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Case Report

Diabetes mellitus with severe insulin resistance in a young male patient with a heterozygous pathogenic *IRS1* frameshift variant

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Highlights

- Severe insulin resistance may be associated with *IRS1* frameshift mutation.
- Metformin is well tolerated and effectively maintains optimal glycemic control.

Abstract. We present the case of a young male patient (height, 158.1 cm [+3.3 standard deviation (SD)]; weight, 63.7 kg [body mass index, 25.5]) with diabetes mellitus and severe insulin resistance associated with a heterozygous pathogenic insulin receptor substrate 1 (*IRS1*) frameshift mutation. The patient also had severe acanthosis nigricans. Notably, the patient's father was undergoing treatment with high doses of insulin for diabetes mellitus, and had been experiencing angina pectoris. Laboratory data showed a fasting plasma glucose level of 88 mg/dL, hemoglobin A1C (HbA1c) of 7.4%, fasting insulin level of 43.1 μg/mL, and a homeostasis model assessment-insulin resistance (HOMA-IR) score of 9.36, indicating hyperinsulinism. Oral glucose tolerance test revealed a diabetic pattern and insulin hypersecretion. In addition, the patient had hyperlipidemia. Genetic studies revealed a heterozygous frameshift variant of *IRS1* [NM_005544.3:c.1791dupG:p.(His598Alafs*13)] in the patient and his father, which can impair the binding and activation of phosphoinositide 3 (PI-3) kinase and defectively mediate the translocation of glucose transporter type 4 (GLUT4) in adipose tissues, possibly leading to glucose intolerance. Therefore, this variant may be disease causing. After confirming *IRS1* mutation, metformin was administered, and physical exercise and dietary management were initiated; metformin was well tolerated, and optimal glycemic control was maintained.

Key words: insulin resistance, diabetes mellitus, phosphoinositol 3-kinase

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Introduction

Hepatic insulin resistance leads to chronic hyperinsulinemia and increased hepatic glucose production, resulting in postprandial hyperglycemia and development of chronic hyperglycemia and diabetes mellitus (1). Hepatic insulin resistance may impair lipid synthesis and hyperlipidemia (2). Impaired insulin receptor substrate (IRS) protein signaling is associated with hepatic insulin resistance. The insulin receptor (IR) is crucial for the activation of downstream kinase cascades, such as phosphoinositol 3-kinase (PI3K) and mediation of the translocation of glucose transporter 4 (GLUT4) in adipose tissue (2). Two major IRS isoforms, IRS1 and IRS2, are associated with hepatic insulin signaling (3). IRS1 undergoes tyrosine phosphorylation and binds to PI3K and Grb2-Map kinase (4). A previous study reported that IRS-1 knockout (KO) mice showed growth retardation and mild insulin resistance with compensatory hyperinsulinemia and β-cell hyperplasia, suggesting impairment of IR- and IGF-1 receptor mediated signal transduction (5). Notably, naturally occurring amino acid substitutions and silent polymorphisms of *IRS1* have been found in patients with and without diabetes (6–9); some of these mutations are rare variations or low-frequency polymorphisms with a similar prevalence in patients with and without diabetes. However, several studies have investigated whether these mutations are associated with impaired insulin activity. A common mutation in *IRS1* (*IRS1* G972 variant) is associated with impaired insulin activity (10–13) and secretion (14, 15). Several clinical studies have demonstrated that *IRS1* mutations are associated with human metabolic disorders (16) such as polycystic ovary syndrome and type 2 diabetes mellitus (17–22).

Herein, we report the case of a young male patient who had inherited diabetes associated with a heterozygous pathogenic *IRS1* frameshift variant and presented with severe insulin resistance. We evaluated the clinical characteristics and clinical course of the patient after diagnosis.

Case Report

A 10-yr-old boy visited a local clinic with primary complaints of thirst and polyuria. The examination revealed postprandial hyperglycemia (330 mg/dL) and a high glycosylated hemoglobin (HbA1c) level of 7.4%, which was consistent with the diagnosis of diabetes mellitus. He was referred to the Department of Pediatrics, Kobari General Hospital, Chiba, Japan, for assessment and treatment. The patient had been delivered normally at 41 wk and 3 d of gestation, and his birth weight and length were 2,868 g and 50.0 cm, respectively. He was diagnosed with autism spectrum disorder and attention deficit hyperactivity disorder at 8 yr of age, and was treated by a pediatric psychiatrist. The patient had no mental delay (Wechsler Intelligence Scale for Children – Fourth Edition (WISC-IV): IQ, 90). Moreover, his father was undergoing treatment with high doses of insulin for diabetes mellitus and had been experiencing angina pectoris. There was a family history of diabetes mellitus and cardiovascular disease among first- and second-degree relatives (**Fig. 1**). The patient became overweight at 8 yr of age because of excessive food intake, with a preference for sweet beverages and snacks. He had a sedentary lifestyle and did not engage in playing because of autism spectrum disorder.

At the first visit to Kobari General Hospital, his height was 158.1 cm (+3.3 SD) and weight was 63.7 kg with body mass index of 25.5, which is > 97 percentile for sex- and age-matched Japanese children (23). In addition, he had a blood pressure of 126/57 mmHg. The patient's general condition was good and consciousness was clear. Chest and abdominal findings were normal. The Tanner scale revealed genitalia (2), pubic hair (2), and testicular volume (6 mL). Furthermore, the characteristic symptoms of acanthosis nigricans were observed in the neck and axillary regions (**Fig. 2**). Laboratory data showed a fasting plasma glucose (FPG) level of 88 mg/dL, HbA1c level of 7.4%, and fasting immunoreactive insulin (IRI) level of 43.1 μg/mL with a homeostasis model assessment-insulin resistance

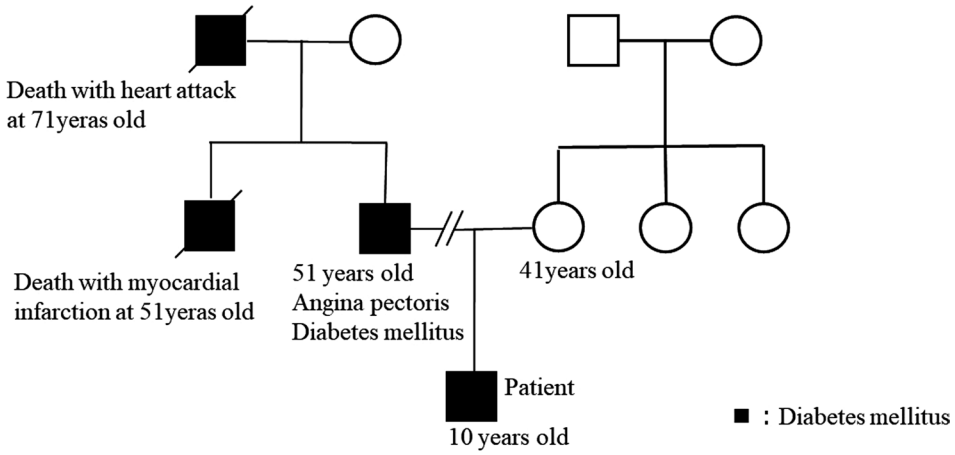

Fig. 1. Family history among first- and second-degree relatives.

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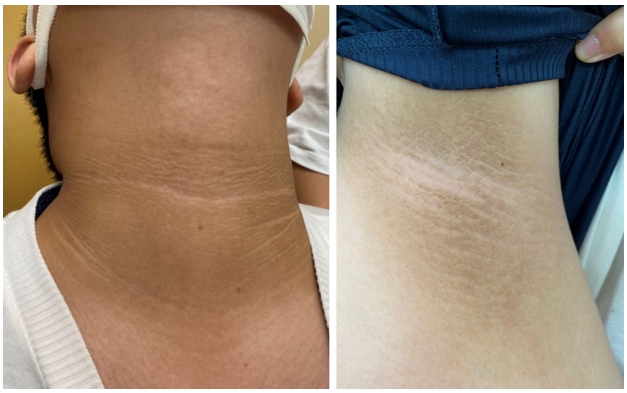
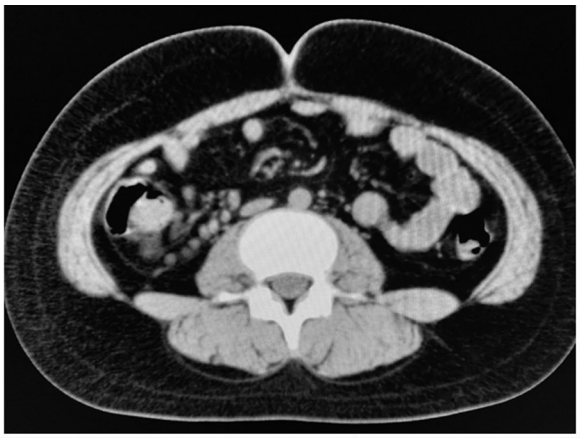


Fig. 2. Significant signs of acanthosis nigricans on the neck and axillar regions.

(HOMA-IR) score of 9.36, indicating hyperinsulinism. Furthermore, laboratory data indicated liver dysfunction (aspartate aminotransferase (AST) 207 IU/L, alanine transaminase (ALT) 442 IU/L, lactate dehydrogenase (LDH) 325 IU/L) and hyperlipidemia (triglycerides, 293 mg/dL; total cholesterol, 252 mg/dL, low-density lipoprotein (LDL)-cholesterol 140 mg/dL, and high-density lipoprotein (HDL)-cholesterol 53 mg/dL) in the fasting state. We performed an oral glucose tolerance test (OGTT; 75 g glucose), which revealed a diabetic pattern and hypersecretion of insulin (**Table 1**) (24). Abdominal computed tomography at the umbilical level (Canon Medical Systems Corporation, Tochigi, Japan), which is the gold standard for measuring intra-abdominal fat volume, showed significant accumulation of visceral and subcutaneous fat (**Fig. 3**).



total fat area	**340.87 m^2**
visceral fat area (normal; <60 m^2 for children)	**92.39 m^2**
subcutaneous fat area	**248.48 m^2**
the visceral-to-subcutaneous fat ratio (normal; <0.3)	**0.37**

Fig. 3. Subcutaneous and visceral fat volumes and their distribution evaluated using abdominal computed tomography (CT) at the umbilical level.

Table 1. Results of OGTT

	PG (mg/dL)	IRI (μg/mL)
0 min	88	43.1
30 min	165	426.0
60 min	172	383.0
90 min	150	493.0
120 min	168	927.0

HOMA-IR	9.36
ΔIPI/ΔPG (30 min)	4.97

Seventy-five grams of glucose was orally administered. PG, plasma glucose; IRI, immunoreactive insulin. Diabetes was assessed according to the Japanese Diabetes Society Guideline (43). Insulin resistance was defined as a homeostasis model assessment of insulin resistance (HOMA-IR) score > 2.5. HOMA-IR was calculated as fasting plasma glucose (FPG) (mg/dL) × fasting immunoreactive insulin (IRI) (μU/mL)/405.

Genetic study

This genetic study was approved by the Institutional Review Board Committee of the Hamamatsu University School of Medicine and was performed after obtaining written informed consent from the patient's parents. To identify the underlying genetic cause, we performed whole-exome sequencing (WES) with leukocyte genomic DNA samples from the patient and his parents using SureSelectXT Human All Exon v6 (Agilent Technologies, Santa Clara, Carifornia, USA). The captured libraries were sequenced using a NextSeq500 (Illumina, San Diego, Carifornia, USA) with 150-bp paired-end reads. Exome data processing, variant calling, and variant annotation were performed as described previously (24). Human GRCh38/hg38 (UCSC Genome Browser) was used as the reference genome.

A heterozygous frameshift variant (NM_005544.3:c.1791dupG: p.(His598Alafs*13)) was identified in *IRS1* of the patient and his father, as confirmed by Sanger sequencing (**Fig. 4**), which is rare to find in the public and in-house databases used in this study. It was assessed as a "disease-causing" variant using MutationTaster, although other *in silico* pathogenic predictions could not evaluate the pathogenicity of this frameshift variant. Based on the American College of Medical Genetics and Genomics (ACMG)/American Association of Molecular Pathology (AMP) criteria (positive for PVS1 alone), this variant was assessed as a "variant of uncertain significance (VUS)" (25). No other rare variants (minor allele frequency < 0.01) were identified in the genes involved in the insulin signaling pathway.

Treatment

After confirming the *IRS1* mutation, 750 mg of metformin was administered daily as an initial

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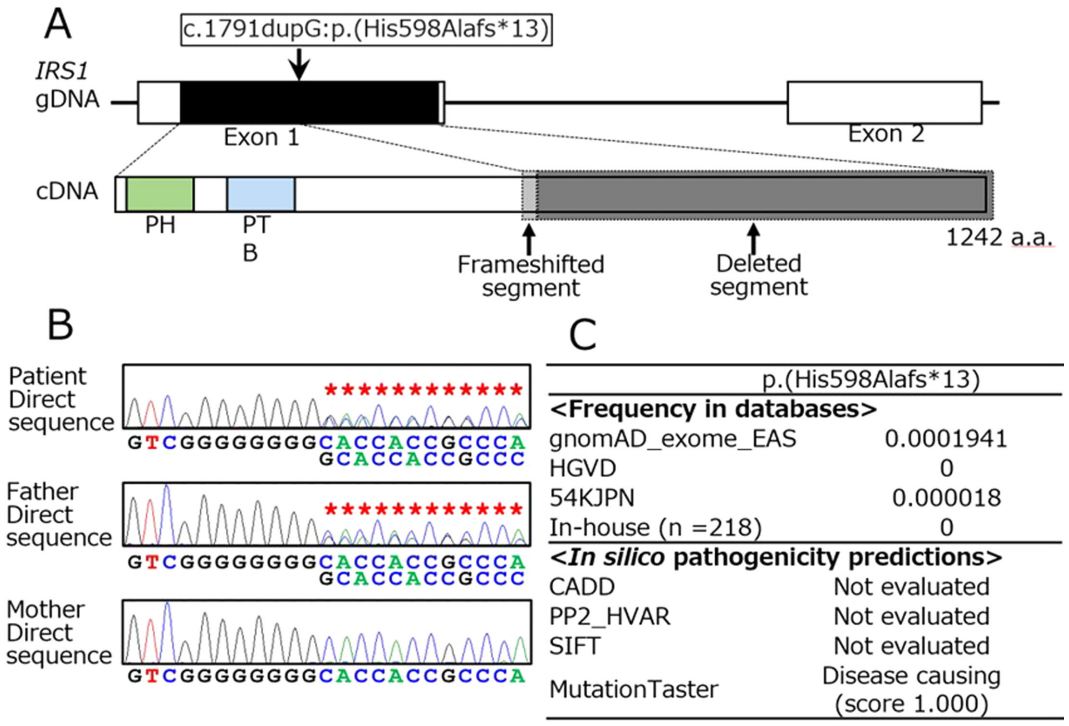

Fig. 4. Summary of genetic studies. A: The gDNA and cDNA structures of *IRS1* and the position of the frameshift variant were identified. This variant is predicted to produce a truncated protein, associated with a 12 amino acid frameshifted segment (highlighted in light gray), lacking a large part of the *IRS1* protein (highlighted in dark gray). PH: pleckstrin homology domain, PTB: phosphotyrosine-binding domain, gDNA: genomic DNA, cDNA: complementary DNA. B: Electrochromatograms showing the frameshift variant in the patient and his father. Frameshifted sequences are indicated by red asterisks. The forward primer was TGGATAATCGGTTCCGAAAG, and the reverse primer was ATCTGCTGTGGGGCAGATAC. For the prediction of frequency and *in silico* pathogenicity, the following URLs were used: (1) Genome Aggregation Database (gnomAD): http://gnomad.broadinstitute.org; (2) Human Genetic Variation Database (HGVD): http://www.hgvd.genome.med.kyoto-u.ac.jp; (3) Whole-genome sequences of approximately 54,000 healthy Japanese individuals and construction of the highly accurate Japanese population reference panel (54KJPN): https://jmorp.megabank.tohoku.ac.jp; (4) Combined Annotation–Dependent Depletion CADD): http://cadd.gs.washington.edu/score; Phred scores of > 10–20 are regarded as deleterious, and scores > 20 indicate the 1% most deleterious; (5) Polyphen-2 Hum Var: http://genetics.bwh.harvard.edu/pph2; Hum Var scores were evaluated as 0.000 (most probably benign) to 1.000 (most probably damaging); (6) Sorting Intolerant From Tolerant (SIFT): http://sift.jcvi.org; scores ≤ 0.05 and scores > 0.05 are assessed as damaging and tolerated, respectively; (7) MutationTaster: http://www.mutationtaster.org (MutationTaster2, GRCh37/Ensembl 69); alterations are classified as disease-causing or polymorphisms, and a high score of ~1.00 indicate the high probability of disease-causing variant or polymorphism.

treatment concomitant with physical exercise and dietary management. Metformin treatment was well tolerated without gastrointestinal side effects, and the patient maintained optimal glycemic control with HbA1c levels of 5.5–7.4%. Fasting IRI level and HOMA-IR score after metformin administration were 18.2–42.1 μg/mL and 3.7–9.7, respectively. Liver dysfunction and hyperlipidemia gradually improved after metformin treatment, and laboratory data, including FPG and HbA1c levels, normalized (**Fig. 5**).

Discussion

We identified a frameshift variant of *IRS1* in a young male patient with diabetes mellitus and insulin resistance. This variant is not associated with nonsense-mediated mRNA decay (26), and may therefore produce a severely truncated protein. Furthermore, the clinical findings in the patient and his father primarily resulted from the *IRS1* variant. Although it was assessed as a VUS based on the ACMG/AMP criteria, it may be a disease-causing variant. The *IRS1* frameshift variant impairs tyrosine phosphorylation and binds to PI 3-kinase and Grb2-Map kinase (4), possibly leading to glucose intolerance. However, IRS-1 KO mice do not spontaneously develop diabetes because of compensatory hyperinsulinemia and β-cell hyperplasia (5). Some studies have demonstrated that IRS-1 mutations impair the metabolic action of insulin (10, 11, 27, 28) and insulin secretion (29, 30). There are various clinical reports showing that *IRS1* mutations can cause human metabolic disorders, including diabetes mellitus (16–22). Therefore, the *IRS1* mutation identified in our patient may have caused diabetes mellitus. Obesity, puberty,

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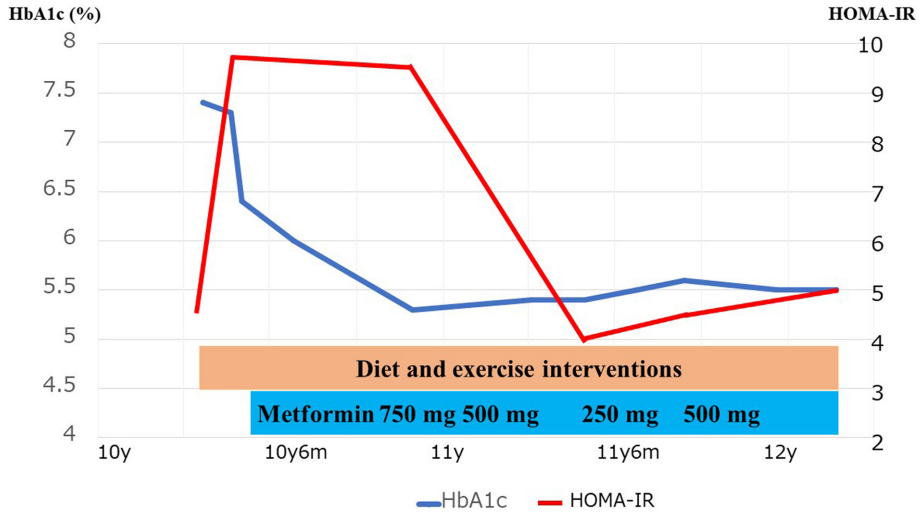

Fig. 5. Clinical course and treatment. The homeostasis model assessment-insulin resistance (HOMA-IR) score was calculated as fasting plasma glucose (FPG) (mg/dL) × fasting immunoreactive insulin (IRI) (μU/mL)/405.

and environmental factors, such as excessive food intake and sedentary life, together with a genetic background, can also lead to the development of diabetes.

Metformin increases insulin sensitivity in insulin-resistant conditions such as obesity, diabetes mellitus, and polycystic ovary syndrome. Furthermore, metformin increases peripheral glucose utilization by inducing GLUT4 expression and increasing its translocation to the plasma membrane. Additionally, metformin-mediated improvements in insulin sensitivity may be associated with several mechanisms, including increased insulin receptor tyrosine kinase activity and enhanced glycogen synthesis (31). Metformin treatment improved whole-body insulin sensitivity in various insulin-resistant conditions, including obesity (32, 33), metabolic syndrome (34), polycystic ovary syndrome (35), prediabetes mellitus (36), and diabetes mellitus (37–39). Therefore, treatment with metformin alone, or in combination with diet and physical activity, or lifestyle modifications can reduce visceral obesity, insulin resistance, hyperinsulinemia, hyperlipidemia, hypertension, and glucose intolerance. The patient responded well to treatment with metformin alone and showed improved glycemic control.

In addition, the patient showed moderate but not severe insulin resistance at diagnosis, possibly because of the young age and early stage of insulin resistance syndrome, which may be the basis for the efficacy of metformin as monotherapy. Thiazolidinediones are insulin-sensitizing drugs currently used for treating patients with insulin resistance and provide an opportunity for effective combination therapy with two insulin-sensitizing agents. This combination treatment improves glucose control, reduces metabolic risk, and improves the outcomes of cardiovascular diseases (40). These oral hypoglycemic drugs, insulin (high doses), and recombinant human IGF-1 are administered to treat congenital insulin resistance syndromes, such as Mendenhall syndrome (41). In contrast, sodium-glucose cotransporter 2 (SGLT2) inhibitors, which modulate elevated glucose levels through a unique mechanism independent of insulin action, are potential candidates for treating patients with severe insulin resistance (42); SGLT2 inhibitors are effective in patients with severe insulin resistance associated with mutations in insulin receptor (*INSR*) or phosphoinositide-3-kinase regulatory subunit 1 (*PIK3RI*) (43–45). Although their long-term safety remains to be assessed, SGLT2 inhibitors are potential candidates for use in patients with insulin resistance syndromes, which could be administered as the disease progresses.

Furthermore, mutations in *IRS1* are associated with psychiatric diseases, such as Alzheimer's disease and autism; the frequency of the rs1801123 polymorphism in *IRS1* is significantly higher in Korean men with autism (46). Notably, autism in our patient was possibly associated with a mutation in *IRS1* (*IRS1* c.1791dupG:p.His598Alafs*13). In contrast, metformin has been reported to improve cognitive and mood functions in patients with type 2 diabetes (47). The mechanism underlying metformin in the central nervous system is not fully understood, but it appears to regulate synaptic transmission or plasticity in pathological conditions and the balance of excitation and inhibition in neural networks (47); this may enable pharmacological efficacy in improving or preventing neurological disorders (48). No clinical study has reported the efficacy of metformin in autism, and we found no alterations in autism following metformin treatment.

Conclusions

Herein, we report the case of a young male patient with diabetes mellitus who presented with severe insulin resistance associated with a heterozygous pathogenic *IRS1* frameshift variant [NM_005544.3:c.1791dupG:p.(His598Alafs*13)]; this frameshift variant of *IRS1* can

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impair the binding and activation of PI3K and defectively mediate the translocation of GLUT4 in adipose tissues. Therefore, we considered this to be a disease-causing variant. Metformin monotherapy effectively sustained optimal control. However, other drugs, including thiazolidinediones, recombinant human IGF-1, and SGLT2 inhibitors, may be administered if insulin resistance progresses.

Conflict of interests: The authors declare no conflict of interest.

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