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Case Report

# Pseudo-hypertriglyceridemia in a 2-year-old male with global developmental delay, myopathy and adrenal hypoplasia

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ABSTRACT

Pseudo-hypertriglyceridemia is an overestimation of serum triglyceride levels due to laboratory assays that measure free glycerol concentrations instead of triglycerides directly. Consequently, conditions presenting with elevated levels of endogenous or exogenous free glycerol, such as glycerol kinase deficiency, result in an overestimation of serum triglycerides. Glycerol kinase deficiency (GKD) is caused by pathogenic variants of the *GK* gene on chromosome Xp21. GKD is characterized biochemically by hyperglycerolaemia and glyceroluria. We herein report a 2-year-old male presented with a history of global developmental delay, axial hypotonia, poor head control and inability to sit unassisted or walk with elevated triglycerides at 683 (normal 44-157 mg/dL). Organic acid analysis showed abnormal accumulation of glycerol. Chromosomal microarray results showed a 4.2 Mb deletion of Xp21.3p21.1 (29296579–33551038) including complete copies of *GK*, *DMD*, and *NR0B1* genes as well as multiple exons of *IL1RAPL1*. This confirmed his glycerol kinase deficiency (GKD) as part of the Xp21 continuous gene deletion syndrome. Elevated triglycerides were then recognized as pseudo-hypertriglyceridemia after the diagnosis. The younger sister and the mother have presented with developmental delay, and have been found to have same mutation. This family highlights the importance recognizing pseudohypertriglyceridemia and diagnostic challenges. Earlier identification through urine organic acid analysis could have been made. The combination of clinical presentations and increased glycerol should cause suspicion for GKD

## Background and case description

Pseudo-hypertriglyceridemia is an overestimation of serum triglyceride levels due to laboratory assays that measure free glycerol concentrations instead of triglycerides directly [1]. Consequently, conditions presenting with elevated levels of endogenous or exogenous free glycerol, such as glycerol kinase deficiency, result in an overestimation of serum triglycerides. Glycerol kinase deficiency (GKD) is a rear X-linked recessive disorder caused by pathogenic variants of the *GK* gene on chromosome Xp21. Three forms of GKD are recognized, characterized as infantile, juvenile, and adult forms. While the juvenile and adult forms are recognized as isolated GKD, the infantile form manifests itself as part of the Xp21.3 contiguous gene deletion syndrome, also known as complex GKD [2]. This syndrome is composed of genes associated with Duchenne muscular dystrophy (*DMD*), X-linked congenital adrenal hypoplasia (*NR0B1*), and intellectual disability (*IL1RAPL1*). GKD is characterized biochemically by hyperglycerolaemia and glyceroluria.

A 2-year-old ex-35 week old male, born to a primigravida mother with an uncomplicated pregnancy and birth, a birth weight of 7lbs 7oz, and an uneventful postnatal period, presented to the genetics clinic with a history of global developmental delay. This includes gross motor delay, speech delay, axial hypotonia, poor head control, and inability to sit unassisted or walk. There was also a family history of developmental delay and intellectual disability in the patient's mother and younger sister. Significant findings on examination included a startled appearance, absent eyebrows and temporal thinning, high forehead, frontal bossing, axial hypotonia and peripheral hypertonia.

Laboratory findings included elevated creatine kinase (CK) of 14,809 units/L (normal 27–160 units/L), aspartate aminotransferase (AST) of 307 (normal 20–60 units/L), alanine aminotransferase (ALT) 265 (normal 15–45 units/L), Adrenocorticotropic Hormone (ACTH) 156 (normal 6–48 pg/mL), and elevated triglycerides at 683 (normal 44–157 mg/dL). Family history: The younger sister of the proband was 20

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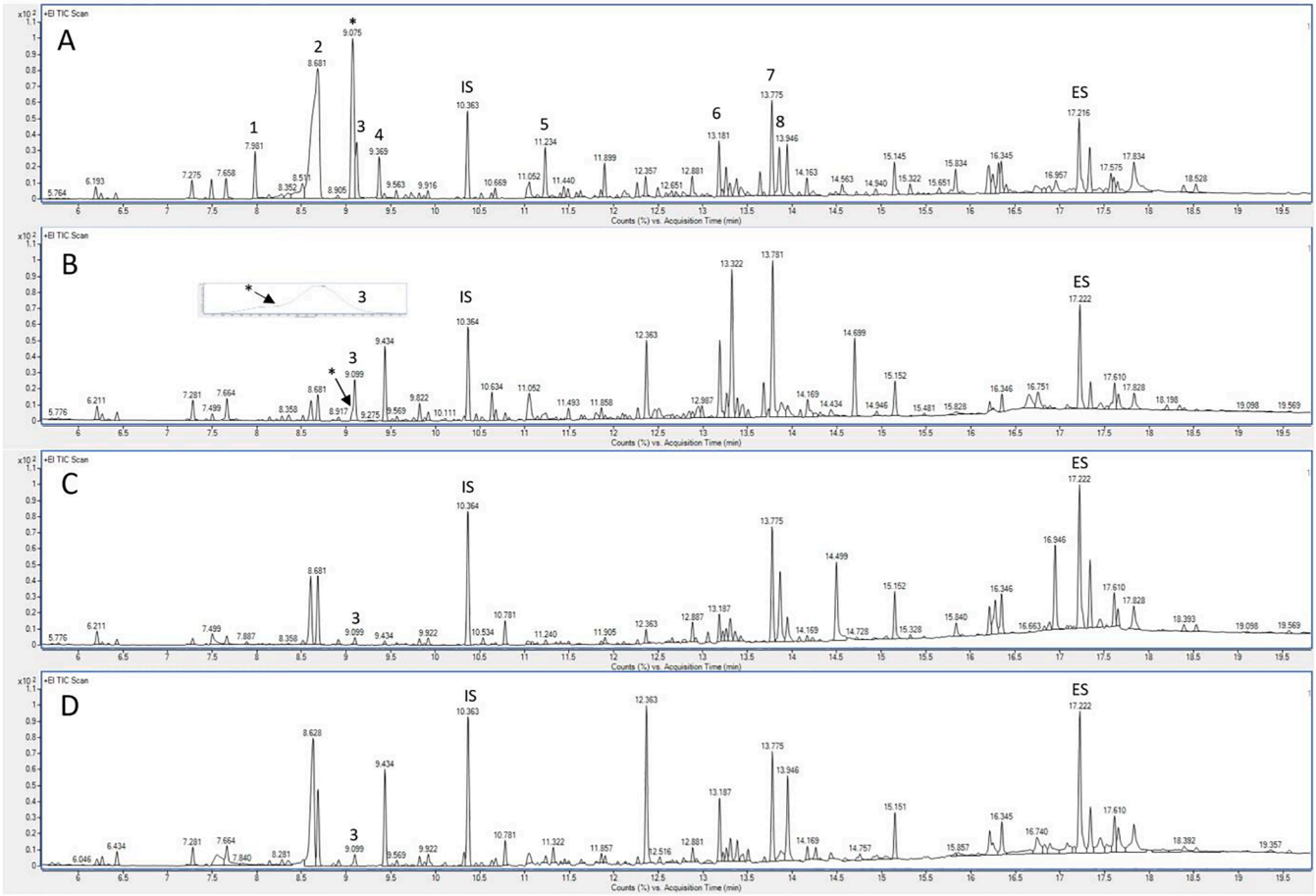


**Fig. 1.** Total Ion Chromatography of urine organic acid by GC/MS on male proband (A), the younger sister (B), the mother (C), and an age-matched 2-year-old normal individual (D). The peak intensity of glycerol (*): male proband 6055442; younger sister 176009; mother 2225, and a normal individual 9763. Major compounds identification: *: glycerol; 1 and 2: urea; 3: phosphate and ethylmalonic acid; 4: succinic acid; 5: adipic acid; 6: aconitic acid; 7: citric acid; 8: hippuric acid; IS (Internal Standard): 4-chlorobenzoic acid; ES (External Standard): tetracosane.

months old when she was diagnosed. She presented with global developmental delay. Her laboratory findings included an elevated creatine kinase (CK) of 544 units/L (normal range: 24–175 units/L) with normal triglycerides at 136 mg/dL (normal range: 42–155 mg/dL) and ACTH at 17.3 pg/mL (normal range: 7.2–63.3 pg/mL). The mother of the proband has a history of learning difficulties. She had normal creatine kinase (CK) of 92 units/L (normal range: <=150 units/L) and normal triglycerides at 92 mg/dL (normal range: 55–170 mg/dL) when she was 38 years old.

## Method

Triglycerides are commonly measured indirectly using different enzyme reagents. In this case, the triglycerides in this boy were measured by an automated chemistry analyzer based on a colorimetric serial enzymatic reaction that includes lipase, glycerol kinase, and L-α-glycerol-phosphate oxidase [3].

To analyze the urinary organic acid profile, including glycerol, acidified urine (pH 1–2) equivalent to 10 mg/dL creatinine was extracted using diethyl ether. The organic extract was then evaporated to dryness under nitrogen, trimethysilyl derivatized by the addition of N, O-bis (trimethylsilyl) trifluoroacetamide with 1% trimethylchorosilane (BSTFA/TMCS), and then analyzed by gas chromatography-mass spectrometry (GC–MS).

## Results and discussion

Urinary organic acid analysis was performed by GC–MS and chromosomal microarray for further evaluation of the male proband. The organic acid analysis revealed marked excretion of glycerol (Fig. 1A). The chromosomal microarray results showed a 4.2 Mb deletion of Xp21.3p21.1 (29296579–33551038). which includes complete copies of the *GK, DMD*, and *NR0B1* genes, as well as multiple exons of *IL1RAPL1*. This confirmed his diagnosis of glycerol kinase deficiency (GKD) as part of the Xp21 continuous gene deletion syndrome. The elevated triglycerides were then recognized as pseudo-hypertriglyceridemia following the diagnosis. Urine was collected from the younger sister and the mother for analysis. The urinary organic acid (UOA) profile showed moderate excretion of glycerol in the sister and normal excretion of glycerol in the mother (Fig. 1B and C). The proband's mother and sister were found to have the same deletion, confirming the inheritance to be of maternal origin.

Glycerol kinase (GK) performs the phosphorylation of glycerol using ATP, producing glycerol 3-phosphate and ADP. Subsequently, glycerol 3-phosphate dehydrogenase converts glycerol 3-phosphate to dihydroxyacetone phosphate, which is involved in glycolysis and gluconeogenesis. Glycerol 3-phosphate serves as an important substrate for gluconeogenesis during prolonged fasting or periods of stress [4]. Mutation or deletion of the *GK* gene leads to GKD resulting in increased plasma and urinary glycerol concentrations. GKD is inherited in an X-linked manner, affecting males, while female carriers may exhibit intellectual disability [5].

Clinical presentations can vary widely in patients with the complex infantile form of GKD. In addition to hyperglycerolemia and glyceroluria, symptoms may include vomiting, lethargy, ketotic hypoglycemia, metabolic acidosis, seizures, increased creatine kinase (hyperCKemia), hypotonia, and developmental delay. These symptoms are related to the loss of multiple neighboring genes on the deleted chromosome segment (Xp21). This includes conditions such as Duchenne muscular dystrophy, congenital adrenal hypoplasia, and *IL1RAPL1*-related intellectual disability disorder. Dysmorphic facial features may also be present [6]. Pseudohypertriglyceridemia is commonly observed in these patients, as triglycerides are quantitated based on the amount of free glycerol present after triglyceride lipolysis [4]. Therefore, UOA analysis by GC–MS is necessary to identify elevated glycerol levels. The combination of increased glycerol and pseudohypertriglyceridemia should raise suspicions of GKD [6].

Treatment of GKD involves managing the diet to include frequent carbohydrate-rich meals, particularly during illness or strenuous exercise [7]. Additionally, careful management and surveillance of other manifestations of the gene deletion syndrome, such as adrenal insufficiency and myopathy, are important.

The male proband's initial and ongoing symptoms were attributed to his muscular dystrophy, global developmental delay, and confirmed adrenal insufficiency based on ACTH stimulation testing. As a result, he requires continuous follow-up with a multidisciplinary team consisting of genetics specialists, metabolic dieticians, developmental pediatricians, speech therapists, physical therapists, endocrinologists, and neuromuscular specialists. Mild glyceroluria was observed in the sister of the male proband through GC/MS analysis (Fig B). Testing for affected family members was recommended, and these individuals are currently being monitored and followed up on.

## Conclusion

This family case reinforces the significance of recognizing pseudohypertriglyceridemia and the challenges it presents in terms of diagnosis. Early detection through UOA analysis could have been established for both the proband and his sister, considering their shared presentation of developmental delay. The presence of clinical symptoms combined with increased levels of glycerol should raise suspicions of GKD. Female carriers of this X-linked disorder should undergo evaluation for intellectual disability and other potential abnormalities, as observed in the younger sister and mother of this patient.

## Points of interest

1. Pseudo-hypertriglyceridemia can be caused by Glycerol kinase deficiency (GKD).
2. Infantile form GKD manifests itself as part of the Xp21.3 continuous gene deletion syndrome, which includes Duchenne Muscular Dystrophy (*DMD*), X-linked congenital adrenal hypoplasia (*NR0B1*) and a gene involved with intellectual disability (*IL1RAPL1*).
3. It is important to understand the significance of recognizing pseudohypertriglyceridemia, and one way to identify this condition is through urine organic acid analysis.

## CRediT authorship contribution statement

**Xiaowei Fu:** Conceptualization, Writing – original draft, Writing – review & editing, Investigation, Supervision, Resources. **Claire P. Williamson:** Writing – original draft. **Kerri Bosfield:** Conceptualization, Writing – original draft, Writing – review & editing.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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