Leprechaunism (Donohue syndrome): A case bearing novel compound heterozygous mutations in the insulin receptor gene

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Abstract. Leprechaunism (Donohue syndrome) is the most severe type of insulin receptor (INSR) gene anomaly with the majority of patients surviving for only 2 years. We report a surviving 2-year-old male with leprechaunism, bearing novel compound heterozygous mutations in the INSR. The patient is a Japanese boy with acanthosis nigricans, lack of subcutaneous fat, hirsutism, thick lips, gum hypertrophy and extremely high insulin levels (6702 mU/mL). He was as having identified novel compound heterozygous mutations in INSR (p.T910M and p.E1047K). At 24 day-old, recombinant human insulin-like growth factor 1 (rh-IGF1) treatment was started because of poor weight gain. At 2 years old, the patient’s serum glucose level and HbA1C value had worsened, and both a bolus of rh-IGF-1 and a subcutaneous injection of a rapid-acting insulin analog after meals, in addition to α-glycosidase inhibitor, were initiated from 2 years onward. Oxygen administration and biphasic positive airway pressure treatment were also initiated from 2 years old due to upper airway obstruction with adenoidal hypertrophy. In the experiments conducted using COS7 cells homozygously transfected with the INSR mutation, T910M INSR failed to process the proreceptor and decreased insulin-stimulated tyrosine phosphorylation. E1047K INSR resulted in a complete absence of insulin-stimulated tyrosine phosphorylation. These findings suggest the near absence of INSR in this patient. We consider that the rhIGF1 treatment contributed to his long survival, but it was not able to prevent his diabetic condition. Our report provides important insights into the function of INSR, and for the treatment of leprechaunism.

Keywords: Leprechaunism, Insulin receptor, Recombinant IGF1

LEPRECHAUNISM (DONOHUE SYNDROME) is an extremely rare, autosomal recessive disorder caused by a defective insulin receptor (INSR). It is characterized by insulin resistance and distinct clinical and facial features [1]. Leprechaunism is the most severe type of INSR disorder with the majority of patients surviving for only 2 years [1-4]. It was found that the exogenous supplementation of recombinant human insulin-like growth factor 1 (rh-IGF1) can rescue the defective INSR insulin pathway via IGF1 receptor (IGF1R) activation, resulting in prolonged patient survival [5]. In addition, functional analyses of the INSR gene revealed that the disease phenotype is directly correlated with the severity of the gene mutation [2, 3, 6]. Here, we report the case of surviving Japanese boy with leprechaunism who had novel, compound heterozygous mutations in the INSR gene (p.T910M and p.E1047K).

Case Report

The patient is a Japanese boy (first-born child) who was two years old at the time of this writing. His parents were healthy, and there was no family history of diabetes. At 8 days old, he was referred to our hospital because of poor sucking and low activity levels. He was delivered at 41 weeks of gestation with a birth weight of 1920 g (-3.7 SD). His appearance was characteristic of leprechaunism, with acanthosis nigricans, lack of subcutaneous fat, decreased muscle, hirsutism, prominent eyes, low-set large ears, thick lips, and gum hypertrophy (Fig. 1A). Plasma glucose levels ranged

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from 38 mg/dL in the fasting state to over 200 mg/dL at the postprandial state. The fasting plasma insulin level was significantly above average at 6702 mU/mL. The results of all other laboratory tests were within normal range. From these findings, the patient was clinically diagnosed with leprechaunism, and we subsequently analyzed the patient’s \(INSR\) gene.

At 24 days old, his weight gain was extremely poor and we began rhIGF1 treatment: rh-IGF1 was subcutaneously injected, at a low concentration, twice a day and was gradually increased up to four times per day, and finally injected continuously at the dose of 1.0 mg/kg/day. Although the patient's serum insulin/glucose ratio and body weight improved, his weight was not normalized (Fig. 2). At 172 days old, we began a continuous rh-IGF1 subcutaneous infusion. At 1 month old, the patient exhibited pale stool, macrohematuria, extremely low levels of heparplastin test (5%), hyperbilirubinemia (direct bilirubin: 1.6 mg/dL), and liver dysfunction (Aspartate transaminase: AST,100-235 IU/L and alanine aminotransferase: ALT, 15-80 IU/L). At 5 months old, scintigraphy tests revealed delayed biliary excretion, and a liver biopsy at 6 months old showed mild hepatitis, partial fibrosis, and accumulation of glycogen in the hepatocytes (Fig.1B). From these findings, the patient was diagnosed with cholestasis and coagulation disorder due to cholestasis. With the rhIGF1 injection treatment, the patient's vitamin K and ursodeoxycholic acid, his cholestasis and his liver dysfunction were improved (AST 97 IU/L , ALT 35 IU/L, T-bil 0.4 mg/dL) at 1 year old.

At 2 years old, the patient’s serum glucose level after meals and HbA1C value (Japan Diabetes Society) had worsened (> 600 mg/dL and 8.6 %-9.4%, respectively) despite the increased bolus of rh-IGF1 [0.03 mg/kg] after meals and the continuous subcutaneous infusion of rh-IGF1 [0.6 mg/kg/day]. Accordingly, the both bolus of rh-IGF-1 and a subcutaneous injection of a rapid-acting insulin analog (8 IU) after meals, in addition to the continuous subcutaneous infusion of rh-IGF1 [0.6 mg/kg/day] and \(\alpha\)-glycosidase inhibitor, were initiated from the patient’s age of 2 years onward. His HbA1C value remains high (9.7 % at 2 years + 1 month of age), and hypoglycemia in the fasting state continues. In addition, the patient suffered from pneumonia and asthmatic bronchitis several times and, developed an upper airway obstruction due to adenoidal hypertrophy. Oxygen administration and biphasic positive airway pressure treatment were initiated from 2 years onward. After the patient underwent a tonsillectomy at 2 years and 6 months old, his respiratory problems improved.

Fig. 1  A: Photograph of the patient with leprechaunism, at 8 days of age. The photo is approved by his parents in written form. B: Light micrographs of Periodic acid-Schiff (PAS) staining of liver tissues from the patient.
Leprechaunism with novel mutations

Materials and Methods

Sequence analysis of the INSR gene
Genomic DNA was isolated from peripheral blood lymphocytes of the patient and his parents, and amplified by PCR as described [7, 8].

Plasmid construction
Flag tagged-pCMV-human INSR cDNA was provided by Dr. Masanori Iwaki (The University of Osaka, Osaka, Japan). A mutated INSR expression vector (p. T910M) was constructed by a 2919 C to T (NM_000208.2, c. 2919 C>T) point mutation using primers (5′T C T T G G A T G G A A3′, where T is the mutation site), and a mutated INSR expression vector (p. E1047K) was constructed by a 3269 G to A and 3271G to A (NM_000208.2, c.3269G>A, C.3271G to A) point mutation using the primer (5′A A T A A G C C T C3′, where A is the mutation site). All mutations were confirmed by DNA sequencing and performed by Life Technologies Japan (Tokyo, Japan).

Immunoblotting analysis
COS-7 cells (0.5 -1.0 × 10⁶) were transiently transfected with either the WT, T910M, or E1047K INSR construct with Lipofectamine 2000 (Invitrogen, Carlsbad, CA), according to the manufacturer’s protocol, and seeded into 60-mm dishes with 10% Dulbecco’s modified Eagle’s medium (DMEM) (Wako Pure Chemical Industries, Ltd, Osaka, Japan) 6 h after transfection. Cells were washed twice with Hanks’ balanced salt solution 12 h after seeding and starved for 2 h in a serum-free medium [DMEM]. The preparation of cell lysates, immunoprecipitation, and immunoblotting were performed as described [7]. We conducted each experiment at least three times, and the mean relative intensity of tyrosine phosphorylation from three different experiments was obtained (Fig. 4B).

Statistical analysis
Statistical analysis was performed using the Excel software and Student’s t-test. The results are shown as mean ± SE. P < 0.05 was considered statistically significant.

Results

Missense mutation (T910M or E1047K) of the INSR gene
In the patient, we identified a heterozygous C to T substitution at position 2919 (NM_000208.2, c. 2919 C>T) that resulted in a threonine (ACG) to methionine (ATG) substitution at residue 910 (mature peptide numbering), and a heterozygous G to A mutation at position 3269 and 3271 (NM_000208.2, c.3269G>A, C.3271G to A) that resulted in a glutamine (GAG)
to lysine (AAA) substitution at residue 1047 (mature peptide numbering) in the INSR β subunit (Fig. 3). Although the INSR T910M mutation has been reported in patients with leprechaunism [9], this is the first report of an INSR E1047K mutation in a patient with leprechaunism. The E1047 mutation was not detected in our patient’s parents, but a heterozygous T910M mutation was detected in his mother (Fig. 3). Accordingly, the E1047 mutation is considered a de novo mutation. Since the patient’s case presents the characteristic clinical findings of leprechaunism, his case is considered to be a compound heterozygous INSR gene mutation (p.T910M and p. E1047K).

**T910M and E1047K gene mutations diminish the insulin-dependent phosphorylation of INSR**

To understand the functions of these mutations, we studied the insulin-dependent autophosphorylation of the INSR β subunit. In cells expressing E1047K, insulin-dependent autophosphorylation of E1047K INSR did not occur (10 ng/mL, 0.00 ± 0.01 vs. 0.86 ± 0.16, p < 0.05; 100 ng/mL, 0.01 ± 0.01 vs. 0.97 ± 0.01, p < 0.05), and the autophosphorylation of T910M INSR was significantly decreased compared to WT (10 ng/mL, 0.24 ± 0.05 vs. 0.86 ± 0.16, p < 0.05; 100 ng/mL, 0.22 ± 0.01 vs. 0.97 ± 0.05, p < 0.05) (Fig. 4). Moreover, cells expressing T910M INSR had lower INSR levels compared to WT and E1047K-expressing cells, despite higher levels of the proreceptor, consistent with a previous report that T910M results in the failure to process the INSR prore-

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**Fig. 3** The chromatogram of the INSR mutation in the proband and that of his parents
This chromatogram, obtained by direct sequencing of PCR products reveals compound heterozygous missense mutations in the proband. A C to T mutation at position 2919 (NM_000208.2, c. 2919 C>T) resulted in the substitution of threonine (ACG) with methionine (ATG) in residue 910 (mature peptide numbering), and another heterozygous mutation G to A at position 3269 and 3271 (NM_000208.2, c.3269G>A, C.3271G to A), resulted in the substitution of glutamine (GAG) with lysine (AAA) in residue 1047 (mature peptide numbering) of the INSR β subunit.

**Fig. 4** Insulin-stimulated autophosphorylation of the INSR β subunit in transiently transfected cells
Cells were starved for 2 h; stimulated with 0, 10, or 100 ng/mL Insulin for 5 min; and lysed. Then, cell lysates were subjected to immunoprecipitation (IP) and immunoblotting (IB) analysis using the indicated antibodies. A: Representative results of immunoblotting. B: Relative intensity of tyrosine phosphorylation of INSRβ, Results are shown as mean ± SE. *, p < 0.05.
In light of these findings, it appears that these compound heterozygous mutations lead to dysfunctional insulin signaling that results in leprechaunism.

**Discussion**

We have described the case of a surviving patient with leprechaunism (Donohue syndrome) bearing novel compound heterozygous mutations in the *INSR* gene (p.T910M and p.E1047K), who is being treated with rh-IGF1.

We newly identified an *INSR* E1047K mutation in our patient, and we found that the mutation led to absence of insulin-dependent INSR autophosphorylation. Since the Glu 1047 insulin receptor has been reported to be an important residue for ATP binding and, as such, the phosphorylation of INSR [10, 11], our findings follow this line. However, the *INSR* T910M mutation has been reported in patients with leprechaunism [9], and has been reported that T910M INSR leads to a failure of processing the proreceptor, as is consistent with our data. Accordingly, these findings suggest that the patient’s INSR function is nearly absent.

It is well known that the insulin and IGF receptors are structurally related and share common post-receptor signaling pathways. The rh-IGF1 treatment for leprechaunism exploits these commonalities, thus rescuing the failed INSR signaling found in leprechaunism [5, 12] and resulting in prolonged patient survival [5]. With the present patient, we also started rh-IGF1 treatment early, with a resulting survival over 2 years. However, the rhIGF1 treatment was not able to prevent the development of diabetes in the patient, therefore we started rapid insulin treatment and α-glycosidase inhibitor. In addition, our patient is suffering from upper airway obstruction due to adenoidal hypertrophy, which might be related to a side effect of rh-IGF1 treatment. This sequence of events explains why IGF1 is not able to compensate for INSR.

Severe fasting hypoglycemia, as our patient also showed, is one of the characteristics of leprechaunism. This suggests an accelerated fasting state with rapid depletion of hepatic glycogen [13, 14]. In contrast, other studies have found that patients with leprechaunism do not exhibit glycogen depletion, and they propose that, since insulin has a low affinity to IGF1R, the high insulin levels in humans may cause hypoglycemia by binding to IGF1R [15]. Consistent with this, the hepatic histological finding in our patient also revealed an accumulation of glycogen in hepatocytes. Notably, in our patient, hypoglycemia might have been induced by high insulin levels that result in IGF1R binding.

In conclusion, we report a surviving case of leprechaunism, bearing a novel heterozygous compound missense mutation (p.T910M and p.E1047K), who is being treated with rh-IGF1. The rhIGF1 treatment is thought to have contributed to the patient’s survival, but it did not prevent diabetes. We suggest that to improve the condition of patients with complete INSR dysfunction, another treatment along with rhIGF1 may be effective.

**Disclosures**

All authors have no disclosures to report.