Case Report

Syndrome of Congenital Insulin Resistance Caused by a Novel \textit{INSR} Gene Mutation

Rojek A et al. Syndrome of Congenital Insulin Resistance

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What is already known about this topic?

- Leprechaunism (Donohue syndrome) is the most severe form of insulin resistance caused by autosomal recessive mutations in the insulin receptor gene and is characterized by extreme insulin resistance leading to hyperinsulinemia, impaired glucose homeostasis and prenatal and postnatal growth retardation.
- Moreover, dysmorphic features, hypertrichosis, acanthosis nigricans, macrogenitosomia, and polycystic ovaries and breast enlargement in females are also present, and common complications include hypertrophy of internal organs. Patients with leprechaunism usually die in the first year of life.

What does this study add?

- The study describes a novel mutation in the \textit{INSR} gene in the patient with Donohue Syndrome
- This case highlights the importance of genetic testing of the \textit{INSR} gene, which is crucial for genetic counseling as well as for improving the prognosis of patients with severe insulin resistance syndromes; the prognosis may strongly depend on the type and location of the \textit{INSR} gene mutation.
- Moreover, until now, there have been no reports of fatty liver disease in patients with proven loss of function of the insulin receptor, which raises the question of whether it plays a role in prognosis.
- The study also highlights the challenges faced by clinicians in the management of this complex, rare condition.

Abstract

Mutations in the insulin receptor gene result in rare inherited syndromes causing insulin resistance, such as leprechaunism (Donohue syndrome), Rabson-Mendenhall syndrome, and insulin resistance type A. Leprechaunism is an autosomal recessive disorder associated with extreme insulin resistance that leads to hyperinsulinemia, impaired glucose homeostasis, fasting hypoglycemia and postprandial hyperglycemia, impaired insulin action causes prenatal and postnatal growth retardation. Lipodystrophy, dysmorphic facies, hypertrichosis, macrogenitosomia and hypertrophy of internal organs are also present. A male infant with congenital insulin resistance was born at term after a normal pregnancy with a weight of 1905 g (<3c), a length of 48 cm (<3c), and an Apgar score of 10. Intrauterine growth retardation, transient hypoglycemia, pneumonia, urinary tract infection and heart defects (PFO – \textit{patent foramen ovale}; PDA - \textit{patent ductus arteriosus}) were diagnosed after birth. At 5 weeks of age, he was admitted to the regional hospital with severe fever, diarrhea and dehydration. Hyperglycemia was observed (672 mg/dl), and insulin was administered. He was referred to a hospital at 7 weeks of age for suspected neonatal diabetes and hypertrophic cardiomyopathy. The physical examination revealed a loud systolic heart murmur, tachycardia, tachypnea, dysmorphic facies, hypertrichosis, acanthosis nigricans, hypotonia, swollen nipples and enlarged testicles. Glycemic fluctuations (50-250 mg/dl) were observed. The serum insulin concentration was high (maximum 1200 IU/ml) at normoglycemia. Ultrasound of the heart confirmed progressive hypertrophic cardiomyopathy. Leprechaunism was confirmed by genetic analysis of the insulin receptor (\textit{INSR}) gene, in which a novel c.320C>G; p. Thr107Arg homozygous missense mutation in exon 2 was found.

Keywords: Insulin receptor, insulin resistance, leprechaunism

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Introduction

Insulin participates in a variety of biochemical processes, such as the metabolism of carbohydrates, lipids and proteins. It also influences cell proliferation, differentiation and apoptosis. It fulfills the role of a growth factor and a regulator of gene expression and contributes to protein biosynthesis (1).
Insulin acts through a specific insulin receptor (INSR), which is encoded by a gene (Gene ID: 3643, OMIM: *147670) located on the short arm of chromosome 19 (19p13.3) composed of 22 exons (2). The mature insulin receptor functions as a heterotrimer consisting of two dimers of two subunits (α and β, respectively). The α subunit is an extracellular ligand binding domain, whereas the intracellular β subunit functions as the catalytic domain of the receptor (3,4,5,6). Mutations in the INSR gene result in rare inherited syndromes of insulin resistance, such as leprechaunism (Donohue syndrome; OMIM: #246200), Rabson-Mendenhall syndrome (RMS; OMIM # 262190), insulin resistance type A (OMIM ID: 610549) and lipoatrophic diabetes mellitus (7,8,9,10,11).

Donohue syndrome is the most severe form of insulin resistance. It is a rare autosomal recessive disorder associated with extreme insulin resistance leading to hyperinsulinemia, impaired glucose homeostasis, fasting hyperglycemia and postprandial hyperglycemia. Impaired insulin action causes prenatal and postnatal growth retardation and lifelong insulin resistance. Dysmorphic features, such as prominent eyes, thick lips, a flattened nasal bridge with upturned nostrils, low-set posteriorly rotated ears and thick skin with a lack of subcutaneous fat are present. Hypertrophy of internal organs, including cardiomegaly, hepatosplenomegaly, and hypertrophy of the ovaries, is also a common complication. Moreover, hypertrichosis, acanthosis nigricans, macrogenitosomia, and polycystic ovaries and breast enlargement in females are also characteristic for children with Donohue syndrome, and in some of them, mental retardation is observed. Patients with leprechaunism usually die in the first year of life (10,12,13,14,15).

Patients with Rabson-Mendenhall syndrome (RMS) develop constant hyperglycemia, diabetic ketoacidosis and other complications of diabetes mellitus, which lead to death in the 1st or 2nd decade of life (16,17,18,19,20,21).

**Case report**

A male infant, which was the child of consanguineous, young, healthy parents from the Roma population, was admitted to the Department of Pediatric Endocrinology at the age of 7 weeks with suspected neonatal diabetes and hypertrophic cardiomyopathy. He was born at term after a normal pregnancy (Apgar 10). He was small for his gestational age, with a birth weight of 1905 g (SDS -3.73) and length of 48 cm (SDS -1.2). Intrauterine growth retardation, transient hypoglycemia, neonatal pneumonia, urinary tract infection and heart defects (PFO – patent foramen ovale; PDA - patent ductus arteriosus) were diagnosed just after birth. He was released from the neonatology unit twelve days after birth. At the age of 5 weeks, he was admitted to the regional hospital in a severe state with fever, diarrhea and dehydration. Hyperglycemia (672 mg/dl) was observed; therefore, insulin (i.v.) was administered for several days. Fluctuations in the blood glucose levels were observed afterwards (40-410 mg/dl) but unexpectedly normalized spontaneously thereafter. The child was referred to the Department of Pediatric Endocrinology at the age of 7 weeks with suspected neonatal diabetes. His body weight was 3000 g (SDS -2.5), and physical examination revealed a loud systolic heart murmur, tachycardia, tachypnea, and inspiratory-expiratory dyspnea. He had multiple phenotypic anomalies, including low-set large ears, coarse facial features, a flattened nasal bridge, thickened lips, generalized hypertrichosis, acanthosis nigricans in the skin folds and over the knees, decreased subcutaneous fat, hypotonia, prominent nipples and enlarged testicles (4 ml). Glycemic fluctuations (50-250 mg/dl) were initially observed. Postprandial hyperglycemia normalized spontaneously. Subsequently, at the age of 8 weeks, recurrent episodes of hypoglycemia occurred, and the patient required intravenous infusions of 10% glucose. The serum insulin concentration was elevated (maximum 1200 IU/ml) at normoglycemia (Table 1). Glycated hemoglobin AlC was at the lower normal limit (4.1%; normal 4.0-6.2%), which demonstrates the predominance of hypoglycemic episodes over postprandial hyperglycemic episodes. Serum liver enzymes and lipid concentration fluctuated around the upper range of normal limits. Moreover, serum IGF-1 and IGFBP3 were decreased (<25 ng/ml (normal 55-327) and <0.5 ug/ml (normal 0.7-3.6), respectively). Inherited metabolic disorders (amino acid and acylcarnitine profiles) were excluded based on the mass spectrometric results. Echocardiography confirmed hypertrophic cardiomyopathy with obstruction of the left ventricular outflow. A radiogram of the chest showed cardiomegaly, hepatomegaly, and hypertrophy of the ovaries, is also a common complication. Moreover, hypertrichosis, acanthosis nigricans, macrogenitosomia, and polycystic ovaries and breast enlargement in females are also characteristic for children with Donohue syndrome, and in some of them, mental retardation is observed. Patients with leprechaunism usually die in the first year of life (10,12,13,14,15).

Patients with Rabson-Mendenhall syndrome (RMS) develop constant hyperglycemia, diabetic ketoacidosis and other complications of diabetes mellitus, which lead to death in the 1st or 2nd decade of life (16,17,18,19,20,21).
electrophoresis on a 1.2%-1.5% agarose gel in the presence of ethidium bromide (Merck), purified from the gel using a QIAquick® Gel Extraction Kit (QIAGEN) and directly sequenced with the same primer pair used for PCR. All sequencing reactions were performed using the BigDye Terminator v 3.1 cycle sequencing kit (Applied Biosystems) on an ABI Prism 3130XL Genetic Analyzer (Applied Biosystems). Finally, the sequences were analyzed using VectorNTI 9.0 Software (Invitrogen) with the reference sequence NC_000019.08 (accession date: 03.03.2008).

Results
The glucose and insulin values for the patient serum are presented in Table 1.

Results of the genetic analysis of the INSR gene
The sequence analysis revealed the presence of a novel homozygous missense mutation, c.320C>G, in exon 2 that changed the polar threonine at position 107 into a basic arginine (p. Thr107Arg) (Figure 1). This variant was not found in control samples in the current version of the ESP6500 dataset deposited in the NHLBI Exome Variant Server, which comprises a set of 2203 African-American and 4300 European-American unrelated individuals with 6503 samples (13,006 chromosomes) in total (accession date: 25.01.2013), or in the ExAC and 1000G databases.

Three predictive tools were used to establish the overall effect of the p. Thr107Arg mutation in the presented patient. Analysis using SIFT (Sorting Intolerant From Tolerant, http://sift.jcvi.org/), which predicts the possible impact of a substitution based on sequences of similar peptides, assigned a score of 0 to the p. Thr107Arg mutation. This indicates that the mutation has a damaging effect on the INSR protein (the SIFT scale designates scores of less than 0.05 as deleterious, while scores of 0.05 or greater are predicted to be tolerated).

Furthermore, analysis using Mutation Taster (http://www.mutationtaster.org/) predicted the p. Thr107Arg mutation to be “disease causing” (score: 1.94; probability p=0.99).

Similarly, PolyPhen-2 (Polymorphism Phenotyping v2) software, which predicts the effects of a substitution based on the structure and function of a human protein using straightforward physical and comparative considerations (http://genetics.bwh.harvard.edu/pph2/), predicted this mutation to be “probably damaging” based on scores of 1.00 (HumDiv, sensitivity: 0.00; specificity: 1.00) and 0.999 (HumVar, sensitivity: 0.09; specificity: 0.99).

Discussion
In this study, we present an infant boy with clinical features and laboratory data typical of leprechaunism whose diagnosis was confirmed by genetic analysis of the insulin receptor gene, in which a novel mutation was found.

Donohue syndrome (leprechaunism) was first identified in 1948 by W. L. Donohue (22). The incidence of leprechaunism has been estimated to be at least 1 in 4 million live births (7). The molecular background of insulin resistance in leprechaunism has been associated with recessive mutations of the INSR gene. Due to such mutations, alternate receptors can no longer serve their function. Mutations in the INSR gene are extremely rare, which is why most cases of inherited severe insulin resistance result from consanguineous parents (12,14,23) as was the case for the infant described here.

Molecular studies carried out for our patient led to the identification of the novel homozygous missense mutation c. C320G in exon 2 in the INSR gene. Only one single nucleotide polymorphism C/T (SNP) at position 320 in the INSR gene (rs140762552, c.320C>T; p. Thr107Met) has been deposited in the SNP database (dbSNP, http://www.ncbi.nlm.nih.gov/snp) to date, but the variant described is of unknown significance. The impact of the mutation c.320G>G on receptor function is unknown; thus, the clinical diagnosis of leprechaunism was confirmed by in silico analysis. This novel mutation was predicted to disrupt a single-stranded right-handed beta-helix in the L-domain of the α subunit of INSR. This domain contains a cysteine-rich region composed of eight disulfide bonds. The three L-domains located in the α subunit of INSR surround a central space that is large enough to accommodate a hormone. Although the protein fragment comprising residues 1-462 does not bind insulin on its own, this central site exhibits many of the features crucial for insulin binding and ligand specificity (24). Therefore, the p. Thr107Arg mutation may disrupt the ability of INSR to bind its ligand.

Longo et al. (23) investigated several patients with inherited insulin resistance syndromes and different survival times that ranged from a few weeks to several years. They distinguished two phenotypes in their patients, leprechaunism and Rabson-Mendenhall syndrome, and tried to establish the genotype-phenotype correlation. They identified new mutations in the INSR gene and analyzed the correlation between these mutations and the survival rate. Mutations that completely or markedly impaired insulin binding to the receptor resulted in the most severe phenotype with early death (leprechaunism), while mutations resulting in residual insulin-binding activity were associated with a longer lifespan (RMS).

We studied a patient with severe insulin resistance who had a phenotype consistent with the descriptions of patients with leprechaunism reported in the literature (14,22,25). Our patient demonstrated all of the relevant disorders of carbohydrate metabolism: fasting hypoglycemia, postprandial hyperglycemia, hyperinsulinemia and severe insulin resistance. A combination of decreased hepatic glucose output in the fasting state and decreased hepatic glycogen synthesis during feeding due to a postreceptor defect in insulin action leads to fasting hypoglycemia and postprandial hyperglycemia (26).

Another consequence of insulin resistance is defects in fatty acid metabolism, which are responsible for the pathogenesis of fatty liver disease (26). The postmortem examination of our patient showed the presence of fatty liver. To date, there have been no reports of fatty liver disease in patients with proven loss of function of the insulin receptor. Donohue and Uchida presented various results of liver histological examinations, including the absence of abnormalities, nonspecific lesions consisting of focal degeneration or necrosis, and focal increases in glycogen and iron deposits (14).

Despite extreme insulin resistance, patients with primary defects at the level of the insulin receptor (generalized insulin resistance) did not manifest metabolic dyslipidemia. Despite higher plasma free fatty acids and glucose levels and massively increased plasma insulin levels, liver fat measurements were normal in the patients with insulin receptor mutations. Theoretically, this could be a result of either reduced hepatic lipogenesis or increased oxidation or excretion of liver triglycerides (27,28). This patient could still have a rudimentary INSR activity and therefore could develop fatty liver disease.

Intrauterine growth retardation and postnatal failure to thrive are part of the clinical picture of congenital insulin resistance. Psiauch et al. (29) suggested that the primary defect in leprechaunism is in the insulin receptor gene and that a secondary defect is probably responsible for the impaired response to endogenous GH and growth retardation. According to Kadowaki
et al. (30), the insulin receptor gene may regulate the function of IGF-I receptors. They suggested that the defect in the INSR gene impairs the functioning of receptors for other growth factors. Our patient had serum levels of IGF-1 and IGFBP-3 below the normal limits. IGF-1 plays an important role in prenatal and postnatal growth. Thus, both the defects in insulin action and the impaired synthesis of IGF-1 and IGFBP-3 resulted in growth retardation in this child. Nakae et al. proved that treatment with rhIGF-1 normalized glucose metabolism and was effective in preventing postnatal growth retardation in a patient with leprechaunism (31). Other authors, however, reported that administration of recombinant human growth hormone (rHGH) and human insulin-like growth factor 1 (rhIGF-1) had little or no influence on glucose homeostasis and none on growth stimulation. They hypothesized that this could be due to a postreceptor defect in IGF-1 signaling caused by the absence of insulin function (13).

There is evidence that insulin at high concentrations acts as a growth factor through IGF-I receptors. IGF-I receptors are present in the ovaries, kidneys and heart. This fact could explain the enlargement of these organs reported in patients with leprechaunism (30).

Recurrent bacterial infections presented an additional problem in our patient. Data from the literature show that infections occur with increased frequency in patients with leprechaunism. It is supposed that a congenital leptin deficiency due to defective insulin activity in the adipose tissue negatively influences T-cell function (23,32).

There is no effective therapy for severe forms of inherited insulin resistance. The life expectancy of children with leprechaunism is poor, and early death is certain. Most patients die during the 1st year of life (14,22,25). Some patients with milder forms of the disease have lived longer, which may be related to partial activity of INSR (33).

**Conclusions**

In summary, we found a novel homozygous mutation in the INSR gene in a patient with severe clinical manifestations of insulin resistance, which confirmed the diagnosis of Donohue syndrome (leprechaunism). We also found the presence of fatty liver in the patient and so far, there have been no reports of fatty liver disease in patients with proven loss of function of the insulin receptor. The fatal outcome in this child demonstrated the high risk in subjects with the most severe form of the disease if the mutation occurs in the ligand-binding domain of the receptor. This rare disorder represents a challenge for further new clinical trials that may improve the prognosis of such patients.

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The authors have nothing to disclose.

**Authorship Contribution:**
All the authors have read the manuscript, accept responsibility for its entire content and approve its submission.

AR: study concept and design, critical revision of the manuscript for important intellectual content, funding procurement.

BW: study concept and design, clinical evaluation of the patient, manuscript drafting.

EG: clinical evaluation of the patient.

AN: study concept and design, critical revision of the manuscript for important intellectual content, funding procurement, study supervision.

**References**


### Table 1. The glucose and insulin values for the patient serum

<table>
<thead>
<tr>
<th>Age (weeks of life)</th>
<th>7th</th>
<th>7th</th>
<th>8th</th>
<th>8th</th>
<th>10th</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fasting</td>
<td>Postprandial</td>
<td>Fasting</td>
<td>Postprandial</td>
<td>Postprandial</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>2.9 (n.&lt;5.6)</td>
<td>13.9 (n.&lt;140)</td>
<td>2.7 (n.&lt;100)</td>
<td>10.1 (n.&lt;140)</td>
<td>4.7 (n.&lt;140)</td>
</tr>
<tr>
<td>Insulin (mIU/L)</td>
<td>300 (n.&lt;12)</td>
<td>-</td>
<td>-</td>
<td>453 (n.&lt;75)</td>
<td>1200 (n.&lt;75)</td>
</tr>
</tbody>
</table>

**Figure 1.** The sequence analysis of the INS R gene
Reference Sequence (NC_000019.8)

- ACG (Thr) → AGG (Arg)