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Molecular Case Studies

RESEARCH REPORT

Neonatal diabetes mellitus due to a novel variant in the *INS* gene

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Abstract Neonatal diabetes mellitus (NDM) is a rare condition that presents with diabetes in the first few months of life. The treatment of NDM may differ depending on the genetic etiology, with numerous studies showing the benefit of sulfonylurea therapy in cases caused by mutations in *KCNJ11* or *ABCC8*. Mutations in the insulin gene (*INS*) have also been identified as causes of NDM; these cases are generally best treated with insulin alone. We report a case of a female infant born small for gestational age (SGA) at late preterm diagnosed with NDM at 7 wk of life who was found by rapid whole-genome sequencing to harbor a novel de novo c.26C>G (p.Pro9Arg) variant in the *INS* gene. She presented with diabetic ketoacidosis, which responded to insulin therapy. She did not respond to empiric trial of sulfonylurea therapy early in her hospital course, and it was discontinued once a genetic diagnosis was made. Early genetic evaluation in patients presenting with NDM is essential to optimize therapeutic decision-making.

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INTRODUCTION

Neonatal diabetes mellitus (NDM) is a rare disorder that is defined by the onset of diabetes before age 6 mo. Its incidence has been estimated at 1 in every 90,000–400,000 births (Polak and Cave 2007; Iafusco and Massa 2012). Infants typically present with small size for gestational age (SGA), dehydration, failure to thrive, and hyperglycemia with or without ketonuria or ketoacidosis. NDM can be either permanent or transient and is likely to have an underlying genetic cause. The most common genetic etiologies of NDM in children born to nonconsanguineous parents include mutations in *KCNJ11* or *ABCC8*, the genes encoding the potassium ATP-sensitive channel subunits Kir6.2 and SUR1, respectively; mutations involving the insulin (*INS*) gene; or methylation abnormalities of Chromosome 6q24 (De Franco et al. 2015). Both autosomal recessive and autosomal dominant *INS* mutations have been described. Patients with defects in *KCNJ11* or *ABCC8* can often be managed well with sulfonylurea therapy (Babenko et al. 2006; Pearson et al. 2006). In cases involving other genetic mutations, insulin remains the mainstay of treatment. Here, we present a case of NDM caused by a de novo variant in the *INS* gene that highlights the utility of rapid whole-genome sequencing (WGS) in the clinical care of NDM.

CASE PRESENTATION

The patient is a female born at 36 wk and 5 d gestation via Cesarean section because of placenta previa. The history was notable for classification of the infant as SGA by weight (birth weight of 2.01 kg, <1st percentile) and for report of enlarged kidneys on prenatal ultrasound. The postnatal history included a 4-d NICU stay, with initial respiratory distress (resolved by discharge) and sepsis evaluation; note was also made of transient hyperglycemia presumed due to an exaggerated stress response, which also resolved. In the outpatient setting, she was followed by her pediatrician for poor weight gain. She subsequently presented at the age of 7 wk to an urgent care facility with tachypnea and a single episode of emesis. She was lethargic, tachycardic, dehydrated, and in obvious respiratory distress. She was transferred to the pediatric emergency department for further care.

On arrival to our facility, the patient was again noted to be in respiratory distress, and her weight was again noted to be low (2.9 kg, <1st percentile for age). Laboratory studies showed a high anion gap of 22 mmol/L (reference range 5–20 mmol/L), metabolic acidosis (venous pH 7.096 [reference range 7.31–7.41]), serum bicarbonate (6 mmol/L [reference range 18–27 mmol/L]), and profound hyperglycemia (initial serum glucose 734 mg/dL [40.7 mmol/L], reference range 60–110 mg/dL [3.3–6.1 mmol/L]). Isotonic fluid resuscitation was initiated with a total of 30 mL/kg of normal saline, and once serum blood glucose decreased below 600 mg/dL (33.3 mmol/L), a regular insulin infusion was begun at 0.05 units/kg/h and soon after was increased to 0.1 units/kg/h. The infant was admitted to the pediatric intensive care unit (PICU).

During the early portion of her PICU stay, blood glucose was monitored hourly and venous blood gas every 2 h per protocol. Maintenance i.v. fluids were provided, with the addition of dextrose as appropriate with decreasing blood glucose. Ketoacidosis was deemed resolved within ~8 h of admission, at which time feeding with breast milk was allowed every 3 h and subcutaneous insulin started. The initial regimen included low doses of glargine insulin and correctional dilute lispro insulin, the doses of which required frequent adjustment because of widely variable blood glucose values. Additional laboratory results from the PICU stay included a hemoglobin A1c (HbA1c) of 6.3% (normal range 4.2%–5.6%), very low C-peptide (0.16 ng/mL, reference range 0.8–3.1 ng/mL), and low insulin level (1 mU/mL, reference range <17 mU/mL), which were inappropriately low for her hyperglycemic state, and negative insulin autoantibody (titer <0.4 U/mL). She was nominated for rapid WGS by our facility's genomics institute while in the PICU on hospital day 5. She was able to be transferred out of the PICU by hospital day 6, and a sample for WGS was collected that day.

Over the next few days, she continued to have widely variable blood glucose levels despite adjustments in dosing and timing of both glargine and dilute lispro insulin. With the knowledge that the majority of neonatal diabetes is caused by mutations in either *ABCC8* or *KCNJ11* that may be sulfonylurea-responsive, she was empirically started on twice-daily glyburide on day 7 of admission, which did not improve her hyperglycemia. On hospital day 8, genomic test results were available and showed a de novo heterozygous c.26C>G (p.Pro9Arg) variant in the *INS* gene (Table 1).

Table 1. Variant table

Gene	Chromosome	HGVS DNA reference	HGVS protein reference	Variant Type	Predicted effect (substitution, deletion, etc.)	dbSNP/ dbVar ID	Genotype (heterozygous/ homozygous)	ClinVarID
INS	11:2182176 (NM_001185098.1)	c.26C>G	p.Pro9Arg	Missense	Likely pathogenic	None	Heterozygous	SCV000898466.1

The p.Pro9Arg variant in *INS*, which has not been previously reported in the literature, is absent from the Exome Aggregation Consortium (ExAC) and Genome Aggregation (gnomAD) population databases. It involves a highly conserved amino acid (phyloP-Vertebrate: 3.35) and is predicted by multiple in silico tools to have a deleterious effect on protein function (MutationTaster: 0.991; SIFT: 0.006; PolyPhen-2: 0.917). Analysis of the parental samples was negative for the c.26C>G (p.Pro9Arg) variant, indicating that it likely occurred as a de novo event in the patient. Therefore, the variant was classified as likely pathogenic. Glyburide was stopped within 36 h after it was started based on the molecular diagnosis, which indicated that sulfonylurea therapy would not be an effective adjunct.

In an effort to lessen glycemic variability and decrease the frequency of correctional dilute lispro insulin injections in an infant with multiple daily feeds, intermediate-acting insulin (NPH) was added and insulin glargine was discontinued, allowing the family to give lispro correctional injections less frequently. With dose titration over the next few days, blood glucose values were generally within the acceptable range, although some variability continued. She was able to be discharged on hospital day 12 on a regimen of NPH 1.5 units every 8 h (1.55 units/kg/d for admission weight of 2.9 kg) and dilute lispro given for hyperglycemia as needed. Recognizing her increased risk for hypoglycemia and glycemic variability because of her very young age and small size, we applied for a continuous glucose sensor (DexCom) during her hospital stay, which the family received within 2 wk of hospital discharge. The family remained in close communication with the endocrinology team following hospital discharge for review of blood glucose values and insulin adjustments as appropriate. At follow-up at age 5 mo, her HbA1c was 6.8% and weight had improved (weight for length at the 93rd percentile). Her regimen included twice-daily NPH insulin and twice-daily short-acting insulin (total of 0.46 units/kg/d) for carbohydrate coverage, with correctional insulin given in between those times as needed. At follow-up at age 9 mo, her HbA1c was 7% and weight for length was at the 97th percentile. Her regimen was adjusted to discontinue NPH insulin and reintroduce once-daily insulin glargine along with short-acting insulin with all meals in a typical basal-bolus regimen (total of 0.77 units/kg/d).

DISCUSSION

There is significant genetic heterogeneity in NDM, an entity that may be transient or permanent. In permanent neonatal diabetes (PNDM), the most common genetic mutations seen are in *KCNJ11* and *ABCC8*, accounting for close to 50% of PNDM; *INS* gene mutations account for ~20% of PNDM, with mutations in other genes (such as *GCK* and *PDX1*) occurring less commonly (De León et al. 2008). Both missense and loss-of-function variants in the *INS* gene have been described in individuals with PNDM (Edghill et al. 2008; Liu et al. 2015), with different mutation types affecting different steps in insulin biosynthesis and function but all producing the phenotype of PNDM. The *INS* gene is translated into the precursor preproinsulin, which undergoes removal of its signal peptide to generate proinsulin, after which further intracellular modifications including excision of C-peptide result in the mature form of insulin (Støy 2014). The novel p.Pro9Arg missense variant detected in our patient occurs in the signal peptide of the preproinsulin molecule, in an amino acid residue that is highly conserved.

Although it could be hypothesized that the p.Pro9Arg variant disrupts insulin biosynthesis very early in the process, given the heterozygous nature of the mutation in this case, it is difficult to attribute development of overt, early-onset diabetes to insulin haploinsufficiency alone. Indeed, the molecular mechanisms underlying the development of PNDM in the setting of heterozygous *INS* mutations are not yet completely understood. Increased endoplasmic reticulum (ER) stress, with or without increased β-cell apoptosis, have been implicated (Oyadomari et al. 2002; Balboa et al. 2018). Recently, Balboa et al. (2018) reported that

iPSC-derived INS-mutant β-like cells transplanted into mice increased ER stress, led to defective β-cell mass expansion, and reduced insulin secretion. Liu et al. (2010, 2015), in reviewing the diversity of the *MIDY* (mutant *INS*-gene-induced diabetes of youth) phenotype, describe the hypothesis of "gain of toxic function," which is felt to be heavily implicated in the development of diabetes and which may be initiated by abnormal interactions between mutant and wild-type proinsulin molecules in the ER of β cells.

Beyond providing information useful in understanding the pathogenesis of NDM in a case such as this, results of rapid genetic sequencing can be key in guiding management. In particular, it is well-known that NDM resulting from mutations in *KCNJ11* and *ABCC8* may respond well to sulfonylurea (SU) therapy. In general, it is felt that SU therapy is ineffective in cases of NDM due to *INS* gene mutations. In our case, SU therapy was empirically started while genetic testing results were pending. When these became available in the span of just 2 d, therapy was able to be appropriately tailored to the patient, as it was evident that SU therapy would be ineffective based on the mechanism of disease. Moreover, although empiric therapy while awaiting genetic results is not uncommon, one case of insulin resistance emerging after addition of SU therapy in a patient with transient NDM due to a mutation in the promoter region of *INS* has been described (Yildiz et al. 2018). Although adverse effects with adjunctive SU therapy have not been commonly described, and although there may be significant genetic and clinical differences between patients with transient or permanent NDM, we feel that whenever possible it is important to prioritize ancillary genetic testing that may substantially impact management. Although other testing methods (e.g., targeted panel testing, whole-exome sequencing) would also have provided an accurate diagnosis in this case, WGS performed at our facility has a shorter turnaround time than most commercially available gene panel and exome sequencing tests. In fragile patients requiring intensive care, rapid diagnosis is crucial for timely, specific treatment, which results in improved outcomes and decreased length of stay (Farnaes et al. 2018).

A clinical feature of interest in this case was the diagnosis of bilateral kidney enlargement made on prenatal ultrasound. Her postnatal course has been notable for ultrasound during admission for DKA showing right kidney length of 5.5 cm and left kidney length 6.2 cm (both high-normal in size [Konuş et al. 1998]) as well as moderate left hydronephrosis, SFU (Society for Fetal Urology) Grade 3. Nephromegaly and/or hydronephrosis are not commonly described in individuals with neonatal diabetes in the available literature; we speculate that this could be related to renal hyperfiltration due to hyperglycemia and polyuria in utero.

An additional clinical feature worth noting in this case includes the HbA1c of 6.3% at diagnosis; alternative estimates of glycemic control, such as fructosamine or glycated albumin, were not measured in our patient. It has been described (Suzuki and Koga 2014; Wirth et al. 2018) that despite the widespread clinical use of HbA1c to estimate glycemic control in diabetes mellitus and because of the persistence of high levels of fetal hemoglobin in neonates, HbA1c is not a reliable measure of glycemic control in NDM. We could have measured fructosamine if the diagnosis of diabetes was in doubt and/or performed hemoglobin subtype analysis to correct the HbA1c (Suzuki and Koga 2014), but there was no clinical justification for the additional testing in light of the clinical presentation and significant ketoacidosis.

When it comes to method of insulin delivery, multiple different regimens of subcutaneous insulin therapy by injection were utilized in this case (including different combinations of long-, intermediate-, and short-acting insulin). Early on, a combination of intermediate- and short-acting insulin (with transition to typical basal-bolus regimen later in infancy) was effective in avoiding hypoglycemia and reducing (although certainly not eliminating) glycemic variability; early availability of a continuous glucose sensor was crucial in allowing for frequent adjustments to the patient's insulin regimen. The successful use of insulin pump therapy (continuous subcutaneous insulin infusion [CSII]) has been well described in cases of NDM (Tubiana-Tufi

2007), even being introduced during initial hospital stay in a case of transient NDM (Park et al. 2013). Challenges common to both injection and pump therapy include frequent feedings, limited subcutaneous fat stores, and need for minute doses of insulin in patients with NDM. CSII was discussed with the family, and they felt they would prefer to continue with multiple daily injections for the time being, but strong consideration is being given to introducing CSII in the near future, particularly as her size and total daily insulin dose increase.

In summary, we present a case of neonatal diabetes due to a heterozygous de novo *INS* variant c.26C>G (p.Pro9Arg), a variant affecting the signal peptide of preproinsulin that has not previously been described in cases of NDM due to *INS* mutations. The development of diabetes in heterozygous *INS* mutations has been linked to a potential gain-of-toxic-function mechanism as well as to increased ER stress in the setting of (pre)proinsulin misfolding. From the clinical standpoint, this case highlights the utility of genetic testing with rapid turnaround time in therapeutic decision-making. Practical challenges remain in treating our very youngest diabetic patients to achieve glycemic targets without hypoglycemia, which may be mitigated by immediate and widespread availability of continuous glucose sensors to such patients.

METHODS

Consent for rapid WGS was obtained for the proband and her parents on hospital day 5. Following DNA extraction from whole blood, sequencing libraries were constructed using the TruSeqDNA PCR-Free Library Prep kit according to the manufacturer's instructions. Paired-end sequencing (2 × 100 bp) was performed with Illumina chemistry on a NovaSeq 6000 to a mean depth of coverage of greater than 40× for the patient and greater than 30× for parental samples (Table 2). The Edico Dragen processor (Illumina) was used for rapid alignment and nucleotide variant calling. Variant analysis and interpretation were performed using the Opal Clinical Interface (Fabric Genomics), which employs proprietary algorithms to prioritize candidate variants by integrating phenotypic and genomic data. Variants were filtered to retain those with allele frequencies of <0.5% in multiple population databases including gnomAD, Exome Variant Server, 1000 Genomes, and ExAC. A custom gene panel was then created in Phenolyzer (Yang et al. 2015) and used to prioritize review of variants detected in genes known to be associated with hyperglycemia (HPO: 0003074). Manual curation was performed and the c.26C>G (p.Pro9Arg) variant in the *INS* gene was classified as likely pathogenic (one strong, one moderate, and one supporting criteria) according to the ACMG/AMP guidelines for the interpretation of sequence variants (Richards et al. 2015). This variant was orthogonally confirmed by Sanger sequencing using the following custom designed forward (F) and reverse (R) primers: F: TTAAAAAAGTGCACCTGACCC CCT and R: TCCAAGGGCCTTTGCGTCAG. Sanger sequencing confirmed the variant was heterozygous in the patient and negative in the parental samples.

ADDITIONAL INFORMATION

Data Deposition and Access

The causative variant has been deposited at ClinVar (https://www.ncbi.nih.nlm.gov/clinvar/) with accession number SCV000898466.1. All data associated with this study are present in the paper or are available at the Longitudinal Pediatric Data Resource under a material transfer agreement or data use agreement, as appropriate, and subject to the limitations of the informed consent documents for each subject (Accession Number nbs000003.v1.p; https://www.nbstrn.org/research-tools/longitudinal-pediatric-data-resource).

Table 2. Sequencing coverage

Trio	R18AA922	R18AA923	R18AA924	Units
Date sample was run	Jun 4 2018	Jun 1 2018	Jun 1 2018	
Sex	F	F (mother)	M (father)	
Yield: raw/bulk	179.5	116.2	125.4	
% mapped	98.50%	98.50%	98.20%	%
% duplicates	7.90%	7.10%	8.10%	%
Yield	176.8	114.4	123.1	Gbp
Insert size: mean	423.6	413.1	407.3	bp
Average and median coverage across genome	49.6	32.3	34.3	×
Average coverage over OMIM genes	46.7	31.0	32.6	×
# of OMIM genes with coverage at <10×	314	703	977	
# of OMIM genes with 100% coverage at ≥10×	97.8%	95.0%	93.1%	%
# of OMIM genes with 100% coverage at ≥20×	90.0%	21.2%	35.0%	%
# of OMIM genes with 100% coverage at ≥30×	37.3%	2.3%	2.7%	%
# of genes with 100% coverage at ≥40×	4.0%	0.5%	0.9%	
Variation (VCF) metrics				
# of calls total	4,968,231	4,962,138	4,837,935	
# of PASS calls	4,887,184	4,903,387	4,775,895	
# of calls total coding	24,742	24,702	24,319	
Total # of SNVs	3,969,838	4,008,954	3,897,489	
Total # of Indels	917,346	894,433	878,406	
Hom/Het ratio (in coding regions)	0.55	0.54	0.60	ratio
Ti/Tv ratio (in coding regions)	1.96	1.97	1.97	ratio
# of het calls (# of hom call)	3,186,900 (1,781,331)	3,208,560 (1,753,578)	3,007,910 (1,830,025)	units

Ethics Statement

Written research consent obtained from patient's parents. Molecular genetic testing was performed under an IRB-approved research protocol with Dr. Stephen Kingsmore as the PI: "Prenatal Precision Medicine (NSIGHT2): A Randomized, Blinded, Prospective Study of the Clinical Utility of Rapid Genomic Sequencing for Infants in the Acute-care Setting," ClinicalTrials.gov ID: NCT03211039.

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Competing Interest Statement

The authors have declared no competing interest.

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Author Contributions

S.E.L. was the primary author and was involved in the patient's clinical care. C M., L.N., and D.S. were involved in the patient's clinical care and in writing and review of the manuscript. M.S.W., M.T., D.D., and S.F.K. contributed the genetic testing and interpretation and review of the manuscript. N.G.C. and R.S.N. were involved in the patient's clinical care and review and editing of the manuscript.

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