity Caucasian	16+6 weeks	Multicystic kidney dysplasia (HP:0000003)Unilateral renal agenesis (HP:0000122) Abnormality of the myocardium (HPO:0001637) Oligohydramnios (HP:0001562) Single umbilical artery (HP:0001195) Increased nuchal translucency (HP:0010880)	G1 P1; 1 previous normal vaginal delivery of female healthy child	Unremarkable	Termination of pregnancy

Table 1 B. Genetic Findings

Procedure (gest age)	Direct/ culture?	Performed Test	Secondary confirmatory test	Gene (name; REFSEQ)	Known disease (OMIM)	Variant	ACMG classify-cation	Criteria applied	Inheritance & zygosity	Interpretation
17	Direct	Trio exome sequencing	n.d.	*NONO* NM_007363.5	Intellectual developmental disorder, X-linked syndromic 34 (#300967)	c.355C>T, p.Arg119Ter	Pathogenic (class 5)	PVS1 PM2 PS2	XL hemizygous	Pathogenic

Abbreviations: ACMG, American College of Medical Genetics and Genomics; OMIM, Online Mendelian Inheritance in Man; REFSEQ, the Reference Sequence database.