Coincidence of a novel KCNJ11 missense variant R365H with a paternally inherited 6q24 duplication in a patient with transient neonatal diabetes

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Objective– Neonatal diabetes mellitus (NDM) is a heterogeneous group of disorders with diabetes manifestation in the first 6 months of life. The most common etiology in permanent NDM are mutations of the K\textsubscript{ATP} channel subunits; in transient NDM chromosome 6q24 abnormalities are the most common cause.

History And Examination– We report a sporadic case of diabetes diagnosed on the 4\textsuperscript{th} day of life without ketoacidosis.

Investigations- Analysis of the \textit{KCNJ11} gene found a novel R365H mutation in the proband and her unaffected father. The functional analysis did not support pathogenicity of this variant. When the patient’s diabetes remitted in the 7\textsuperscript{th} month of life, the 6q24 region was analyzed and a paternally inherited duplication was identified.

Conclusions- Our case reports a coincidental novel \textit{KCNJ11} variant in a patient with transient NDM due to a 6q24 duplication, illustrating the difficulty in testing neonates before the clinical course of NDM is known.
Neonatal Diabetes Mellitus (NDM) is a heterogeneous group of disorders with diabetes manifestation before 6 months of life, which most frequently has a monogenic etiology (1). In patients with permanent NDM (i.e. without remission (PNDM)) mutations of the K$_{\text{ATP}}$ channel subunits (encoded by the KCNJ11 and ABCC8 genes) and mutations of the insulin gene are the most common etiology (1). Transient Neonatal Diabetes Mellitus (TNDM) usually resolves by 6 months of age, but more than 50% of TNDM patients relapse into diabetes during childhood or adulthood (2). Abnormalities of the imprinted region, 6q24, which encompasses the ZAC and HYMAI genes, cause approximately 70% of TNDM cases (3). Mutations of K$_{\text{ATP}}$ channel subunits are the second most common etiology (3).

**HISTORY AND EXAMINATION**

We report a patient who was born with a birth weight of 2320g at 38 weeks gestation (< 3rd centile). Due to the low birth weight she required hospitalization at the newborn care unit. She was diagnosed with diabetes on the 4th day of postnatal life (blood glucose 19.5 mmol/l) without ketoacidosis. The initial treatment was intravenous insulin (0.04 U/kg/h), followed by subcutaneous injections of NPH insulin (0.9 U/kg/day).

**INVESTIGATIONS**

At the age of 2 weeks and following a clinical diagnosis of NDM, analysis of the KCNJ11 gene was undertaken (3). We found a novel, heterozygous missense mutation R365H (c.1094G>A; p.Arg365His) in the proband. The mutation was present in the unaffected father and paternal grandfather (Figure 1). Standard OGTT’s revealed a normal glucose tolerance with normal insulin and C-peptide values in the parents and paternal grandmother, however the paternal grandfather had impaired fasting glycemia (IFG) combined with impaired glucose tolerance (IGT) (0-hr 5.8; 2-hr glycemia: 9.4 mmol/l).

K$_{\text{ATP}}$ channel mutations causing TNDM in the proband and adult-onset diabetes in a parent and/or grandparent have been previously reported (3). The R365H mutation identified in our family was thought likely to be pathogenic as the arginine residue at codon 365 is conserved in dog, rat and mouse, was not present in 298 control chromosomes, and has not been previously reported in patients with hyperinsulinism. Therefore, *in vitro* functional studies of this mutation were undertaken. Channels containing the R365H mutation were expressed in *Xenopus* oocytes and their surface density, activation by the metabolic inhibitor azide and block by the sulphonylurea tolbutamide were measured (4). Unexpectedly there was no difference between the wild-type and mutant channels: questioning the pathogenicity of this mutation.

In the meantime, the patient developed hypoglycaemia on insulin treatment and at the age of 7 months the insulin therapy was discontinued. The 9-value 24 hour glycemic profile 7 months after stopping insulin administration was normal (4.7-4.1-5.5-7.8-7.6-7.1-3.9-5.3-5.2 mmol/l) and the HbA1c was 4.3% (DCCT range, HPLC, Variant II, Biorad, US). Currently at the age of 2 years the HbA1c is 5.1%. No glycosuria has been noted on repeated testing.

Thus we had to revise our initial diagnosis of PNDM due to a KCNJ11 mutation(3). Genetic analysis of the methylation status within the TNDM critical region on the chromosome 6 was performed, using the methylation specific PCR (MS-PCR) method, as previously described (5). A 6q24 duplication was identified and family member testing demonstrated that the
Coincidence of KCNJ11 mutation and 6q24 duplication was also present in the unaffected father and paternal grandmother (Figure 1).

CONCLUSIONS

We report a novel KCNJ11 variant (R365H) and a 6q24 duplication in a proband with TNDM and her unaffected father. The paternally-inherited 6q24 duplication is likely to be the aetiological mutation as a consequence of over-expression of paternal genes within the duplicated chromosome 6q24 region. Expression of genes in this region is regulated by imprinting. The maternal allele is methylated and therefore inactive: only genes on the paternally derived chromosome are transcribed. Normal development depends on normal doses of gene transcripts. The proband’s father is unaffected because his 6q24 duplication is maternally inherited, and therefore inactive (2). The impaired fasting glycemia and IGT in the paternal grandfather may be influenced by his age (53 years), weight (BMI 28.5 kg/m²) and maternal history of type 2 diabetes. We conclude that the R365H is likely to be a rare variant of no clinical significance.

In the PNDM patients, screening of the KCNJ11 mutations is recommended and - if negative - mutations in other genes should be investigated (e.g. insulin gene, ABCC8 etc.) (1,6). In case of TNDM, K_{ATP} channel genes should be tested if 6q24 analysis is negative. Our case report highlights the difficulty in testing neonates before the clinical course (transient vs permanent) is known and when no supportive signs (e.g. macroglossia and umbilical hernia in 6q24 abnormalities (2)) are present. Thus, in these patients we recommend screening for KCNJ11 gene mutations and 6q24 abnormalities simultaneously as making the genetic diagnosis earlier may influence treatment (patients with K_{ATP} channel mutations are often sensitive to sulfonylureas whilst patients with 6q24 abnormalities are usually treated with insulin (2,3)). Finally, when a novel mutation in a known gene is found, functional studies and investigations of other neonatal diabetes genes can play an important role.

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REFERENCE
Figure 1. Pedigree of the family with coincidence of the novel R365H variant of the KCNJ11 gene and the 6q24 duplication.

Nature of hyperglycemia: TNDM (black filled), IFG+IGT (stripes). The KCNJ11 genotype is shown under each symbol; NN denotes no mutation identified. The 6q24 status is showed below. An arrow points to the proband. Proband, proband’s father and paternal grandfather are carriers of the R365H mutation. Proband has also a paternally inherited 6q24 duplication. Proband’s father has a maternally derived 6q24 duplication. The paternal grandfather has both FG (Impaired Fasting Glucose) and IGT (Impaired Glucose Tolerance) which seem to be associated with age or type 2 DM, and not with the R365H mutation of the KCNJ11 gene.