Transient neonatal diabetes due to a missense mutation (E227K) in the gene encoding the ATP-sensitive potassium channel (KCNJ11)

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Key Clinical Message
Neonatal diabetes is a monogenic form of diabetes. Herein, we report on a newborn presenting diabetic ketoacidosis at 17 days of life. A KCNJ11 mutation was identified. In such cases, insulin can be replaced by sulfonylurea with a successful metabolic control, as an example of how molecular diagnosis may influence the clinical management of the disorder.

Keywords
Insulin, K<sub>ATP</sub> sensitive channels, KCNJ11 gene, neonatal diabetes mellitus, sulfonylureas.

Introduction
Neonatal diabetes mellitus (NDM) is a rare monogenic form of diabetes starting within the first 6 months of life [1–3]. The disease has an incidence of about 1:100,000–260,000 live births and can be permanent (PNDM), requiring lifelong treatment, or may be transient (TNDM), in which case the diabetes may spontaneously remit (or be so mild as not to require treatment), but will often relapse, usually during adolescence [3–5].

TNDM comprises approximately 50% of children with neonatal diabetes [6]. A genetic diagnosis has been made in up to 90% of these patients [7]. In the majority of them (around 70%), it was found that a genetic or epigenetic alteration in the TNDM locus on chromosome 6q24 causing the overexpression of two imprinted genes [7]. Less frequently, activating mutations of the genes KCNJ11 and ABCC8 (accounting for 10% and 13% of the cases, respectively) may result in TNDM [7]. Mutations in these genes lead to a gain-in-function of the pancreatic ATP-sensitive potassium (K<sub>ATP</sub>) channel, which is critical in the regulation of insulin secretion by the beta cell [3, 6, 8, 9].

The beta cell K<sub>ATP</sub> channel is an octameric complex composed of four pore-forming subunits: channel-building inwardly rectifying potassium-channel subunits (Kir6.2) encoded by the KCNJ11 gene, and four regulatory sulfonylurea-receptor subunits (SUR1) and encoded by the ABCC8 gene [10, 11]. These subunits regulate the metabolic activity of the channel, which is shut down in response to an increase in intracellular ATP, leading to insulin secretion. Gain-in-function mutations of either of these genes keep the channel in open conformation and impair insulin secretion [10–12].

Most patients with KCNJ11 mutations treated with insulin can be transferred to sulfonylurea (SU) with a remarkable improvement in metabolic control and patient’s quality of life [13]. Sulfonylureas close K<sub>ATP</sub> channels, through an ATP-independent route, improving insulin secretion and representing a suitable therapeutic alternative for patients with KCNJ11 mutations [10]. For these rea-
sons, identification of $K_{ATP}$ channel mutation can have a major impact on the treatment’s choice. This is an example about how molecular diagnosis can influence the clinical management of the patients.

Herein, we report on a case of NDM in a Caucasian boy, who presented severe diabetic ketoacidosis (DKA) at 17 days of life. The disease remitted 4 months later. The genetic screening showed a heterozygous missense mutation (c.679 G>A) in the KCNJ11 gene which leads to the replacement of lysine with glutamic acid at position 227 (E227K) of the ATP sensitive potassium channel.

**Case Report**

An 11-day-old Caucasian boy was admitted to the neonatal care unit with complaints of poor weight progression, suppurative conjunctivitis, and mucoral candidiasis. He was the second child of a 21-year-old woman (gravidia 2, para 1) without history of diabetes, and was born through cesarean section at 38 weeks of pregnancy due to pre-eclampsia. APGAR score was 5/9/10; birth weight was 2890 g (p15), length 47 cm (p15) and head circumference 34.5 cm (p50) [14]. On physical examination, the infant exhibited axial hypotonia and weak suction reflex, demanding a nasogastric tube in order to be feed. At 17 days old, a sudden deterioration of his general condition was noticed. The child became irritable, drowsy, dehydrated, tachypneic, tachycardic with poor peripheral perfusion; rectal temperature was 37.8°C. He was started on intravenous vancomycin after isolation of a methicillin-resistant staphylococcus from the axillary suppurative adenitis. Blood tests revealed a glycemia of 1412 mg/dL with high levels of ketonemia; serum sodium was 172 mmol/L; potassium 3.9 mmol/L, and the pH was 7.0. After initial treatment with intravenous 0.9% saline serum, he was started on intravenous insulin perfusion (0.01 U/kg/h) in a 0.45% saline serum supplemented with 15 mEq/L of potassium chloride. Blood glucose was brought to normal levels and the acidosis was corrected.

Three days later, the insulin perfusion was stopped and the child was transferred to a subcutaneous protocol of intensive insulin therapy, consisting in a once daily administration of insulin glargine (1 U/day), and insulin lispro every 6 h. Further investigations revealed that there was no evidence of pancreatic exocrine failure. Transfontanellar and abdominal ultrasounds; electroencephalography and brain magnetic resonance imaging showed no relevant findings. An intratratial communication “ostium secundum”, associated with enlargement of right cavities was found in the echocardiogram. C-peptide was 0.37 ng/mL (0.80–4.20); auto antibodies against islet cell (ICA), decarboxylase of glutamic acid (GAD), and insulin were all negative. Thyroid function test was normal.

At the age of 2 month, the infant was referred to a tertiary hospital due to instability of his metabolic control, alternating episodes of hypoglycemia with hyperglycemia. He was put on an insulin pump device with continuous glucose monitoring. There was a progressive normalization of his glycemia and the insulin needs declined gradually. Two months later, insulin administration was stopped, given that glycemia was always within normal levels with minimal amounts of insulin. At this stage, the C-peptide was already normal. At the age of 9 months, his growth had declined from p10 to $p<1$. Simultaneously, his body weight decreased from p15 to $p<1$. He was extubated by this time, but his neurodevelopment is still abnormal.

**Discussion**

The E227K mutation found in our patient is a gain-function mutation that results in both impaired ATP sensitivity and higher intrinsic ‘open probability’ of the $K_{ATP}$ channel, causing impairment in insulin secretion [11]. The E227K mutation has been reported in several other patients with TNDM but also in a few with PNDM reflecting the phenotypic variability of KCNJ11 mutations (Table 1). The reason why the same mutation causes a relapsing/remitting form of diabetes in some patients whereas in others it produces a permanent diabetes is unclear [15]. It was not demonstrated a clear relationship between the clinical phenotype and the magnitude of the impairment of the ATP-sensitive potassium (KATP) channels. TNDM may result from a reduction in insulin requirements at the time of remission due to changes in beta cell turnover or to compensatory alterations (at the level of the beta cell, pancreas, or whole body), overcoming the lower effectiveness of the ATP sensitive potassium channel. Therefore, the genetic background of the patient as well as other still unrecognized environmental factors may play an important role in the phenotypic expression of the mutation. On the other hand, the apparent clinical variability may result from confounding factors: patients diagnosed during puberty or early adulthood may have had a period of hyperglycemia that was missed during the neonatal period.

The E227K may be inherited from affected parents, or occurs as de novo mutation. In our study, the mutation is present in the affected child but also in his asymptomatic mother, suggesting that there was not a complete co-segregation of the mutation with diabetes. This situation has also been described previously (Table 1).

In the majority of TNDM and PNDM cases caused by KCNJ11 mutations [3, 13], including E227K mutations [11], metabolic control was achieved by replacing insulin therapy with sulfonylureas, which are well-known $K_{ATP}$ channel inhibitors (Table 1). This finding supports the
idea that if our patient has a relapse of diabetes, he may achieve optimal glycemic control with oral sulfonylurea treatment, strengthening the importance of the molecular diagnosis even if neonatal diabetes remits.

The expression of $KCNJ11$ in the central nervous system and skeletal muscle explain the neurological features associated with syndromic forms of PNDM, such as developmental delay, muscle weakness, and epilepsy [16, 17]. However, neurological features were also identified in patients with nonsyndromic forms of PNDM and TNDM who carried $KCNJ11$ mutations as speech delay, autistic spectrum disorder, and learning disability. Until now it is

Table 1. Clinical data of patients with heterozygous E227K mutation.

| E227K Mutation KCNJ11 gene | Age at onset | Clinical presentation | Evolution | Initial treatment | Transition to sulfonylurea |
|-----------------------------|--------------|-----------------------|------------|------------------|--------------------------|
| Edghill, et al. [19]        |              |                       |            |                  |                          |
| Family – ISPAD 57: German Maternal allele |              |                       |            |                  |                          |
| Patient 1 (Index case, male) | 6 wks        | ?                     | Transient Remission 31 wks | Insulin | No |
| Patient 2 (Maternal half sister) | 8 wks        | ?                     | Permanent | Insulin | No |
| Patient 3 (Maternal half sister) | — —         | —                     | Asymptomatic carrier | — — | — |
| Patient 4 (Mother) | Birth | —                     | Transient Remission 36 wks Relapse 25.5 yrs | Insulin | No |
| Family- ISPAD 114: Canada Paternal allele |              |                       |            |                  |                          |
| Patient 1 (Index case, male) | 13 wks       | ?                     | Transient Remission 52 wks Relapse 6 yrs | SU | |
| Patient 2 (Father) | 23 yrs | ?                     | Diabetic (neonatal? permanent/transient?) | SU | |
| Flanagan, et al. [6], UK |              |                       |            |                  |                          |
| Family 1 |              |                       |            |                  |                          |
| Patient 1 (Index case, male) | 6 wks        | ?                     | Transient Remission 31 wks | Insulin | No |
| Patient 2 (Maternal half sister) | 13 wks       | ?                     | Permanent | Insulin | No |
| Patient 3 (Maternal half sister) | — —         | —                     | Asymptomatic carrier | — — | — |
| Patient 4 (Mother) | Birth | —                     | Transient Remission 36 wks Relapse 25.5 yrs | Insulin | No |
| Family 2 |              |                       |            |                  |                          |
| Patient 1 (Index case, male) | 93 days      | Hyperglycemia         | Transient Remission –195 days | Insulin | No |
| Patient 2 (Father) | 5 wks        | DKA                   | Transient? | Insulin | — |
| Patient 2 (Father) | 23 yrs       | ?                     | Diabetic (neonatal? permanent/transient?) | — No |
| Rica, et al. [20], Spain |              |                       |            |                  |                          |
| Patient 1 (Index case, female) | 4 mth        | DKA                   | Transient Remission- 6 mth | Insulin | Successful |
| Patient 2 (Sibling, male) | 2 mth        | Poor weigh gain, hyperglycemia | Permanent | Insulin | Successful |
| Patient 2 (Sibling, male) | 15 yrs       | Polyurea Polydipsia | Developed proliferative retinopathy | Insulin | Successful |
| Patient 3 (Father) | 93 days      | DKA                   | Transient Remission 9 mth | Insulin | Not tried |
| Patient 2 (Female) | 2 mth        | DKA                   | Transient Remission 7 mth | Insulin | Successful |
| Azores, 2013 |              |                       |            |                  |                          |
| Patient 1 (Index case, male) | 17 days      | DKA                   | Transient Remission- 4 mth | Insulin | Not tried |
| Patient 2 (Mother) | ?            | ?                     | Asymptomatic carrier | ? | |

Wks, weeks; mth, months; yrs, years; DKA, diabetic ketoacidosis; MODY, maturity-onset diabetes of the young.
difficult to assure whether these complications are a consequence of the mutation or whether environmental and/or other genetic factors are involved [6]. In the case herein reported, the infant exhibited axial hypotonia and weak suction reflex, that may be caused by the mutation or/and may be due to exposure to hyperglycemia and ketosis during the first days of the child’s life [1, 3, 18]. Further studies are necessary to clarify the etiology of neurological impairment in TNDM patients who carried KCNJ11 mutations and to access the effectiveness of SU in improving the neurological development.

In conclusion, KCNJ11 activating mutations may be an important cause of TNDM. Molecular diagnosis should be performed in order to identify those patients who may benefit from SU therapy. Further investigation is required to understand clinical heterogeneity and the incomplete co-segregation of the KCNJ11 mutation with diabetes, and also the molecular mechanisms underlying the biphasic course of TNDM.

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Conflict of Interest

None declared.

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