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Case Report

Initial clinical manifestations in a young male with *RFX6*-variant-associated diabetes

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Highlights

- The initial presentation of *RFX6*-variant-associated diabetes includes occasional hyperglycemia and hypoglycemia.
- The initial stage of *RFX6*-variant-associated diabetes includes occasional hyperglycemia that responds to lifestyle changes.

Abstract. To date, heterozygous loss-of-function variants of *RFX6* have been identified in 13 families with diabetes. Here, we present initial clinical information regarding a young male with diabetes who carried a heterozygous nonsense variant of *RFX6* (p.Arg377Ter) previously reported in his family with diabetes. At 11 yr and 7 mo of age, the patient experienced severe thirst and hyperglycemia (331–398 mg/dL). Laboratory tests revealed elevated levels of glycated hemoglobin (HbA1c) (47 mmol/mL, 6.5%) and the Homeostatic Model for Insulin Resistance (HOMA-IR) (3.4). Blood glucose self-monitoring demonstrated grossly normal blood glucose levels, together with occasional postprandial hyperglycemia, and a few episodes of hypoglycemia. An oral glucose tolerance test revealed mild hyperglycemia and a delayed peak insulin level. His laboratory indices improved over two years with self-control of diet and exercise. These results indicate that the initial presentation of *RFX6*-variant-associated diabetes includes occasional hyperglycemia and hypoglycemia in response to changes in lifestyle. The possible association between *RFX6* variants and mild insulin resistance requires further validation in future studies.

Key words: insulin secretion, insulin resistance, *RFX6*

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Introduction

RFX6 (NM_173560.4) encodes a 928-amino acid transcription factor involved in the differentiation of pancreatic endocrine progenitor cells into beta cells (1). In 2017, Patel *et al.* identified five heterozygous loss-of-function variants of *RFX6* in patients with diabetes (1). Subsequently, seven patients with diabetes and heterozygous *RFX6* variants have been reported (2–5). Furthermore, Walker *et al.* reported that reduced *RFX6* expression with minor environmental factors is associated with the risk of type 2 diabetes (6). However, the phenotypic characteristics of patients with pathogenic *RFX6* variants, particularly those with early-stage diabetes, remain unclear. Here, we report the clinical features of a young male with early-stage *RFX6*-variant-associated diabetes.

Patient

This study was approved by the Institutional Review Board Committee of the National Center for Child Health and Development and was performed after obtaining written informed consent. The patient was a Japanese adolescent male born as the second child to non-consanguineous parents. His elder sister, mother, and grandmother were diagnosed with *RFX6*-variant-associated diabetes, as reported in our previous study (2). These individuals harbored a heterozygous nonsense variant of *RFX6* (c.1129 C>T, p.Arg377Ter).

Given the family history of diabetes, close monitoring for symptoms was conducted. However, he showed no signs of diabetes until 11 yr and 7 mo of age. Annual health check-ups at the school did not reveal glucosuria. At 11 yr and 7 mo of age, he experienced severe thirst. As self-glucose monitoring by his mother revealed hyperglycemia (331–398 mg/dL), he visited our hospital. He was otherwise healthy and was not obese (**Table 1**, **Fig. 1**). His exercise regimen consisted of an hour and a half of tennis once weekly. He exhibited bilateral testes with a volume of 10 mL without other pubertal signs. Acanthosis nigricans was absent. Laboratory tests revealed mildly increased levels of glycated hemoglobin (HbA1c) and postprandial glucose with a normal level of serum C-peptide (**Table 1**). The Homeostatic Model for Insulin Resistance (HOMA-IR) values were slightly above the reference range (**Table 1**). Four-months of self-monitoring (1–6 times per day) showed that his blood glucose level remained mostly within the normal range (70–139 mg/dL, 129 of 169 measurements), although hyperglycemia (140–331 mg/dL) was observed 31 times (**Fig. 2a**). In addition, mild hypoglycemia before lunch and dinner was recorded nine times. An oral glucose tolerance test (OGTT) showed hyperglycemia of 242 mg/dL and a delayed insulin peak of 102.9 μIU/mL, both at 120 min after glucose load (**Fig. 2b**). Autoantibodies against glutamic acid decarboxylase, islet antigen 2, insulin, and zinc transporter 8 were absent. Based on this information and family history, he was suspected of developing *RFX6*-variant-associated diabetes. Sanger sequencing confirmed the presence of the same *RFX6* variant as his relatives (c.1129 C>T, p.Arg377Ter). As his glucose level remained mostly within the normal range, we followed this patient without medical intervention. He started self-management of food intake and daily exercise, and his weight gradually decreased (**Fig. 1**). At 12 yr and 6 mo of age, he underwent a repeated clinical examination. Three-months of self-monitoring revealed reduced frequencies of hyperglycemia and hypoglycemia (3 and 5 of 159 measurements, respectively). Blood levels of HbA1c and HOMA-IR were within the reference ranges (**Table 1**). An OGTT showed mild hyperglycemia and the peak of insulin was 60 min after the glucose load (**Fig. 2b**). At 14 yr and 10 mo of age, he was in a good health condition. He noticed no symptoms of hyperglycemia or hypoglycemia, and occasional self-monitoring revealed no hyperglycemia or hypoglycemia. An OGTT confirmed improved glucose metabolism; we observed a glucose peak of 195 mg/dL and an insulin peak of 70.9 μIU/mL, both at 30 min after glucose loading (**Fig. 2b**).

Discussion

We identified a patient with early stage *RFX6*-variant-associated diabetes. At diagnosis, the patient exhibited occasional postprandial hyperglycemia and a mildly increased HbA1c level. This phenotype is among the mildest of those reported in patients with *RFX6*-variant-associated diabetes, considering that almost all previously reported patients exhibited obvious hyperglycemia and a high HbA1c level and were treated with oral hypoglycemic agents or insulin (1–5). Given the low penetrance of *RFX6*-variant-associated diabetes, many mild cases may have been overlooked. More importantly, the clinical features of the patient improved with self-controlled food intake and daily exercise. These findings are consistent with previous observations that mild weight loss improves glucose metabolism in some individuals with type 2 diabetes (7). The present case provides evidence that the onset of *RFX6*-variant-associated diabetes can be influenced by minor environmental factors, and that the initial stage of diabetes can manifest as occasional hyperglycemia, which responds to lifestyle interventions.

At diagnosis, the patient experienced episodes of mild hyperglycemia, mostly after meals. The OGTT showed hyperglycemia and a delayed insulin peak. In this regard, Chandra *et al.* proposed that RFX6 of adult mice transactivates the *Ins* gene and regulates the P/Q-type and L-type calcium channels of beta cells that are involved in insulin secretion (8). Thus, RFX6 is likely to participate in the insulin response to glucose stimulation. Notably, our patient experienced a few episodes of mild hypoglycemia before lunch and dinner. Such hypoglycemia has also been described in individuals with type 1 and type 2 diabetes and may reflect perturbed insulin secretion (9, 10).

The elevated HOMA-IR, together with the lack

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Table 1. Patient clinical findings

	Patient	Reference range
At birth		
Birth weight	3,352 g (+0.78 SD)	
Gestational weeks	37 wk 5 d	
At diagnosis (11 yr 7 mo of age)		
Height	154.1 cm (+1.13 SD)	
Body weight	54.1 kg (+1.45 SD)	
Body mass index	22.8 kg/m^2 (+1.38 SD)	
HbA1c	**47 mmol/mL (6.5%)**	4.6%–6.2%
Blood glucose	162 mg/dL	
Serum C-peptide	6.83 ng/mL	3.02–9.06 ng/mL †
AST	26 IU/L	13–30 IU/L
ALT	26 IU/L	10–42 IU/L
TC	159 mg/dL	< 220 mg/dL
TG	172 mg/dL	< 175 mg/dL †
At 11 yr 7 mo of age		
Height	155.2 cm (+1.18 SD)	
Body weight	54.2 kg (+1.41 SD)	
Body mass index	22.5 kg/m^2 (+1.41 SD)	
HbA1c	**47 mmol/mL (6.5%)**	4.6%–6.2%
Blood glucose	101 mg/dL	< 110 mg/dL §
Insulinogenic index	0.6	> 0.4
HOMA-β	127.9%	> 30%
HOMA-IR	**3.4**	< 2.5 ‡
At 12 yr 6 mo of age		
Height	164.1 cm (+1.39 SD)	
Body weight	51.1 kg (+0.73 SD)	
Body mass index	19.0 kg/m^2 (+0.20 SD)	
HbA1c	39 mmol/mL (5.8%)	4.6%-6.2%
Blood glucose	90 mg/dL	< 110 mg/dL §
Serum C-peptide	1.23 ng/mL	0.61–2.09 ng/mL §
Insulinogenic index	0.6	> 0.4
HOMA-β	65%	> 30%
HOMA-IR	1.1	< 2.5 ‡
At 14 yr 10 mo of age		
Height	172 cm (+0.87 SD)	
Body weight	66.1 kg (+0.97 SD)	
Body mass index	22.3 kg/m^2 (+0.73 SD)	
HbA1c	38 mmol/mL (5.7%)	4.6%–6.2%
Blood glucose	97 mg/dL	< 110 mg/dL §
Serum C-peptide	1.54 ng/mL	0.61–2.09 ng/mL §
Insulinogenic index	0.7	> 0.4
HOMA-β	76%	> 30%
HOMA-IR	1.7	< 2.5 ‡

ALT, alanine aminotransferase; AST, aspartate aminotransferase; HbA1c, hemoglobin A1c; HOMA-β, Homeostatic Model Assessment of beta cell function; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; SD, standard deviation; TC, total cholesterol; TG, triglycerides. Blood glucose and serum C-peptide levels were measured simultaneously. Values above the reference range are in boldface. † Reference range in the postprandial state. ‡ Reference range in adults. § Reference range in the fasting state.

of obesity, indicated possible insulin resistance in our patient. Previous studies have suggested that RFX6 contributes to the secretion of gastric inhibitory peptide (GIP) (1). The serum GIP level is lower in patients with *RFX6* pathogenic variants than in control individuals (1). Considering that GIP mediates glucose uptake in adipocytes (11), *RFX6* pathogenic variants may induce mild insulin resistance by reducing secretion of GIP. However, given that the HOMA-IR level, which reflects insulin resistance, tends to increase during puberty (12), this assumption needs to be evaluated in future studies.

Conclusion

In summary, the results of this study indicate that the initial presentation of *RFX6*-variant-associated diabetes

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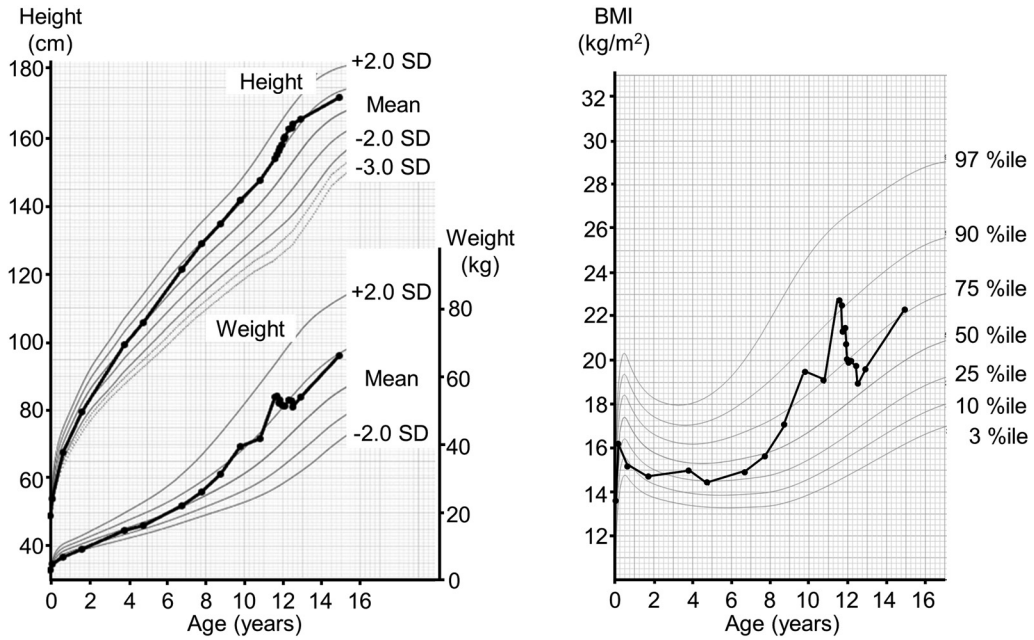

Fig. 1. Patient growth chart and body mass index (BMI) percentile curve. Height and weight are plotted against the reference curves for Japanese children (http://jspe.umin.jp/medical/chart_dl. html). SD, standard deviation.

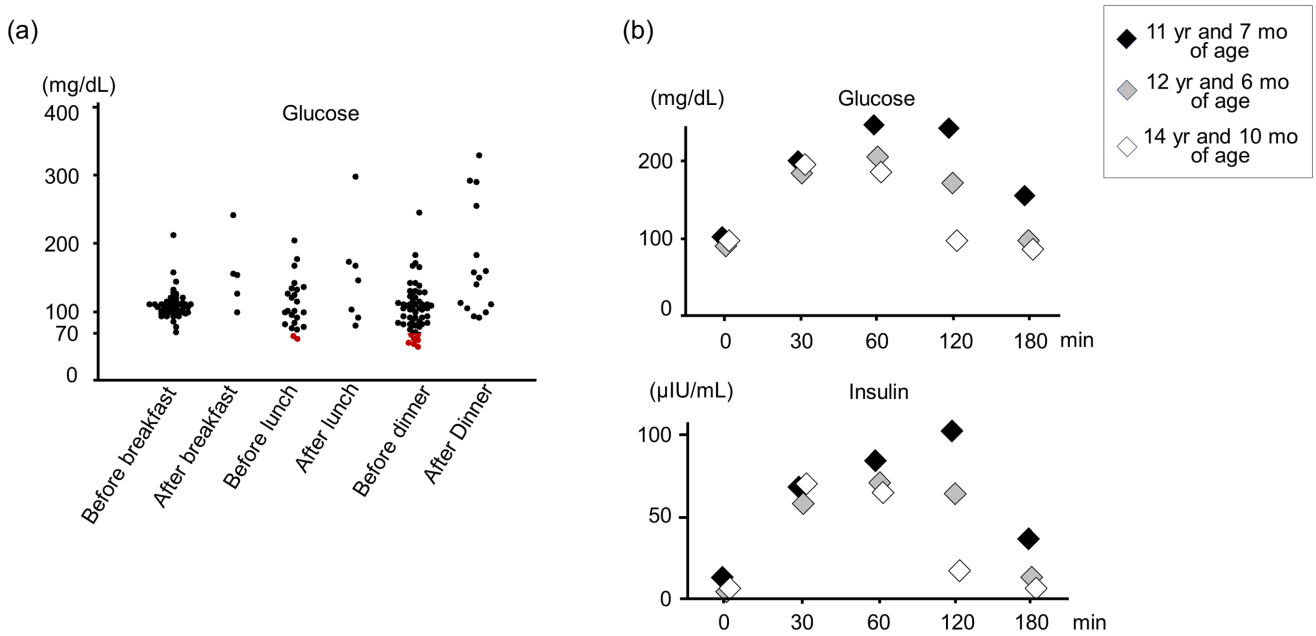

Fig. 2. Patient laboratory data. (a) The record of self-monitored blood glucose levels from ages 11 yr and 7 mo to 11 yr and 10 mo. The red dots indicate hypoglycemia (< 70 mg/mL). (b) Results of the oral glucose tolerance test (OGTT). The black, grey, and white plots represent results at 11 yr and 7 mo, 12 yr and 6 mo, and 14 yr and 10 mo of age, respectively.

includes occasional hyperglycemia and hypoglycemia that responds to lifestyle changes. Furthermore, our findings raise the possibility that *RFX6* pathogenic variants are associated with mild insulin resistance. However, these results require further validation.

Conflict of interests: Y.H. received seminar speaker fees from Novo Nordisk.

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