The Effectiveness of Sirolimus Treatment in Two Rare Disorders with Nonketotic Hypoinsulinemic Hypoglycemia: The Role of mTOR Pathway

Zeynep Şıklar1, Tuğba Çetin1, Nilgün Çakar2, Merih Berberoğlu1

1Ankara University Faculty of Medicine, Department of Pediatric Endocrinology, Ankara, Turkey
2Ankara University Faculty of Medicine, Department of Pediatric Reumatology, Ankara, Turkey

What is already known on this topic?
Nonketotic-hypoinsulinemic hypoglycemia (NkHH) is a very rare problem of glucose consumption increase without hyperinsulinism. In these cases, there is no effective therapy other than frequent feeding to counter hypoglycemia.

What this study adds?
Sirolimus treatment may function as a further therapeutic option in NkHH. It could be a lifesaving tool for those kind of disorders as sirolimus appears to control the persistent hypoglycemia effectively in patients with NkHH, by inhibition of mammalian target of rapamicin.

Abstract
Nonketotic-hypoinsulinemic hypoglycemia (NkHH) is a very rare problem characterized by increase in glucose consumption without hyperinsulinism. This disorder has mainly been reported in cases with AKT2 mutation and rarely in cases with PTEN mutation. In cases with PTEN or AKT2 mutation, there is no effective therapy other than frequent feeding to counter hypoglycemia. The mammalian target of rapamicin (mTOR) inhibitor, sirolimus, has been used in hyperinsulinemic hypoglycemia that was unresponsive to other medical treatment. In the insulin signaling pathway, both AKT2 and PTEN function upstream of mTOR. However, the role of Sirolimus on hypoglycemia in AKT2 and PTEN mutations is unknown. Case 1: Six month-old female with AKT2 mutation [c.49G>A (p.E17K)] and evidence of NkHH. Frequent feeding was unsuccessful in correcting hypoglycemia and her proptosis continued to worsen. Sirolimus treatment was started at three years of age. Subsequently, blood glucose (BG) levels increased to normal levels. Case 2: In a male with PTEN mutation (p.G132V (c.395G>T), persistent NkHH started at 16 years of age (fasting BG: 27 mg/dL, fasting insulin 1.5 mmol/L, while ketone negative). Sirolimus treatment was started and hypoglycemia was successfully controlled. NkHH is a very rare and serious disorder which is challenging, both for diagnosis and treatment. Additionally, AKT2 and PTEN mutations may result in NkHH. Sirolimus treatment, through mTOR inhibition, appeared to be effectively controlling the persistent hypoglycemia and may be a life-saving therapy in this NkHH due to AKT2 and PTEN mutations.

Keywords: AKT2, PTEN, sirolimus, hypoglycemia, treatment

Introduction
Recurrent/persistent fasting hypoglycemia is a life-threatening condition in childhood and is frequently related to either hyperinsulinism or inborn errors of metabolism impairing hepatic glucose production (1). Hyperinsulinism is the most common cause of persistent hypoketotic, hypo-fattyacidemia, hypoglycaemia in infancy and childhood. In this situation, excessive insulin secretion suppresses the mobilisation of fatty acids from adipose tissue, preventing ketone body synthesis in the liver (2,3). Another well known cause of nonketotic hypoglycemia are fatty acid oxidation defects (4).
Recently, a few cases with unexplained, recurrent and severe fasting hypoglycemia without hyperinsulinism or fatty acid oxidation defects have been reported (1,5,6,7). We preferred to use the term of “nonketotic-hypoinsulinemic hypoglycemia (NkHH)” in these cases.

NkHH is a very rare problem of glucose consumption increase without hyperinsulinism. In 2011, the first case with genetic defects of AKT2 leading to NkHH was published. AKT2 is a serine/threonine kinase that plays an important role in insulin signal transduction (7). Normally, when insulin combines with its receptor at target tissue, it requires phosphatidylinositol-3,4,5-trisphosphate (PIP3) to accumulate at the plasma membrane to facilitate insulin transmission within the cell. Gain of function mutation of AKT2 cause PIP3 accumulation without the need for insulin. The biochemical profile of AKT2 activating mutation is very similar to hyperinsulinism (7).

Similar to AKT2 activating mutation, a defect in other molecules that have a role in insulin signalling are expected to be a cause of NkHH. Rarely, NkHH can also develop with mutation of the tumor suppressor gene PTEN (8).

Treatment of hypoglycemia in NkHH can be very difficult, because there is no beneficial medical therapy to counteract insulin synthesis or secretion. In these cases, no effective therapy is available other than frequent feeding to prevent hypoglycemia. Thus, there is no available therapy at all in patients who cannot be fed for any reason, including vomiting, gastrointestinal problems, or anorexia.

The mammalian target of rapamycin (mTOR) inhibitor, sirolimus, has been used in hyperinsulinemic hypoglycemia which was unresponsive to other medical treatment (3,9). In the insulin signaling pathway, both AKT2 and PTEN function upstream of mTOR. Thus inhibition of mTOR should counter activating mutations in AKT2 and PTEN. However, the effect of sirolimus in hypoglycemia due to AKT2 and PTEN mutations is unknown.

In this paper, clinical and biochemical characteristics of two rare cases with NkHH are presented. Additionally, the effect of sirolimus on hypoglycemia is reported in these cases.

**Case Reports**

**Case 1**
A seven month-old female patient was brought to clinic by her family because she had recurrent hypoglycemia for one month. She was born at term with a history of polyhydramnios. On physical examination there was bilateral proptosis, hypertrichosis, hypertelorism, a flat nasal bridge, macroglossia and acanthosis nigricans. At the time of admission her height was 68 cm (50th percentile) with a weight of 7700 g (25-50th percentile) and a head circumference of 44 cm (75th percentile). She had hypoinsulinemia (<0.2 miU/mL, C-peptide <0.1 ng/mL) and was nonketotic during hypoglycemia when blood glucose (BG) was 27 mg/dL. Other biochemical and hormonal analysis showed normal results. Due to hypoglycemia occurring during fasting, frequent feeding and addition of cornstarch to foods was implemented. Whenever severe hypoglycemia occurred, intravenous glucose infusion was also given.

Genetic analysis revealed a de novo AKT2 mutation [c.49G>A (p.E17K)] in the patient (5). During follow-up, frequent feeding was unsuccessful in treating all the hypoglycemic episodes. Clinically, acanthosis nigricans and proptosis continued to worsen (Figure 1). After informed consent was given by her parents, sirolimus treatment was started at three years of age. On sirolimus treatment BG levels increased to normal levels (mean BG before treatment: 48-52 mg/dL/day, after treatment 77-108 mg/dL/day). Prior to starting sirolimus treatment, it was observed that she could not fast longer than 3 hours although this increased to 4 to 5 hours with the treatment. Neurological evaluation revealed normal language, cognitive, social, and fine motor development with a slight delay in gross motor development.

![Figure 1. Case 1 with AKT2 mutation](image.png)
Case 2
A male with multiple systemic involvement was diagnosed with hamartoma-tumor syndrome before his admission to the endocrinology department. He had verrucous epidermal nevus and adrenal hemorrhage at birth, and at five month of age renal vein and inferior vena cava thrombosis with hypertension. At 16 months of age, he developed pelvic and retroperitoneal lipomatosis, multiple polyps of the colon and focal segmental glomerulosclerosis. Total colectomy for polyps was carried out due to the recurrent bleeding. He also had macrocephaly, delayed motor mental development and epileptic seizures (Figure 2). These symptoms suggested PTEN hamartoma-tumor syndrome (PHTS), so mutation analysis was conducted. Results revealed a PTEN mutation [p.G132V (c.395G>T)] (10). Due to the PTEN mutation, he carried a high risk of thyroid malignancy. Due to this risk prophylactic thyroidectomy was performed and thyroxine replacement was started.

During his follow-up, at 16 years of age, severe recurrent hypoglycemia (fasting BG: 6 to 27 mg/dL) was noted. Fasting insulin was low (1.5 mmol/L), while ketone was negative. Hypoglycemia was persistent even though there was no known causes of hypoglycemia, with no detection of congenital metabolic disorders.

A tentative diagnosis of NkHH was reached and frequent feeding was offered, but this was ineffective in resolving the hypoglycemic attacks. In addition, feeding was not always possible due to occasional anorectic episodes.

We were aware that PTEN may have a role in the insulin signalling pathway and there were reports suggesting that a few patients with PTEN mutation also had hypoglycemic events (7,8).

After informed consent was given, sirolimus treatment was started. Follow-up examinations and evaluations were done at three-monthly intervals. These included complete blood count, serum BUN, creatinine, electrolytes, aminotransferase measurement (aspartate aminotransferase and alanine aminotransferase), lipid profile and hemoglobin A1c. Sirolimus treatment also resulted in an improvement in duration of fasting time in this patient, although the effect was not as marked. Fasting time for case 2 increased to 3 to 4 hours, up from no more than 2 hours, prior to sirolimus. Case 2 required an increased dose of sirolimus during the first month of treatment. With increased dosage, the frequency and severity of hypoglycemia reduced: mean BG before treatment was 46-64 mg/dL/day; after treatment this was 62-92 mg/dL/day. The lowest fasting glucose level of 32 mg/dL was experienced by case 2.

Sirolimus Dosing
For both patients, initial sirolimus dose was 0.5 mg/m²/day. The dose of sirolimus was then titrated according to the serum level for both patients (between 4 and 12 mg/dL). Final sirolimus dose was 1 mg/m²/day in case 1 and 4 mg/m²/day in case 2. Of note, transient leucocytosis was observed in case 1 without any other obvious cause. Duration of sirolimus treatment was 42 months in case 1 and nine months in case 2.

Informed consent for the publication of these cases was obtained from both sets of parents.

Discussion
It is accepted that glucose homeostasis is maintained by the action of insulin on muscle, adipose tissue and liver (5). Insulin stimulate energy storage and growth through effects on glucose, lipid and amino acid metabolism. At the cellular
level, insulin effects are mediated by a transmembrane tyrosine kinase receptor that phosphorylates insulin receptor substrate (IRS) and other adaptor proteins. Further downstream, insulin signaling leads to activation of AKT serine/threonine kinases (1).

In recent times, NkHH cases due to activation of the insulin signaling pathway have begun to be published. AKT2 mutation has been shown to be directly causative for this specific condition (1,8,11). AKT2 is critical for the control of glucose and lipid metabolism. It is recruited to the cell surface by phosphoinositide 3-kinase (PI3K) and phosphorylated by pyruvate dehydrogenase kinase 1 and mTOR c2 kinases. It has a transducer effect during insulin signaling to GLUT4 (12). The causes of hypoglycemia in AKT2 mutation are related to the activation of the insulin signaling pathway. The first case with a gain of function of AKT2 causing hypoinsulinemic hypoglycemia was reported in 2011 (1). Since then, a few cases have been reported, including the two presented herein (5,6,7).

AKT2 is a signal transducer in both glucose metabolism and lipid homeostasis (12). In our case, extra-ocular adipose tissue expansion leading to proptosis was prominent. Some deposits of adipose tissue may be more responsive to AKT2 mutation than others. The cause of this difference is unknown although one plausible explanation may be different levels of expression of AKT2 in metabolic tissues (12).

PTEN is one the most important tumour suppressors. Deactivation of PTEN causes the activation of mTOR c1, which in turn leads to the augmented translocation of specific mRNAs which is crucial for cell growth and proliferation (13). The PTEN–PI3K–AKT–mTOR pathway has a central role in the regulation of glucose metabolism. This pathway has downstream effects on the insulin receptor and IRS adaptor molecules. It is known that the PI3K–AKT pathway enhances insulin-mediated glucose uptake and membrane translocation of the glucose transporter GLUT4, and inhibits gluconeogenesis (13). PTEN deficiency results in enhanced activation of the AKT signaling pathway. A mutation of PTEN may augment PI3K signaling to AKT, which then affects the mTOR pathway (14).

Usually, hypoglycemia in a patient with PTEN gene mutation is not noticed. Schmid et al (8) reported a case with PHTS, which was caused by germ line mutations in the PTEN gene. The case was treated with sirolimus for uncontrolled tumor cell proliferation. In this case a fasting glucose level of 1.9 mmol/L (reference: 3.6-5.6 mmol/L; equivalent to 35 mg/dL) was detected at the age of 42 months, although there was no extra information about the course of hypoglycemia. In our PTHS patient (case 2), hypoglycemia was detected at 16 years old. Blood sugar levels were very low at times and frequent feeding was ineffective to resolve hypoglycemia. In particular, the management of hypoglycemia in anorectic periods in this patient were very challenging. This situation prompted the search for alternative or additional treatment modalities.

PTEN loss induces adipogenic-like transformation in hepatocytes and transcription of genes involved in lipogenesis and β-oxidation (13). Additionally, mTOR has a central role in the regulation of cell cycle and initiation of transcription by translating the signalling for growth and proliferation. The administration of rapamycin (mTOR inhibitor) in patients with PTEN mutation has been found to be effective in reducing hamartomatous masses, lipomatous lesions, and thymus hyperplasia with clinical recovery (8,15,16). Hence, both hypoglycemia and uncontrolled cell proliferation in various tissues may be controlled with mTOR inhibition. With sirolimus treatment, hypoglycemia in case 2 was controlled more succesfully.

A dysregulated PTEN–PI3K–AKT–mTOR signaling pathway may result not only in extensive tumor cell proliferation, but also deregulation of glucose metabolism. Increased glucose utilization is consistent with hyperactivation of the PI3K/AKT pathway, being one of the key mediators of increased glucose utilization observed in many cancer cells (17). Kinross et al (18) developed a murine model with Pik3ca H1047R mutation. Pik3ca is the gene encoding p110, a catalytic subunit of PI3K. In this model, a dramatic increase in body weight, which was associated with increased organ size, a reduction in BG levels and undetectable insulin levels, were observed. In the human, mosaic activating mutations in PI3K are known to cause segmental overgrowth. In a study which evaluated the metabolic phenotype of 22 patients with mosaic activating mutations affecting PI3K, three patients were found to have early onset, severe, nonketotic hypoglycemia (11).

With all of these findings, it seems probable that dysregulation of the insulin signalling pathway affecting AKT2, PTEN, or PI3K could cause NkHH with syndromic features. Unexplained hypoglycemia mimicking that of hyperinsulinism with no detectable insulin should alert the physician to a possible insulin transducing defect. An effective treatment option for hypoglycemia in these patients could be to block insulin signalling. The only known, widely-used agent to impact this pathway is the mTOR inhibitor, sirolimus. Until now, sirolimus has been successfully used for the severe, diffuse form of congenital hyperinsulinism (9). In our cases, we decided to give sirolimus to improve hypoglycemia in a patient with AKT2 mutation and another patient with PTHS as sirolimus will inhibit mTOR, the next
downstream step in PTEN/AKT signalling. In both patients hypoglycemia was controlled with sirolimus treatment.

The reported side effects of sirolimus treatment include immunosuppressive effects, oral mucositis, renal dysfunction, pneumonitis, increased serum aminotransferase levels, hepatitis, and dyslipidemia (9,19). Of these, only mild leucocytosis was observed in case 1 without any additional symptoms.

Conclusion

NkHH is a very rare but significant disorder which presented some challenges in both diagnosis and treatment. Activating mutations in AKT2 or PTEN, upstream from mTOR in the insulin signalling pathway, may lead to NkHH. Sirolimus treatment, resulting in mTOR inhibition, appeared to be effective in controlling the persistent hypoglycemia in two cases. Sirolimus may be a life-saving therapeutic option for some of these rare diseases caused by increased activation of insulin signalling.

Ethics

Informed Consent: Informed consent for the publication of these cases was obtained from both sets of parents.

Peer-review: Externally peer-reviewed.

Authorship Contribution

Concept: Zeynep Şıklar, Merih Berberoğlu, Design: Zeynep Şıklar, Merih Berberoğlu, Data Collection or Processing: Zeynep Şıklar, Tuğba Çetin, Nilgün Çakar, Analysis or Interpretation: Zeynep Şıklar, Merih Berberoğlu, Literature Search: Zeynep Şıklar, Writing: Zeynep Şıklar.

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Hussain K, Challis B, Rocha N, Payne F, Minic M, Thompson A, Daly A, Scott C, Harris J, Smillie BJ, Savage DB, Ramaswami U, De Lonlay P, O’Rahilly S, Barroso I, Semple RK. An activating mutation of AKT2 and human hypoglycemia. Science 2011;334:474-477. Epub 2011 Oct 6
2. Hussain K, Bodamer OA, Cameron FJ, Camacho-Hubner C, Soos MA, Jones J, Krywawych S, O’Rahilly S, Aynsley-Green A. Hypoketotic hypofattyacidaemic hypoinsulinemic hypoglycemia in a child with hemihypertrophy? A new syndrome. Horm Res 2004;61:222-227. Epub 2004 Feb 2
3. Demirbilek H, Hussain K. Congenital hyperinsulinism: diagnosis and treatment update. J Clin Res Pediatr Endocrinol 2017;9(Suppl 2):69-87. Epub 2017 Dec 27
4. Houten SM, Wanders RJ. A general introduction to the biochemistry of mitochondrial fatty acid oxidation. J Inherit Metab Dis 2010;33:469-477. Epub 2010 Mar 2
5. Garg N, Bademci G, Foster J, Şıklar Z, Berberoğlu M, Tekin M. MORFAN Syndrome: An Infantile Hypoinsulinemic Hypoketotic Hypoglycemia Due to an AKT2 Mutation. J Pediatr 2015;167:489-491. Epub 2015 May 23
6. Atya VB, Flanagan SE, Schober E, Rami-Merhar B, Ellard S, Hussain K. Activating AKT2 mutation: hypoinsulinemic hypoketotic hypoglycemia. J Clin Endocrinol Metab 2014;99:391-394. Epub 2013 Nov 27
7. Minic M, Rocha N, Harris J, Groeneveld MP, Leiter S, Wareham N, Sleigh A, De Lonlay P, Hussain K, O’Rahilly S, Semple RK. Constitutive activation of AKT2 in humans leads to hypoglycemia without fatty liver or metabolic dyslipidemia. J Clin Endocrinol Metab 2017;102:2914-2921.
8. Schmid GL, Kassner F, Uhlig HH, Körner A, Kretsch M, N, Zepp F, Kowalzik F, Laner A, Starke S, Wilhelm FK, Schuster S, Viehweger A, Hirsch W, Kiess W, Garten A. Sirolimus treatment of severe PTEN hamartoma tumor syndrome: case report and in vitro studies. Pediatr Res 2014;75:527-534. Epub 2013 Dec 23
9. Minute M, Patti G, Torinese G, Faleschini E, Zuliani C, Ventura A. Sirolimus Therapy in Congenital Hyperinsulinism: A Successful Experience Beyond Infancy. Peditrians 2015;136:e1373-e1376.
10. Teksin M, Hismi BO, Fitoz S, Yalcinkaya F, Ekim M, Kansu A, Ertem M, Dedg, Tutar E, Arsan S, Zhou XP, Pilsaraki R, Eng C, Akar N. A germline PTEN mutation with manifestations of prenatal onset and verrucous epidermal nevus. Am J Med Genet A 2006;140:1472-1475.
11. Leiter SM, Parker VER, Welters A, Knox R, Rocha N, Clark G, Payne F, Lotta L, Harris J, Guerrero-Fernández J, González-Casado J, García-Mihaur S, Gordo G, Wareham N, Martinez-Gleiz V, Allison M, O’Rahilly S, Barroso I, Meissner T, Davies S, Hussain K, Temple K, Barreda-Bonis AC, Kummer S, Semple RK. Hypoinsulinemic, hypoketotic hypoglycemia due to mosaic genetic activation of P53-kinase. Eur J Endocrinol 2017;177:175-186. Epub 2017 May 31
12. Kajono E, McGraw TE, Gonzalez E. Development of a new model system to dissect isoform specific Akt signalling in adipocytes. Biochem J 2015;468:425-434. Epub 2015 Apr 9
13. Song MS, Salmena L, Pandolfi PP. The functions and regulation of the PTEN tumour suppressor. Nat Rev Mol Cell Biol 2012;13:283-296.
14. Patel M, Gomez NC, McFadden AW, Moats-Staats BM, Wu S, Rojas A, Sapp T, Simon JM, Smith SV, Kaiser-Rogers K, Davis JJ. PTEN deficiency mediates a reciprocal response to IGFI and mTOR inhibition. Mol Cancer Res 2014;12:1610-1620. Epub 2014 Jul 3
15. Iacobas I, Burrows PE, Adams DM, Suton VR, Hollier LH, Chintagumpala MM. Oral rapamycin in the treatment of patients with hamartoma syndromes and PTEN mutation. Pediatr Blood Cancer 2011;57:321-323. Epub 2011 Feb 25
16. Marsh DJ, Trahair TN, Martin JL, Chee WY, Walker J, Kirk EP, Baxter RC, Marshall GM. Rapamycin treatment for a child with germline PTEN mutation. Nat Clin Pract Oncol 2008;5:357-361. Epub 2008 Apr 22
17. Robey RB, Hay N. Is Akt the “Warburg kinase”?-Akt-energy metabolism interactions and oncogenesis. Semin Cancer Res 2014;12:1610-1620. Epub 2014 Jul 3
18. Lin Xiao, Cao R, Tiongco AM, Cai L, El-Omar EM, Shi XL. PTEN deficiency mediates a reciprocal response to IGFI and mTOR inhibition. Mol Cancer Res 2014;12:1610-1620. Epub 2014 Jul 3
19. Kinross KM, Montgomery KG, Mangoafico SP, Hare LM, Kleinschmidt M, Bywater MJ, Poulton IJ, Vrahinas C, Henneicke H, Malaterre J, Waring PM, Cullinane C, Sims NA, McArthur GA, Andrikopoulos S, Phillips WA. Ubiquitous expression of the Pik3caH1047R mutation. J Clin Res Pediatr Endocrinol 2018;10:279-283. Epub 2017 Dec 8

Şıklar Z et al.

Sirolimus Treatment in Nonketotic Hypoinsulinemic Hypoglycemia