Case report

Diazoxide-unresponsive Hyperinsulinemic Hypoglycaemia in a Preterm Infant with Heterozygous Insulin Receptor Gene Mutation

POON SW-Y et al. Hyperinsulinism due to Insulin Receptor Mutation

Sarah Wing-yiu POON, Brian Hon-yin CHUNG, Mabel Siu-chun WONG, Anita Man-ching TSANG
Department of Paediatrics and Adolescent Medicine, Queen Mary Hospital, The University of Hong Kong

What is already known on this topic ?
Homozygous or compound heterozygous mutations in INSR gene cause severe insulin resistance syndromes Donohue syndrome (DS, also known as leprechaunism) and Rabson-Mendenhall syndrome (RMS) whereas heterozygous INSR gene mutations result in a milder phenotype known as type A insulin resistance syndrome (type A-IR). Adults with type A-IR commonly demonstrate abnormal glucose homeostasis with fasting and postload hyperglycaemia, as well as high testosterone levels compared to age-matched controls. Phenotypes and clinical course in children, especially infants, with heterozygous INSR gene mutations are less reported. There is also lack of literature on how these infants can be managed.

What this study adds?
We report a preterm infant who presented with diazoxide-unresponsive hyperinsulinemic hypoglycaemia. Whole-exome sequencing revealed heterozygous INSR gene mutation in the infant and her father. We postulated that use of diazoxide exacerbated post-prandial glucose excursion by inhibiting insulin release, while hypoglycaemia that follows could be explained by reduced degradation or clearance of insulin due to the underlying mutation. Our study highlights that in situation where mutations could not be identified by targeted sequencing of *ABCC8/KCNJ11* or *GCK* genes in an infant with suboptimal response to diazoxide, sequencing of the *INSR* gene should be considered. Indeed, *INSR* gene should be included in the targeted gene panel for workup of hyperinsulinism.

Abstract
Homozygous or compound heterozygous mutations in insulin receptor gene (*INSR*) lead to marked insulin resistance and hyperglycaemia in Donohue syndrome and Rabson-Mendenhall syndrome, conditions which are associated with significant morbidity early in life. On the other hand, heterozygous *INSR* gene mutations result in milder phenotype known as type A insulin resistance syndrome. While presentation in adults this condition is well reported, phenotypes in infant are less well-characterized. We herein report an infant presenting with hyperinsulinemic hypoglycaemia who did not respond to diazoxide therapy. She was subsequently found to carry heterozygous *INSR* gene mutation. Our patient was a female infant born at 29 weeks of gestation who developed recurrent hypoglycaemia in early infancy. Workup showed hyperinsulinism and she was started on first-line therapy with diazoxide and high-calorie feeds. However, continuous blood glucose monitoring showed post-prandial hyperglycaemia followed by rapid fall to hypogylcaemia. Whole exome sequencing was performed to investigate for diazoxide-unresponsive hyperinsulinism, which revealed a likely pathogenic mutation in the *INSR* gene c.1246C>T p. (R416X). This nonsense mutation was inherited from the father. With the molecular diagnosis, diazoxide was stopped and she followed a diet with low glycaemic-index food. Subsequent monitoring showed stable glucose profile. Our case highlights the importance to consider type A insulin resistance syndrome when no mutation could be identified in the *ABCC8/KCNJ11* genes in diazoxide-unresponsive hyperinsulinism. With autosomal dominant inheritance, cascade screening should be performed in family members to identify those harbouring the mutation as they are at risk of early onset diabetes.
Keywords: Hyperinsulinism, hypoglycaemia, insulin receptor

Sarah Wing-yiu POON, NCB 115, Queen Mary Hospital, 102 Pokfulam Road, Hong Kong
sarahpoonwy@gmail.com
(852)22554482
0000-0003-0818-9699

Introduction

Mutations in the insulin receptor (*INSR*) gene are known to cause insulin resistance and hyperinsulinemia. Homozygous or compound heterozygous mutations in *INSR* gene cause severe insulin resistance syndromes Donohue syndrome (DS, also known as leprechaunism) and Rabson-Mendenhall syndrome (RMS) whereas heterozygous *INSR* gene mutations result in a milder phenotype known as type A insulin resistance syndrome (type A-IR). In both DS and RMS, patients manifest with marked hyperinsulinemia with fluctuating blood glucose levels, impaired muscle and adipose tissue development, growth

failure, characteristic facial features and intellectual disability. Significant hyperglycaemia ensues when β-cells decompensate. Patients with DS, the most severe insulin resistance syndrome, seldom survive beyond infancy whereas patients with RMS can survive into early adulthood and usually die of diabetic ketoacidosis or advanced microvascular complications in the second decade of life (1). On the other hand, patients with type A-IR live beyond middle age and usually present with hypoglycaemic symptoms, hypertrichosis, acanthosis nigricans and hyperandrogenism in the absence of obesity or lipoatrophy. Biochemically, these adults commonly demonstrate abnormal glucose homeostasis with fasting and postload hyperglycaemia, as well as high testosterone levels compared to age-matched controls (2).

Phenotypes and clinical course in children, especially infants, with heterozygous *INSR* gene mutations are less reported. There is also lack of literature on how these infants can be managed. We herein report a preterm infant who presented with diazoxide-unresponsive hyperinsulinemic hypoglycaemia. Whole-exome sequencing revealed heterozygous *INSR* gene mutation in the infant and her father, which helped to guide further investigation and management.

Case presentation

Our proband was a Chinese female infant born at 29 weeks of gestation for threatened preterm labour, weighing 1kg (25th centile) and measuring 37 cm (25th centile) in length. Antenatal history was unremarkable with no gestational diabetes in the mother and there was no family history of endocrine disorders. There was no birth asphyxia and her neonatal course was relatively smooth with mild respiratory distress syndrome requiring 1 day of invasive ventilation, feeding intolerance and grade 1 intraventricular haemorrhage. Newborn screening for inborn errors of metabolism was normal. Parenteral nutrition was given according to standard protocol and enteral feeding was gradually stepped up.

She presented with recurrent hypoglycaemia since 1 month of life when full enteral feeding was established. Investigations showed repeated pre-feed hyperinsulinemic hypoglycemia with low serum levels of free fatty acids and ketone (Table 1). Thyroid function, growth hormone, cortisol and ammonia levels were normal and lactate was not elevated. After cardiac assessment, diazoxide 15 mg/kg/day and hydrochlorothiazide were commenced for neonatal hyperinsulinism. Glucose polymer (Polycal) was added to feeds. However, glucose profile further worsened with more frequent episodes of pre-feed hypoglycemia. Baby was then put onto continuous glucose monitoring system (CGMS) which revealed frequent post-prandial hyperglycaemia ranging from 178.2mg/dL to 309.6mg/dL, followed by rapid fall to hypogylcaemic range with nadir of 37.8mg/dL. Insulin was still detectable (26.9-69.0 pmol/L) during these episodes of hypoglycaemia. Bolus feeding was thus halted and she was commenced on continuous milk feeding with dextrose infusion. To further investigate for diazoxide-unresponsive hyperinsulinism, genetic analysis was performed for the infant and her parents. Whole exome sequencing revealed a likely pathogenic mutation c.1246C>T p. (R416X) in exon 5 of the *INSR* gene, which resulted in a change of the 416th codon from arginine to a premature termination. This nonsense mutation resulted in a truncated protein product. Her father carried the same mutation.

With the molecular diagnosis, diazoxide and Polycal supplement were gradually tapered. Dextrose infusion was weaned and bolus feeding was re-introduced on a three-hourly basis. Less post-prandial excursion, followed by a less severe plunge in blood glucose (BG) level, was observed. She finally passed an 8-hour fasting challenge with BG 82.7 mg/dL at the end of the test and was discharged with bolus feeding at 4 months old. Regular home BG monitoring showed no hypoglycaemia and she adopted a weaning diet with low glycaemic-index food. Subsequent assessment at 20 months old showed normal neurological development.

We also evaluated our proband's father in view of the mutation identified. In retrospect, he reported dizziness and tiredness after large carbohydrate meals but had never required medical attention. Physical examination showed a BMI of 26.6 kg/m2 with no acanthosis nigricans. His fasting BG, hemoglobin A1c, lipid profile and liver function tests were normal. His homeostatic model assessment for insulin resistance (HOMA-IR) was 3.0 which was >95th centile cut off for normal glucose tolerance in southern Chinese (3). A 6-hour OGTT with 75 grams oral anhydrous glucose solution was performed (Table 2). He had normal glucose tolerance, but fasting hyperinsulinemia and elevated insulin-to-C-peptide ratio of 0.42 (normal range for fasting < 0.1)(4). At 210 minutes, he developed asymptomatic hypoglycaemia with BG down to 46.8 mg/dL and paired insulin was 132 pmol/L. He was subsequently referred to the adult endocrine unit for follow up.

The parents of this infant, and the father himself, gave written consent to the writing of this manuscript. The study has been approved by the ethics committee of the Hong Kong West Cluster Clinical Research Ethics Review Board (HKWC-2022-249).

Discussion

We reported an infant with heterozygous mutation in *INSR* gene who presented with hyperinsulinism in the neonatal period, highlighting the importance to consider this entity especially in the setting of excessive post-prandial glucose excursion followed by reactive hypoglycaemia. Apart from treatment implication, cascade screening for family members is crucial in early identification of individuals at risk of young-onset glucose intolerance and insulin-resistant diabetes. These mutation carriers may benefit from dietary modification and use of insulin-sensitizing drugs such as metformin and glitazones (2,5).

Hypoglycaemia associated with heterozygous *INSR* mutations has been described in a few adult studies **(4,6–8)**. Most experienced hypoglycaemic during fasting and more characteristically after meal, which was similar to our case. Diagnosis was made with fasting hyperinsulinism, elevated fasting insulin to C-peptide ratio and hypoglycemia on prolonged OGTT. Further hyperinsulinemic-euglycaemic clamp studies in them showed markedly reduced insulin sensitivity and lowered metabolic clearance rate for insulin compared to control. As a result, there is excessive insulin secretion after meal loading, which persists at high concentrations even with a falling BG level, therefore resulting in suppressed hepatic glucose output and postprandial hypoglycaemia (4,6). This phenomenon was also observed in our proband's father who demonstrated fasting and postload hyperinsulinemia, as well as hyperinsulinemic hypoglycaemia (BG 46.8mg/dL mmol/L, insulin 132pmol/L) at 210 minutes of OGTT. Notably, he also exhibited elevated insulin to C-peptide ratio. In normal physiological conditions, insulin and C-peptide are co-secreted by the pancreas, with insulin rapidly metabolized by the liver and C-peptide slowly eliminated by the kidneys (9). Hence, elevated insulin to C-peptide ratio was suggestive of decreased clearance of endogenous insulin as a result of the underlying *INSR* mutation. While he has not developed frank diabetes, long term follow up of his metabolic profile will be necessary and avoidance of high glycaemic index food may help to ameliorate symptoms

of post-prandial hypoglycaemia.

The *INSR* gene, located on chromosome 19, consists of 22 exons and 21 introns. Exons 1-11 (and part of exon 12) encode the extracellular α-subunits of the receptor that bind insulin, whereas exons 12-22 encode the β-subunits that span the plasma membrane and have an intracellular tyrosine kinase domain. Mutations in the α-subunits lead to decrease in the number of mature INSR or defective insulin binding while mutations in the β-subunits impair autophosphorylation and subsequent activation of downstream signaling transduction. Longo et al demonstrated that mutations markedly impairing insulin binding resulted in the most severe phenotype with early demise, while mutations leaving residual insulin binding activity were associated with longer survival (10). And while there is no definite genotype-phenotype correlation due to rarity of these syndromes, mutations affecting the α-subunit of the receptor are generally associated with a more severe phenotype than those affecting the β-subunit (10,11). Hence majority of patients with Donohue syndrome have mutations in the α-subunit, while type-A IR syndrome is more frequently associated with mutations in the tyrosine kinase domain of the β-subunit (6)(7)(8)(4)(11). The nonsense mutation c.1246C>T identified in our proband and his father is located in the second leucine-rich repeat domain (L2) of the extracellular ligand-binding α-subunit. It was previously reported in a boy diagnosed with Donohue syndrome at 1 month old. Interestingly, this boy only carried a single mutation, same as our proband, but presented early with severe phenotype (12). Unfortunately we were not able to perform functional analysis which could possibly explain the milder phenotype in our case. Nevertheless, this is the first report of a heterozygous α-subunit mutation causing neonatal hyperinsulinism with mild presentation.

Neonatal hyperinsulinimic hypoglycaemia linked to heterozygous *INSR* mutation was first reported in 4 infants from 3 families by Sethi et al (13). All these infants had mutations located in exon 20 of the *INSR* gene, which encode the β-subunit of the receptor. They were all born small for gestational age and developed hypoglycaemia on first day of life. In contrast to our proband, they showed good response to diazoxide therapy (at a dose between 3- 7.5mg/kg/day) and were able to wean off the medication before 1 year of age. Diazoxide acts to open pancreatic β-cell ATP – sensitive potassium (K_{ATP}) channels and inhibit insulin secretion. The mechanism in which hyperinsulinism in these infants responds to diazoxide is not clear. In contrast to the reported cases, glucose profile in our infant worsened after diazoxide. We postulated that use of diazoxide exacerbated post-prandial glucose excursion by inhibiting insulin release while hypoglycaemia that follows could be explained by reduced degradation or clearance of insulin due to the underlying mutation.

The most common form of monogenic hyperinsulinism is caused by inactivating mutations in the *ABCC8* or *KCNJ11* genes, which encode subunits of the K_{ATP} channel. These mutations also account for almost 90% of diazoxide-unresponsive hyperinsulinism cases, followed by activating mutations of glucokinase (*GCK*) gene (14,15). Different from infants with *INSR* mutation, those who harbour *ABCC8* or *KCNJ11* mutations typically present with fasting hypoglycaemia rather than post-prandial hypoglycaemia. In addition, post-prandial hyperglycaemia, a prominent feature in patients harbouring *INSR* gene mutations, helps to differentiate these conditions. Birth weight also provides another important clue to help identify patients with diazoxide-unresponsive hyperinsulinism due to K_{ATP} channel mutations, as these babies are usually born marcrosomic. Therefore, careful history taking and biochemical phenotyping in the evaluation of hyperinsulinism are very helpful in recognizing patients with possible *INSR* gene mutations. CGMS was used to monitor the glucose profile in our infant. While accuracy might be an issue in young infant, CGMS offers great value in the evaluation of glucose fluctuation, thereby helping clinicians consider the diagnosis of *INSR* gene mutations.

In conclusion, the present study details the clinical and biochemical features of an infant with hyperinsulinimic hypoglycaemia caused by heterozygous *INSR* gene mutation. Response to diazoxide therapy was poor and resulted in even more severe post-prandial hyperglycaemia. While further accumulation of clinical experience in managing this group of children is required, accurate genetic diagnosis of the condition is essential to ensure regular monitoring of metabolic control and prompt initiation of intervention when necessary.

Reference

1.   Ben Harouch S, Klar A, Falik Zaccai TC. INSR-Related Severe Syndromic Insulin Resistance. In: Adam MP, Everman DB, Mirzaa GM, Pagon RA, Wallace SE, Bean LJ, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993 [cited 2022 Nov 3]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK476444/

2.   Takasawa K, Tsuji-Hosokawa A, Takishima S, Wada Y, Nagasaki K, Dateki S, et al. Clinical characteristics of adolescent cases with Type A insulin resistance syndrome caused by heterozygous mutations in the β-subunit of the insulin receptor (*INSR*) gene: 胰岛素受体(*INSR*)基因β亚基杂合突变所导致A型胰岛素抵抗综合征的青少年病例临床特征. Journal of Diabetes. 2019 Jan;11(1):46–54.

3.   Lee CH, Shih AZL, Woo YC, Fong CHY, Leung OY, Janus E, et al. Optimal Cut-Offs of Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) to Identify Dysglycemia and Type 2 Diabetes Mellitus: A 15-Year Prospective Study in Chinese. Hu C, editor. PLoS ONE. 2016 Sep 22;11(9):e0163424.

4.   Huang Z, Li Y, Tang T, Xu W, Liao Z, Yao B, et al. Hyperinsulinaemic hypoglycaemia associated with a heterozygous missense mutation of R1174W in the insulin receptor (IR) gene. Clinical Endocrinology. 2009 Nov;71(5):659–65.

5.   Musso C, Cochran E, Moran SA, Skarulis MC, Oral EA, Taylor S, et al. Clinical Course of Genetic Diseases of the Insulin Receptor (Type A and Rabson-Mendenhall Syndromes): A 30-Year Prospective. Medicine. 2004 Jul;83(4):209–22.

6.   Højlund K, Hansen T, Lajer M, Henriksen JE, Levin K, Lindholm J, et al. A Novel Syndrome of Autosomal-Dominant Hyperinsulinemic Hypoglycemia Linked to a Mutation in the Human Insulin Receptor Gene. Diabetes. 2004 Jun 1;53(6):1592–8.

7.   Preumont V, Feincoeur C, Lascols O, Courtillot C, Touraine P, Maiter D, et al. Hypoglycaemia revealing heterozygous insulin receptor mutations. Diabetes & Metabolism. 2017 Feb;43(1):95–6.

8.   Kuroda Y, Iwahashi H, Mineo I, Fukui K, Fukuhara A, Iwamoto R, et al. Hyperinsulinemic hypoglycemia syndrome associated with mutations in the human insulin receptor gene: Report of two cases. Endocr J. 2015;62(4):353–62.

9.   Lebowitz MR. The Molar Ratio of Insulin to C-Peptide: An Aid to the Diagnosis of Hypoglycemia due to Surreptitious (or Inadvertent) Insulin Administration. Arch Intern Med. 1993 Mar 8;153(5):650.

10. Longo N. Genotype-phenotype correlation in inherited severe insulin resistance. Human Molecular Genetics. 2002 Jun 1;11(12):1465–75.

11. Ardon O, Procter M, Tvrdik T, Longo N, Mao R. Sequencing analysis of insulin receptor defects and detection of two novel mutations in INSR gene. Molecular Genetics and Metabolism Reports. 2014;1:71–84.

12. Semple RK, Soos MA, Luan J, Mitchell CS, Wilson JC, Gurnell M, et al. Elevated plasma adiponectin in humans with genetically defective insulin receptors. J Clin Endocrinol Metab. 2006 Aug;91(8):3219–23.

13. Sethi A, Foulds N, Ehtisham S, Ahmed SH, Houghton J, Colclough K, et al. Heterozygous Insulin Receptor (INSR) Mutation Associated with Neonatal Hyperinsulinemic Hypoglycaemia and Familial Diabetes Mellitus: Case Series. Jcrpe. 2020 Nov 25;12(4):420–6.

14. Rosenfeld E, Ganguly A, De Leon DD. Congenital hyperinsulinism disorders: Genetic and clinical characteristics. Am J Med Genet. 2019 Dec;181(4):682–92.

15. Kapoor RR, Flanagan SE, Arya VB, Shield JP, Ellard S, Hussain K. Clinical and molecular characterisation of 300 patients with congenital hyperinsulinism. European Journal of Endocrinology. 2013 Apr;168(4):557–64.

Authorship contribution

Table 1: Biochemical parameters during hypoglycaemia

	Day 37 of life	Day 82 of life
Insulin (pmol/L)	104.2	50.7
Blood glucose (BG) (mg/dL)	25.0	43.2
Free fatty acid (FFA) (mEq/L)	0.11	0.13
Beta-hydroxybutyrate(B-OHB) (mmol/L)	0.05	0.1

Table 2: Extended 6-hour OGTT of proband's father

Time (min)	0	30	60	90	120	150	180	210	240	270	300	330	360
Glucose (mg/dL)	72.0	180.0	144.0	118.8	104.4	122.4	75.6	46.8	66.6	75.6	81.0	82.8	88.2
C-peptide (pmol/L)	280	2500	2020	1920	1630	1520	830	420	310	280	210	230	280
Insulin (pmol/L)	118	1750	1229	1236	819	854	305	132	97	97	76	90	118
Insulin-to C peptide ratio	0.42	0.70	0.61	0.64	0.50	0.56	0.37	0.31	0.31	0.35	0.36	0.39	0.42