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Case Report

Permanent Neonatal Diabetes with High Insulin Requirements Due to a New Variant in the *INS* Gene

Hernández et al. Permanent Neonatal Diabetes Due to New Variant in the *INS* Gene

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What is already known on this topic?
The three most frequent pathogenic variants that cause permanent neonatal diabetes involve the ABCC8, KCNJ11, and INS genes. The latter is responsible for 10-20% of cases and results in variable clinical behavior, associated with intrauterine growth restriction, MODY-type diabetes, and permanent or transient neonatal diabetes.

What this study adds?
This study reports a novel pathogenic variant within the INS gene, not documented in databases. Unlike ABCC8 and KCNJ11 variant, INS variant does not respond to sulfonylurea treatment and requires insulin for glycemic control, posing a challenge in breastfeeding patients. It highlights the need for a clinical approach supported by early molecular diagnosis.

ABSTRACT
Neonatal diabetes is an infrequent disorder that may present as transient, permanent, or syndromic. It is most commonly caused by pathogenic variants involving the ABCC8, KCNJ11, and INS genes. To describe a neonate with permanent diabetes mellitus due to a previously unreported variant in the INS gene, outlining the diagnostic complexities, therapeutic interventions, and related clinical challenges. Neonate with symmetrical intrauterine growth restriction, who presented severe hyperglycemia not associated with ketosis or infectious. He had high insulin requirements and did not respond to sulfonylurea management. Anti-insulin and anti-islet pancreatic antibodies were negative. Genetic sequencing revealed a homozygous missense variant (c.3G>A, p.Met1Ile) in the INS gene, which had not been previously reported in the literature.
Timely molecular diagnosis of neonatal diabetes enables optimization of management strategies, mitigating the long-term impact on growth, neurodevelopment, and the occurrence of hypoglycemic episodes.
Keywords: Neonatal Diabetes Mellitus; Newborn; Insulin Gene.

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Introduction
Neonatal diabetes mellitus (NDM) is a rare genetic condition, with a prevalence ranging from 1/21,000 to 1/300,000 live births, varying by geographical location (1–3). In Europe, the prevalence is estimated to be between 1/90,000 to 1/300,000 live births (3). In regions with high consanguinity, such as Anatolia (the southeastern region of Turkey) and the Middle East, the prevalence may increase to between 1/21,000 and 1/48,000 live births (1). The onset of NDM typically occurs within the first six months of life, although cases with later presentation, between 9 to 12 months, have been documented.
The diagnosis of NDM should be suspected when plasma glucose levels exceed 150-250 mg/dL, particularly after excluding other potential causes of hyperglycemia, including sepsis, low birth weight, or prematurity-associated complications, and medications such as phenytoin, glucocorticoids, ionotropic or high dextrose infusions (4–6). Autoimmune diabetes should also be ruled out through negative antibodies testing against glutamic acid decarboxylase (GAD), insulin, zinc transporter, and tyrosine phosphatase. A key biochemical feature of NDM is reduced levels of basal insulin levels and C-peptide (4,5).
To date, over 40 genes have been implicated in the pathogenesis of NDM, with different inheritance patterns. These genes affect insulin synthesis, action, and secretion by altering beta cell development (aplasia and pancreatic hypoplasia), increasing beta cell destruction by apoptosis or protein misfolding with consequent endoplasmic reticulum stress due to retained proteins, and altering beta cell membrane depolarization leading to failure in the extrusion of synthesized insulin into the circulation (3). The three most frequently involved genes are ABCC8, KCNJ11, and INS. The latter is located on chromosome 11p15.5, is responsible for 6.7 to 18% of cases, and results in variable clinical behavior associated with intrauterine growth restriction and MODY-type diabetes (1,7).
This report presents a case of neonatal diabetes due to a novel pathogenic variant in the INS gene, with strikingly high insulin requirements. We aim to emphasize the role of molecular diagnostics in establishing timely and effective management.

Case Report
A male neonate, aged two days, was admitted to the neonatal unit. The patient's mother, aged 18, was experiencing her first pregnancy. The baby was delivered via cesarean section at 35 weeks gestation, with a birth weight of 1,310 grams, length of 44 cm, and a head circumference of 29 cm (length-for-age Z score -1.05, weight-for-age Z score -2.71, and head circumference-for-age Z score -2.27 according to the Intergrowth 21 standards). There was no familial history of consanguinity.
The neonate initially demonstrated adequate adaptation but developed clinical deterioration within the first two days of life, which was characterized by severe anemia necessitating transfusion, intermittent sinus bradycardia with a normal echocardiogram, and persistent hyperglycemia with blood glucose levels reaching 574 mg/dl. After the exclusion of infectious etiologies, familial medical history, and medication-induced hyperglycemia, a diagnosis of neonatal diabetes mellitus was considered.
The patient was initially managed with an intravenous insulin infusion at a rate of 0.07 U/kg/h. Subsequently, insulin Detemir was introduced at a maximum dose of 0.8 U/kg/every 12 hours, in conjunction with insulin Aspart administered in a flexible scheme. Due to the high prevalence of ABCC8 and KCNJ11 mutations in NDM, a therapeutic trial with sulfonylurea was conducted, but this yielded no improvement in glycaemic control. Following sufficient

weight gain, Detemir was replaced with insulin Glargina, and the patient continued with preprandial insulin Aspart, necessitating a progressive dose increase. He was discharged from the neonatal unit at three months of age, requiring 1.7 U/kg/day of insulin.
At the 18-month follow-up, the child's height-for-age was -1.38 SD, weight-for-height was -0.59 SD, and the glycated hemoglobin level was 4.3%. The insulin requirement had decreased to 0.7 U/kg/day. No C-peptide levels were obtained. Antibody testing for anti-insulin antibodies by EIA (0.8 U/m) and anti-pancreatic islet antibodies (0.5 U/m) was negative. Genetic testing confirmed the presence of a novel homozygous missense variant (c.3G>A, p.Met1Ile) in the INS gene (NM_00101042376.3) with clinical significance Pathogenic. (Reference rs397515521).

Discussion

Neonatal diabetes represents a heterogeneous group of monogenic disorders, with diverse clinical manifestations (3). Its symptoms are nonspecific and include tachypnea, lethargy, irritability, dehydration, failure to thrive, polyuria, convulsions, or hypotonia (8). Biochemical alterations that suggest the diagnosis include glycosuria, ketonuria, and hyperketonemia (4). The diagnosis in this patient was suspected when persistent hyperglycemia and large amounts of insulin requirements were noted. As part of the study, antibodies against pancreatic islets were requested; unfortunately, we could only obtain islet antibodies ICA (against cytoplasmic proteins in the beta cell) and anti-insulin, which results were negative.
The three main genes involved in permanent neonatal diabetes are KCNJ11, ABCC8, and INS, all located on chromosome 11. Pathogenic variants in the first two genes are the most frequent, accounting for 30 to 50% of the cases, and are responsible for encoding the subunits of the ATP-sensitive potassium channels of the beta cell (3,7,9). Pathogenic variants in the INS gene are mostly de novo, and their diagnosis is usually made before six months of age (7). However, some publications, such as that of Ngoc et al., report later ages of diagnosis of 9.7±1.9 months in up to 30% of cases (7).
Heterozygous variants in the INS missense gene have been associated with misfolding of the proinsulin molecule and consequently altered final insulin synthesis; these variants usually appear *de novo* in 80% of cases (3). Homozygous recessive pathogenic variants in this gene, as in our case, can impair insulin biosynthesis through mechanisms such as reduced mRNA stability, misfolding of proinsulin, and defective protein processing, leading to endoplasmic reticulum stress and β-cell apoptosis (2,3,7,8,10). Ngoc et al. report missense variants in exons 2, 3, and intronic region 2 (7). In this case, the location of the variant is in exon 2. It has been reported in silico studies that the methionine residue at this position is at the start of protein translation, which is highly conserved between species and, therefore, supports its pathogenicity.
Other genes less frequently associated with neonatal diabetes are SLC2A2, SLC19A2, EIF2AK3, GCK (Glucokinase), IPF1 (Insulin Promoting Factor), PTF1A (Pancreatic Transcription Factor Subunit 1 Alpha), HNF1B (Hepatocyte Nuclear Factor Homebox 1B), FOXP3 (Forkhead Box P3), ZFP57 (Zinc Finger Protein 57), GLIS3 and GATA6; these should be considered in cases with high suspicion of neonatal diabetes with negative genetic studies for KCNJ11, ABCC8 and INS (2,5,11).
Early genetic study is recommended when hyperglycemia persists longer than 2-3 weeks of life or when serum glucose levels greater than 1000 mg/dl are present without an apparent cause (5). In the present case, having presented hyperglycemia peaks higher than 500 mg/dl for more than two months of duration and high insulin requirements of up to 1.7 IU/kg/day made him a candidate for early molecular study; however, his result was only available at around nine months of age.
Molecular testing by NGS (Next Generation Sequence) or MS-MLPA (methylation-specific multiplex ligation-dependent probe amplification) can provide a timely diagnosis to guide management and define prognosis (8,12). In this patient, since the results of the genetic panel were not available, a management test with sulfonylurea was performed, considering that the most frequent cause of transient and permanent neonatal diabetes is the involvement of the KCNJ11 and ABCC8 genes (3,12–14). The lack of response to treatment with oral medication suggested a different genetic etiology, which was corroborated by molecular testing.
Case series of patients with pathogenic variants for the INS gene report a higher prevalence of intrauterine growth restriction (IUGR). This finding was corroborated in this child by anthropometric data at birth. IUGR is a common finding and is explained by in-utero insulin deficiency, which is directly related to prenatal growth (15–18). In the long term, they may present a risk of low height, as is the current case, or low height, for which it is important to optimize glycemic control (19).
Glycemic control and insulin management are a challenge in neonatal diabetes. The fact that newborns have an irregular amount and frequency of food intake makes glycemic control less stable. The use of continuous glucose monitoring has been described as a method to guide insulin management and maintain blood glucose values within the normal range for a longer time (20). In clinical studies, continuous glucose monitoring has been described as safe, although with some technical limitations due to the small subcutaneous area available for sensor application in newborns and infants, especially those with low weight or low-fat tissue, as well as increased risk of infection at the application site and local skin reactions that can be minimized by rotating the sensor sites (19,21). Despite being a useful tool, its use in children under two years of age is not authorized in our country, which limits this patient's access to this technology. It would have been particularly beneficial, as the patient may have experienced inadvertent hypoglycemia suggested by low glycated hemoglobin levels that could not be detected by the blood glucometer used in this case.
Finally, it is recommended in neonates to start with preprandial short-acting subcutaneous insulin at doses of 0.1-0.15 IU/kg/dose or guided by the response to insulin infusion (21). In this case, it is striking the high doses of insulin required during the first months of life (up to 1.77 U/kg/d), but with a progressive decrease in the requirements, reaching a dose of 0.7 U/kg/d at 18 months of age. Studies such as the one carried out in the Vietnamese population report insulin requirements at not-so-high doses, with the highest insulin need reported at 1.1 U/kg/d, and this may be due precisely to genetic variability and clinical heterogeneity (7).

Conclusion

Neonatal diabetes is a rare condition with transient, permanent, and syndromic presentations.
This case underscores the necessity of early molecular diagnostics, which can inform personalized therapeutic strategies and improve long-term outcomes. In resource-limited settings, increased access to genetic testing may uncover previously unidentified mutations, contributing to global genetic databases and advancing our understanding of this rare condition. In this case, the novel homozygous variant in the INS gene highlights the complexity of NDM and the importance of ongoing research to refine treatment protocols and improve quality of life for affected individuals.

Statements

The authors declare that they have no conflict of interest. Informed consent was obtained from the parents to authorize the publication of this case. It was submitted for review by the ethics committee of the medical school of the Universidad de Antioquia, Medellín, Colombia.

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