JIMD Reports
DOI 10.1007/8904_2011_110

CASE REPORT

Galactokinase Deficiency in a Patient with Congenital Hyperinsulinism

Mashbat Bayarchimeg · Dunia Ismail · Amanda Lam · Derek Burk · Jeremy Kirk · Wolfgang Hogler · Sarah E Flanagan · Sian Ellard · Khalid Hussain

Received: 5 August 2011 / Revised: 14 September 2011 / Accepted: 25 October 2011 / Published online: 13 December 2011

Abstract *Background*: Galactokinase catalyses the first committed step in galactose metabolism, the conversion of galactose to galactose-1-phosphate. Galactokinase deficiency is an extremely rare form of galactosaemia, and the most frequent complication reported is cataracts. Congenital hyperinsulinism (CHI) is a cause of severe hypoglycaemia in the newborn period. Galactosaemia has not previously been reported in a neonate with concomitant CHI.

Aims: To report the first case of a patient with CHI and galactokinase deficiency, and to describe the diagnostic pitfalls with bedside blood glucose testing in a neonate with combined galactokinase deficiency and CHI.

Patients/methods: A 3-day-old baby girl from consanguineous parents presented with poor feeding, irritability and seizures. Capillary blood glucose testing using bedside test strips and glucometer showed a glucose level of 18 mmol/L, but the actual laboratory blood glucose level was only 1.8 mmol/L. After discontinuation of oral feeding (stopping provision of dietary galactose), the bedside capillary blood glucose correlated with laboratory glucose concentrations. *Results*: Biochemically the patient had CHI (blood glucose level 2.3 mmol/L with simultaneous serum insulin level of 30 mU/L) and galactokinase deficiency (elevated serum galactose level 0.62 μmol/L). Homozygous loss of function mutations in *ABCC8* and *GALK1* were found, which explained the patient's CHI and galactokinase deficiency, respectively.

Conclusion: This is the first reported case of CHI and galactokinase deficiency occurring in the same patient. Severe hypoglycaemia in neonates with CHI may go undetected with bedside blood glucose meters in patients with galactokinase deficiency.

Communicated by: Alberto B Burlina.

Competing interests: None declared.

M. Bayarchimeg · D. Ismail · K. Hussain
Department of Endocrinology, Great Ormond Street Hospital for Children NHS Trust, London, UK

M. Bayarchimeg · D. Ismail · A. Lam · D. Burk · K. Hussain (✉)
Clinical and Molecular Genetics Unit, Developmental Endocrinology Research Group, The Institute of Child Health, University College London, 30 Guilford Street, London WC1N 1EH, UK
e-mail: K.Hussain@ich.ucl.ac.uk

A. Lam · D. Burk
Department of Biochemistry, Great Ormond Street Hospital for Children NHS Trust, London, UK

J. Kirk · W. Hogler
Department of Endocrinology and Diabetes, Birmingham Children's Hospital, Birmingham, UK

S. E. Flanagan · S. Ellard
Institute of Biomedical and Clinical Science, Peninsula Medical School, University of Exeter, Barrack Road, Exeter, UK

Introduction

Galactosaemia describes a group of diseases characterised by abnormalities in galactose metabolism. Galactokinase deficiency (OMIM 230200) is a rare type of galactosaemia and causes congenital cataracts during infancy (Hennermann et al. 2011). The enzyme galactokinase catalyses the first committed step in the metabolism of galactose by phosphorylating the galactose at the first carbon. Galactokinase is encoded by the *GALK1* gene which is localised on chromosome 17q24 (Stambolian et al. 1995).

Congenital hyperinsulinism (CHI) is a major cause of severe hypoglycaemia in the newborn period. Delay in the

diagnosis and treatment of the hypoglycaemia is the major reason for the increased risk of brain damage observed in these patients. The most common cause of severe CHI which is medically unresponsive involves defects in the genes regulating the function of the pancreatic ATP sensitive potassium channel (K_{ATP} channel) (Thomas et al. 1995, 1996). Mutations in *ABCC8* are the most common cause of severe medically unresponsive CHI.

Blood glucose test strips in conjunction with glucose meters are used widely to measure capillary blood glucose levels in patients with hypoglycaemia and hyperglycaemia (Meex et al. 2006). The advantages of using these glucose meters with the test strips are that they are readily available, they give immediate results and a minimum amount of blood is required. Despite these advantages, the results can be affected by numerous factors. For example, severe dehydration, hypotension and high haematocrit (>55%) may cause an underestimation of blood glucose concentration (Barreau and Buttery 1987; Atkin et al. 1991). Falsely elevated blood glucose levels may be caused by lipaemic blood, low haematocrit (<25%) and chemicals in the blood that are measured by the meter.

We report the first case of combined CHI and galactokinase deficiency in a human. This baby girl presented with fitting and routine glucose measured by bedside test strips and a glucose meter showed a blood glucose level of 18 mmol/L, but the actual laboratory blood glucose (measured using a glucose oxidase method) level was only 1.8 mmol/L. The falsely elevated blood glucose was due to the increased concentration of galactose which the meter was unable to discriminate from blood glucose.

Case History

The patient was born at term with a birth weight of 4.2 kg to consanguineous parents following a normal vaginal delivery. There was no history of gestational diabetes mellitus, and she was discharged home on day 2 of life with no concerns. On day 3, however, she had generalised seizures (eye rolling, sweatiness and shaking limbs) requiring admission to hospital. Routine blood glucose monitoring (whilst on enteral feeds and intravenous dextrose) showed a marked discrepancy between the bedside glucometer (Roche Accu-Chek Advantage II meter; Roche Diagnostics Limited, Lewes, East Sussex, UK) blood glucose reading and the laboratory blood glucose levels (see Table 1) as measured by the glucose oxidase method. This discrepancy disappeared when the feeds were stopped and the patient maintained on intravenous glucose infusion.

Once the feeds were stopped, she required up to 22 mg/kg/min of intravenous dextrose to maintain normoglycaemia, and further investigations confirmed hyperinsulinaemic hypoglycaemia (laboratory blood glucose 2.3 mmol/L with a simultaneous serum insulin of 30 mU/L, and undetectable serum fatty acid and ketone bodies). Her hypoglycaemia failed to respond to maximal dose of medical therapy with diazoxide (20 mg/kg/day) and octreotide (35 μg/kg/day), thus requiring a near total pancreatectomy. Histology of the resected pancreas confirmed changes typical of diffuse CHI. Ophthalmology examination showed no cataracts but pseudotumour cerebri was noted. She was then managed on a galactose-free diet. At the age of 2 years, this patient has global developmental delay, microcephaly and generalised epilepsy.

Table 1 Discrepancy between the blood glucose levels measured by the bedside using a glucometer with test strips and laboratory blood glucose

Bedside blood glucose reading (mmol/L)	Laboratory blood glucose (mmol/L)	Milk feeds
18	2.9	Yes
12	3.1	Yes
15	2.2	Yes
5.6	5.8	No
4.3	4.6	No
4.7	4.9	No

Methods

Genetic Studies for *ABCC8/KCNJ11* Mutations

Genomic DNA was extracted from peripheral leukocytes using standard procedures. The *KCNJ11* and *ABCC8* genes were amplified and the products sequenced as previously described (Flanagan et al. 2007). The sequences were compared to the published sequences (NM_000525 and NM_000352.2) using Mutation Surveyor 3.24 software (SoftGenetics, PA, USA). Mutation testing was undertaken in parental samples to confirm their carrier status.

Genetic Studies for Galactokinase

Genomic DNA was extracted from peripheral leucocytes using standard procedures and the GALK1 was amplified (primers available on request). Standard PCR conditions were used with the addition of 5 mM betaine and 5% DMSO. PCR products were purified using microClean (Web Scientific) and then sequenced using the ABI Big Dye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems) with standard conditions and then run on the ABI 3730 DNA analyser. Data were analysed using Sequencher 4.9 (Gene Codes Corporation, Ann Arbor, MI, USA).

Enzymology

Galactokinase Assay

Galactokinase activity was assayed in washed lysed erythrocytes as described by Ng et al. (1965). Samples were incubated with 14°C-galactose (Amersham, Bucks, UK) in the presence of ATP and magnesium for 30 min at 37°C. The 14°C-galactose-1-phosphate product, radiolabelled reaction intermediates and unreacted 14°C-galactose were separated by descending paper chromatography using DE81 ion exchange paper (Whatman, Kent, UK). Non-specific radiolabelled contaminants were corrected with a sample blank in which the sample was added immediately before the reaction was terminated. Results were expressed as micromols of 14°C-galactose-1-phosphate generated per hour per gram of haemoglobin (μmol/h/g Hb) following correction of non-specific radiolabelled contaminants and radiolabelled reaction intermediates.

Galactose-1-Phosphate Uridyl Transferase Assay

As galactokinase is a labile enzyme, a second enzyme galactose-1-phosphate uridyl transferase was assayed in washed lysed erythrocytes using the method described by Beutler (Beutler and Baluda 1966). Results are expressed as the amount of uridine diphosphoglucose consumed per hour per gram of haemoglobin (μmol/h/g Hb).

Results

Genetic Studies for CHI

Sequence analysis identified a previously reported missense mutation, E128K (c.382 G>A; p.Glu128Lys) in exon 3 of the *ABCC8* gene (Fig. 1) (Yan et al. 2007). Mutation testing confirmed that the unaffected parents were heterozygous for the mutation.

Galactokinase

All the coding regions of *GALK1* gene from the affected patient were sequenced to investigate the underlying genetic causes of the galactokinase-deficient phenotype. We detected a homozygous R256W (c.766 C>T; p.Arg256Trp) missense mutation in exon 5 of the *GALK1* gene (Fig. 2) which encodes the galactokinase protein and which has been previously identified in a different patient and shown to substantially reduce the enzyme activity (Asada et al. 1999). The mutation occurred at CpG dinucleotides but outside of any conserved regions of the gene. Asada et al. (1999) has shown through COS cell expression analysis that this particular missense mutation completely abolishes the activity of galactokinase.

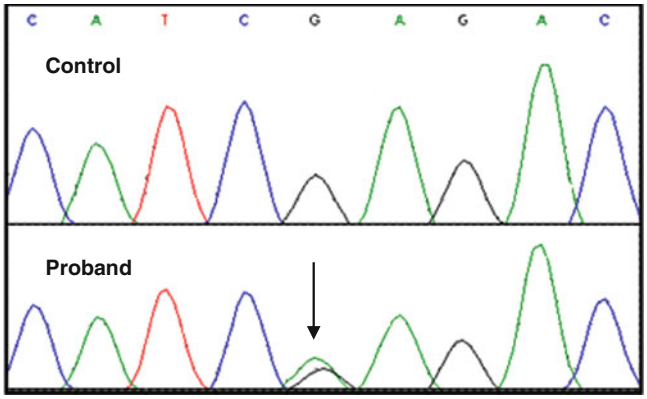

Fig. 1 Electropherograms showing the homozygous *ABCC8* mutation and a normal control. A *black arrow* points to the c.382 G>A mutation which results in the substitution of glutamic acid (GAG) by lysine (AAG) at residue 128 (E128K)

Enzymology

Galactokinase activity in the patient was undetectable and was significantly lower than a simultaneously assayed control erythrocyte sample (2.1 μmol/h/g Hb, reference range 1.0–3.6). The galactose-1-phosphate uridyl transferase activity in the patient was within the unaffected range (33.7 μmol/h/g Hb, reference range 18.0–40.0). The serum level of galactose at the time of diagnosis was 0.62 μmol/L (normal for non-galactosaemic patient <0.1).

Discussion

This is the first patient to be reported with CHI and galactokinase deficiency. The loss of function mutation in the *ABCC8* gene leads to severe hyperinsulinaemic hypoglycaemia, whereas the loss of function mutation in the *GALK1* gene leads to a substantial reduction in the activity of the enzyme galactokinase resulting in the accumulation of galactose in the blood. The functional consequences of the *GALK1* mutation observed in our patient have been previously studied by Asada et al. (1999). This particular *GALK1* mutation completely abolishes the activity of galactokinase and this was confirmed biochemically in our patient.

Hand-held (bedside) blood glucose monitors in conjunction with glucose test strips are widely used in the care of patients with hypoglycaemia (neonatal hypoglycaemia) and hyperglycaemia (diabetes mellitus). Despite the potential advantages, some of these capillary glucose monitors fail to discriminate between blood glucose and other substrates

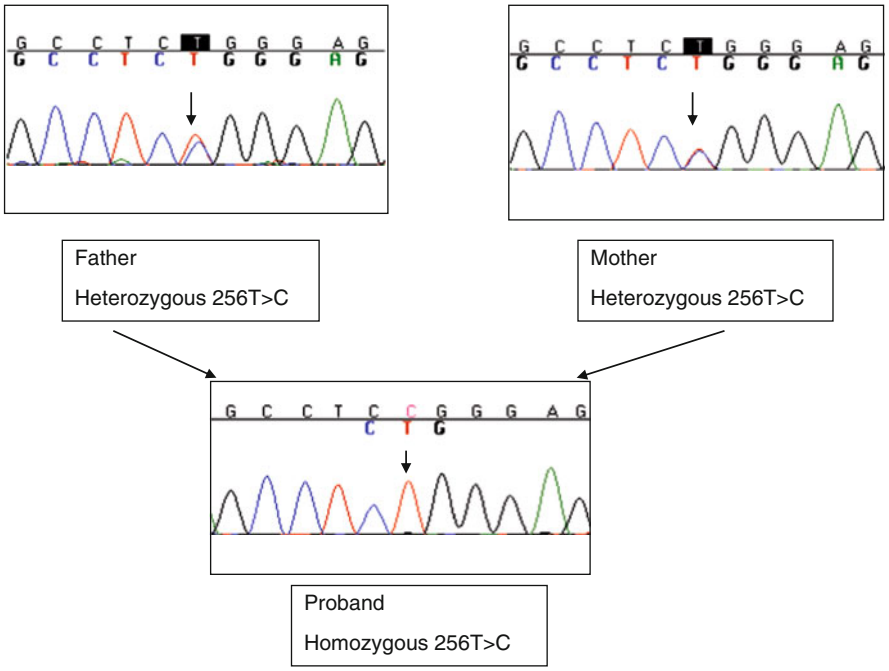

Fig. 2 Electropherograms showing the homozygous *GALK1* mutation in the proband and the heterozygote status of the parents. The *black arrow* points to the homozygous R256W (c.766 C>T; p.Arg256Trp) missense mutation in exon 5 of the *GALK1* gene

such as galactose (Hyde and Betts 2006; Nelson 2008; Newman et al. 2002). The Roche Accu-Chek Advantage II meter used in this patient will detect blood galactose if the galactose concentration is >0.56 mmol/L (Newman et al. 2002). These test strips and Roche Accu-Chek Advantage II meter use the glucose dehydrogenase (GDH) and pyrroloquinolinequinone (PQQ) enzyme system for measuring blood glucose, and this is known to detect galactose as well as glucose. In contrast, the standard laboratory methods used to measure blood glucose levels are based on the glucose oxidase or hexokinase methods.

Galactokinase (GALK) is the first enzyme in the Leloir pathway, converting galactose into galactose-1-phosphate (Gal-1-P). The deficiency of galactokinase leads to an accumulation of galactose in the blood which then interferes with the capillary blood glucose monitoring. In the case of the Roche Accu-Chek Advantage II meter, the increased level of galactose leads to a falsely elevated measurement for blood glucose. There have been several case reports of newborns with classical galactosaemia (due to galactose-1-phosphate uridyl transferase deficiency) who presented with falsely elevated blood glucose levels when measured by the bedside using a Roche Accu-Chek Advantage II meter (Hyde and Betts 2006; Nelson 2008; Newman et al. 2002). Hyde and Betts (2006) reported a 5-week-old galactosaemic baby with failure to thrive and again falsely elevated blood glucose levels. Newman et al. (2002) reported a premature newborn with galactosaemia who had falsely elevated blood glucose levels.

Whilst galactokinase deficiency is rare in comparison to galactosaemia, the gene frequency, however, can vary widely with an east-to-west gradient across Europe (from 1:1,000,000 to 1:52,000). A high incidence of galactokinase deficiency is found among Roma, an endogamous Gypsy population originating from Eastern Europe (16). The high incidence is attributable to a founder effect, as demonstrated by the segregation of a single nucleotide mutation (P28T) which is present in about 5% of the Roma population (Kalaydjieva et al. 1999).

In Galactokinase deficiency, cataracts and pseudotumour cerebri appear to be the major complications, and the outcome for patients with galactokinase deficiency is thought to be much better than for patients with classical galactosaemia (Hennermann et al. 2011). In contrast, in classical galactosaemia long-term follow-up studies of patients have shown that, in spite of a severely galactose-restricted diet, most patients develop abnormalities such as disturbed mental and/or motor development and females develop hypergonadotropic hypogonadism (Bosch et al. 2002). In classical galactosaemia there is also impairment of speech, resulting from disruption in motor planning and programming or motor execution (Potter 2011). In our patient the global developmental delay, microcephaly and generalised epilepsy most likely reflect the combination of hyperinsulinaemic hypoglycaemia and galactokinase deficiency.

In summary, we present a previously unreported combination of a neonate with CHI and galactokinase deficiency. The homozygous *ABCC8* gene mutation led to severe

hypoglycaemia, and the homozygous *GALK1* mutation led to the accumulation of galactose. The elevated serum galactose level resulted in falsely high capillary blood glucose level when measured by a bedside glucometer, and obscured the prevailing hypoglycaemia caused by CHI. Severe hypoglycaemia in neonates with CHI may go undetected with bedside capillary blood glucose meters in the presence of galactokinase deficiency.

Disclosure Summary

No conflict of interest for any authors.

Precis

This is the first report of an infant with combined galactokinase deficiency and congenital hyperinsulinism. Galactokinase deficiency led to falsely elevated blood glucose levels in the presence of severe hypoglycaemia caused by congenital hyperinsulinism.

References

Asada M, Okano Y, Imamura T, Suyama I, Hase Y, Isshiki G (1999) Molecular characterization of galactokinase deficiency in Japanese patients. J Hum Genet 44(6):377–382

Atkin SH, Dasmahapatra A, Jaker MA, Chorost MI, Reddy S (1991) Fingerstick glucose determination in shock. Ann Intern Med 114 (12):1020–1024

Barreau PB, Buttery JE (1987) The effect of the haematocrit value on the determination of glucose levels by reagent-strip methods. Med J Aust 147(6):286–288

Beutler E, Baluda MC (1966) Improved method for measuring galactose-1-phosphate uridyl transferase activity of erythrocytes. Clin Chim Acta 13:369–379

Bosch AM, Bakker HD, van Gennip AH, van Kempen JV, Wanders RJ, Wijburg FA (2002) Clinical features of galactokinase deficiency: a review of the literature. J Inherit Metab Dis 25(8):629–634

Flanagan SE, Patch AM, Mackay DJ, Edghill EL, Gloyn AL, Robinson D, Shield JP et al (2007) Mutations in ATP-sensitive K + channel genes cause transient neonatal diabetes and permanent diabetes in childhood or adulthood. Diabetes 56 (7):1930–1937

Hennermann JB, Schadewaldt P, Vetter B, Shin YS, Mönch E, Klein J (2011) Features and outcome of galactokinase deficiency in children diagnosed by newborn screening. J Inherit Metab Dis 34 (2):399–407

Hyde P, Betts P (2006) Galactosaemia presenting as bedside hyperglycaemia. J Paediatr Child Health 42(10):659

Kalaydjieva L, Perez-Lezaun A, Angelicheva D, Onengut S, Dye D, Bosshard NU, Jordanova A et al (1999) A founder mutation in the GK1 gene is responsible for galactokinase deficiency in Roma (Gypsies). Am J Hum Genet 65:1299–1307

Meex C, Poncin J, Chapelle JP, Cavalier E (2006) Analytical validation of the new plasma calibrated Accu-Chek Test Strips (Roche Diagnostics). Clin Chem Lab Med 44(11):1376–1378

Nelson JR (2008) False bedside hyperglycaemia revealing galactosaemia in a premature twin female baby. Arch Pediatr 15 (8):1359–1360

Newman JD, Ramsden CA, Balazs ND (2002) Monitoring neonatal hypoglycemia with the Accu-chek advantage II glucose meter: the cautionary tale of galactosemia. Clin Chem 48(11):2071

Ng WG, Donnell GN, Bergren WR (1965) Galactokinase activity in human erythrocytes of individuals at different ages. J Lab Clin Med 66:115–121

Potter NL (2011) Voice disorders in children with classic galactosaemia. J Inherit Metab Dis 34(2):377–385

Stambolian D, Ai Y, Sidjanin D, Nesburn K, Sathe G, Rosenberg M, Bergsma DJ (1995) Cloning of the galactokinase cDNA and identification of mutations in two families with cataracts. Nat Genet 10:307–312

Thomas P, Ye Y, Lightner E (1996) Mutation of the pancreatic islet inward rectifier Kir6.2 also leads to familial persistent hyperinsulinemic hypoglycemia of infancy. Hum Mol Genet 5 (11):1809–1812

Thomas PM, Cote GJ, Wohllk N, Haddad B, Mathew PM, Rabl W, Aguilar-Bryan L et al (1995) Mutations in the sulfonylurea receptor gene in familial persistent hyperinsulinemic hypoglycemia of infancy. Science 268:426–429

Yan FF, Lin YW, MacMullen C, Ganguly A, Stanley CA, Shyng SL (2007) Congenital hyperinsulinism associated ABCC8 mutations that cause defective trafficking of ATP-sensitive K + channels: identification and rescue. Diabetes 56(9):2339–2348

http://www.springer.com/978-3-642-28095-5

JIMD Reports - Case and Research Reports, 2012/2
Peters, V. (Ed.)
2012, VII, 130 p. 36 illus., 19 illus. in color., Softcover
ISBN: 978-3-642-28095-5