



Gestational diabetes associated with a novel mutation (378–379insTT) in the glycerol kinase gene



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ABSTRACT

Glycerol kinase deficiency (GKD) is an X-linked inborn error of metabolism at the interface of fat and carbohydrate metabolism. We report a male patient with GKD and a novel insertion of TT in exon 5 at position 378 of the GK cDNA (378–379insTT). This resulted in a premature stop codon and 0.8% normal GK activity. The mother is a carrier for this mutation and had gestational diabetes requiring insulin during this pregnancy but not in her previous pregnancy. Given the association between GKD and type 2 diabetes mellitus, it is interesting that the mother had gestational diabetes while carrying an affected fetus. Therefore, GKD is another disease where there may be a maternal–fetal interaction based on genotype. Further investigations may help elucidate the role of GKD in the carrier mother's gestational diabetes. In addition, these studies will provide better-informed counseling to families with GKD regarding the risk to carrier females.

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1. Introduction

Glycerol kinase (GK) catalyzes the phosphorylation of glycerol to glycerol 3-phosphate [14]. The glycerol 3-phosphate can then be reduced to dihydroxyacetone phosphate which enters the glycolytic pathway, or be used as the backbone for the formation of glycerolipids (triglycerides and plasmalogens) as well as glycerol phospholipids [14]. Therefore GK is at the interface of fat and glucose metabolism.

Glycerol kinase deficiency (GKD; MIM: 307030) is an X-linked inborn error of metabolism that is due to mutations, deletions or insertions within the GK gene on Xp21 [14]. Patients with isolated GKD (iGKD) can be either symptomatic with episodes of metabolic acidosis and central nervous system decompensation or asymptomatic with only pseudo-hypertriglyceridemia [14]. We and others have previously shown that there is no genotype–phenotype correlation in GKD and therefore, we cannot predict by the DNA mutation or the GKD activity, which patients will be symptomatic and which will be asymptomatic [5,22]. This suggests that there is additional complexity in the system and we have hypothesized that this complexity is due in part to other

factors that are inherited independently, including modifier genes, flux through related metabolic pathways, post-transcriptional and post-translational modifications, and the cellular network within which GK functions [2–4].

Given that GK is at the interface of fat and carbohydrate metabolism, it is interesting to note that GK and GKD have links to both obesity and type 2 diabetes mellitus (T2DM). Gaudet et al. reported that 12 out of 18 men carrying a GKD missense mutation (N288D) had obesity and met the criteria for impaired glucose tolerance and/or T2DM [6]. These individuals had the “asymptomatic” form of iGKD. We determined that the lymphoblastoid cell line from a patient with the N288D missense mutation had residual GK enzymatic activity [5]. Several other groups have also reported an association between diabetes and GKD [21,27]. In addition, the thiazolidinediones, common drugs to treat T2DM, increase GK expression in adipocytes and treat insulin insensitivity by making a futile cycle involving GK that ultimately reduces free fatty acids [1,7,12].

GK also plays a role in obesity in mice. In a mouse cross using quantitative traits to identify genes important in abdominal obesity, GK was identified as one of the top ten genes important for the predisposition for abdominal obesity [23]. A *Gyk* (the mouse ortholog of GK) knockout (KO) mouse was made by gene-targeted deletion using a mouse *Gyk* cDNA [9]. The *Gyk* KO male mice are born in the expected numbers and are normal at birth. They become growth retarded and die at days 3–4. The KO mice have a >80 fold increase in plasma glycerol and ~3 fold increase of free fatty acid. The ultimate cause of death of the mice still remains unclear, but we have shown that it is due in part to

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extensive metabolic acidosis [11]. Additionally, we have previously shown that there are altered expression levels of genes in metabolism and insulin signaling pathway in glycerol kinase (*Gyk*) knockout (KO) mouse liver [13], brown fat [18], and muscle [19]. The altered expression of insulin signaling genes in the *Gyk* KO mice suggested that they have reduced glucose uptake, and consequently reduced insulin sensitivity [8].

2. Materials and methods

2.1. DNA mutation analysis

After consent using a UCLA IRB approved protocol, a lymphoblastoid cell line was made from blood isolated from the proband and his mother as previously described [28]. For the proband, his mother and maternal grandmother, we sequenced the 20 exons and intron–exon boundaries of the Xp21 *GK* gene as previously described [28]. The insTT mutation was confirmed by restriction fragment polymorphism with the DdeI restriction enzyme (New England Biolabs, Ipswich, MA). The DNA was digested for 1–3 h at 37 °C with one unit of DdeI enzyme per microgram of DNA per manufacturer's protocol and run on a 1% agarose gel.

2.2. GK activity

We determined the GK activity in a lymphoblastoid cell line from the patient and his mother using a radiochemical assay as previously described [28].

3. Results and discussion

The patient presented with recurrent vomiting. Urine organic acids revealed glyceroluria and blood analysis showed elevated triglycerides (pseudo-hypertriglyceridemia), which are consistent with the symptomatic form of isolated glycerol kinase deficiency (GKD) [14]. He also had gastroesophageal reflux. He was born at 37 weeks gestational age, and his birth weight was 3785 g, birth length 57 cm and head circumference 36 cm. He was born by induced vaginal delivery and the umbilical cord was wrapped around a leg. Otherwise the birth history was unremarkable. There was no hypoglycemia, seizures, mental retardation, or diabetes.

The mother had two pregnancies. The first pregnancy was uneventful and oral glucose tolerance test (OGTT) was normal. With the second pregnancy (when she carried this affected fetus) she had gestational diabetes. Initial OGTT was normal, but she had elevated blood glucose levels. This required 2–3 units of insulin per day. While this is a modest insulin dose, it is striking that she required insulin given that only 10% of pregnancies result in gestational diabetes [20] and many of these are treated with only diet and exercise. The diabetes resolved after the pregnancy indicating she was not one of the many individuals for whom the diagnosis of gestational diabetes was simply revealing type 2 diabetes that was not yet diagnosed [20]. The maternal grandmother had four pregnancies. In the first pregnancy she had gestational diabetes and required insulin therapy starting in the 6th month of gestation. In her second pregnancy, she had trace glucose in the urine but normal blood sugars. In the last two pregnancies there was no diabetes. She did not have diabetes outside of pregnancy.

Sequence analysis of the proband (III-2) revealed an insertion of two thymidine (TT) residues at position 378 of the Xp21 *GK* cDNA (378–379insTT) which was confirmed by allele specific cleavage with the restriction enzyme DdeI (Table 1, Fig. 1). This insertion into exon 5 resulted in a frameshift and premature stop codon. His mother (II-1) was heterozygous for this mutation and therefore a carrier for GKD. The maternal grandmother (I-1) was normal.

The proband's GK activity in the lymphoblastoid cell line was 0.8% of a normal control. The patient's mother had 45.8% GK activity (Table 1). She had gestational diabetes requiring insulin during this pregnancy but

Table 1

Summary of sequence analysis.

The first column denotes the patient and the letters–numbers correspond with the pedigree in Fig. 1. I-1 is the maternal grandmother, II-1 is the mother and III-2 is the proband. The second column shows the glycerol kinase (GK) mutation found in that individual either normal or 378–379insTT. The proband III-2 has only one genotype as he is hemizygous for *GK* which is on the X-chromosome. The third column denotes the GK activity determined using a radio-chemical assay and is expressed as percentage of a normal individual. ND is not determined. The fourth column lists the phenotype in the individual. DM means diabetes mellitus and GKD stands for glycerol kinase deficiency.

Patient	Mutation	Activity	Phenotype
I-1	Normal/normal	ND	Gestational DM
II-1	378-379insTT/normal	45.8%	Gestational DM
III-2	378-379insTT	0.8%	Symptomatic GKD

not in her previous pregnancy. The maternal grandmother's GK activity was not determined.

As noted above, GK is at the interface of fat and carbohydrate metabolism and GKD has been shown to increase risk for obesity, insulin resistance and diabetes [6,13,18,21,23,27]. Recent data suggest that glycerol-stimulated proinsulin biosynthesis and insulin secretion in pancreatic islets after adenoviral-mediated expression of *GK* require the mitochondrial metabolism of glycerol [24]. One mechanism for obesity in GKD may be the need for frequent meals to treat the hypoglycemia. In addition, there is evidence of increased fat storage in liver cells overexpressing *GK* suggesting that GK has a role in lipid storage [25]. While there are many factors increasing risk of gestational diabetes [20], it is intriguing to note that the mother of the proband (II-1) had gestational diabetes when she was pregnant with an affected fetus (III-2), but not with her other pregnancy (III-1). It is likely that some of the risk for the gestational diabetes was due to the fact that the mother was a carrier for this disorder and has only half the normal activity. This risk was increased due to the fact that she was carrying an affected fetus with less than 1% activity. This would add GKD to a host of other diseases where the maternal and fetal genotypes affect risk for pregnancy related complications. This list includes risk of prenatal complications such as preeclampsia, low birth weight and HLA-B maternal–fetal genotype in families with risk for schizophrenia [15]. In addition RHD maternal–fetal genotype incompatibility increases risk for schizophrenia [16]. Other inborn errors of metabolism (IEM) also have been noted to have maternal–fetal genotype effects. In particular pregnancies where the mother is carrying a fetus with an IEM (such as long-chain 3-hydroxyacyl CoA dehydrogenase [LCHAD]), the mother can present with pre-eclampsia, HELLP (hemolysis, elevated liver enzymes and low platelets) syndrome or acute fatty liver of pregnancy [10,26]; in addition, the fetus may have non-immune hydrops [17].

Given the complexity of gestational diabetes [20] and the association of GKD with diabetes [6,21,27], it may be that the maternal (GKD carrier status) and the fetal (GKD male) milieu may be only one component in the etiology of this mother's gestational diabetes. The of the proband had a history of gestational diabetes in one of her four pregnancies and isolated glucosuria in another, but she was not a carrier of the 378–379insTT mutation. Therefore, it is likely that there are other genetic factors predisposing to gestational diabetes in this family and the *GK* mutation and/or glycerol load in her daughter (II-1) and her daughter's fetus (III-2) contributed to manifest this predisposition in her daughter.

In summary, this is a case of symptomatic GKD due to a novel *GK* mutation (insTT) that suggests the possibility of a maternal–fetal effect. Specifically, there may be an increased risk of gestational diabetes in a mother heterozygous for a *GK* frameshift mutation when carrying a hemizygous male fetus with that mutation that may result from the maternal–fetal interaction. The observations that GKD is associated with insulin resistance and diabetes ([6,21,27]), a group of drugs routinely used to treat T2DM increases *GK* expression in fat [7], and GK plays an important role in murine obesity [23] suggest that the possible

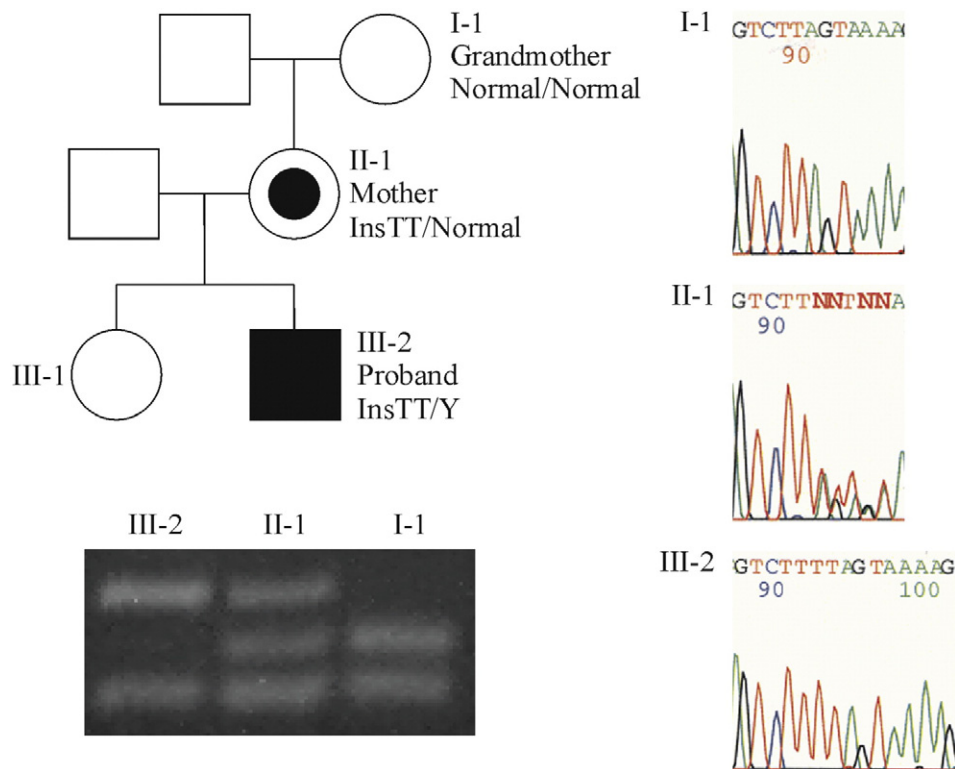


Fig. 1. InsTT causing GKD and associated with gestational diabetes. Panel A (top left): pedigree of GKD family. The proband is III-2 and has a genotype of InsTT/Y. The mother of the proband (II-1) is a carrier (InsTT/Normal) and the maternal grandmother (I-1) has normal GK genotype. Panel B (bottom left): gel electrophoresis of the RFLP using DdeI restriction digestion. Panel C (right): sequence analysis.

maternal–fetal interaction in this maternal–fetal dyad may not be a coincidence. We recommend that other metabolic centers monitor female carriers of GKD for gestational diabetes and see if gestational diabetes occurs more often when a carrier mother has an affected fetus. This will help confirm this possible maternal–fetal interaction. These observations may have important counseling implications for families with GKD.

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