A novel RFX6 heterozygous mutation (p.R652X) in maturity-onset diabetes mellitus: A case report

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INTRODUCTION
Maturity onset diabetes mellitus of the young (MODY) is a monogenic form of diabetes mainly due to gene mutation related to pancreatic β-cell dysfunction¹. Mutations in HNF1A, HNF1B, and GCK are the most common causes of MODY and are responsible for ~40% of its etiology, but unidentified MODY genes are involved¹.

Regulatory factor X6 (RFX6) is a member of the regulatory factor X (RFX) family of transcription factors²-³. RFX6 regulates the differentiation and function of insulin-producing cells²-³. A homozygous RFX6 gene defect proves the Mitchell-Riley syndrome, which is characterized by neonatal diabetes with pancreatic hypoplasia, duodenal and jejunal atresia, and gall bladder agenesis⁴. Recently, heterozygous RFX6 gene mutations have been noted in MODY cases⁵. RFX6-related MODY cases have characteristics such as lack of islet autoantibodies and reduced secretion of insulin and glucose-dependent insulinotropic polypeptide (GIP) in response to glucose ingestion⁵. Here we report a patient with a novel heterozygous RFX6 mutation (p.R652X).

CASE REPORT
A 16-year-old female visited her family doctor after being found positive for urinary glucose in her school medical checkup. She had a family history of diabetes (Figure 1). Her plasma glucose and HbA1c levels were 467 mg/dL and 10.8%, respectively; she was diagnosed with diabetes and referred to our institution for genetic examination. Upon admission, her height, body weight, and body mass index were 142 cm, 42 kg, and 20.8, respectively. She had no islet-specific autoantibodies and showed a reduced meal-induced response of insulin, glucose-dependent insulinotropic polypeptide, and glucagon-like peptide-1, which is consistent with the phenotype of MODY due to heterozygous RFX6 mutation. In conclusion, we report a case of MODY due to a novel heterozygous mutation, p.R652X.

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A meal tolerance test was performed 9 months after her discharge (Figure 2b). Compared with individuals having normal glucose tolerance (NGT)\(^6\), the proband showed higher glucose levels before and after meal ingestion. Insulin and C-peptide were lower in the proband than in those with NGT despite the higher glucose levels before and 30 min after meal ingestion. The insulinogenic index \([\text{insulin}_{30\text{ min}} - \text{insulin}_{0\text{ min}}]/(\text{glucose}_{30\text{ min}} - \text{glucose}_{0\text{ min}})\] was lower in the proband (0.44) than that of NGT (0.83). Fasting and postprandial levels of GIP and GLP-1 were lower than those of NGT. Due to her family history of diabetes (Figure 1) and the early onset of her disease, we sequenced MODY-related genes of the proband, her father, mother, and brother. We found a heterozygous \(\text{RFX6}\) mutation \((\text{RFX6}: \text{NM}_173560: \text{exon17: c.1954C>T: p.R652X})\) in the proband (III-2) and in her mother (II-3) and brother (III-1).

We obtained approval from the ethics committee of Gifu University Graduate School of Medicine (Approval number 29-191). Genetic testing was carried out after counseling by a clinical genetic specialist. Written informed consent was obtained.

**DISCUSSION**

We report a case of MODY due to the novel heterozygous \(\text{RFX6}\) mutation \(\text{p.R652X}\). Our patient had no islet autoantibodies and reduced insulin and GIP response, which is consistent with the characteristics of \(\text{RFX6}\)-related MODY cases\(^5\). Our patient also had a reduced GLP-1 response. These findings are consistent with the reduced levels of GIP and GLP-1 found in \(\text{Rfx6}\)-deficient mice\(^7\) and may underlie the substantial improvement in glycemic control by the GLP-1 receptor agonist liraglutide in this case (Figure 2a). Consistently, it was reported that patients with the \(\text{RFX6}\) mutation show a good therapeutic response to dipeptidyl peptidase-4 (DPP-4) inhibitors\(^8\). Further clinical studies are warranted to determine the efficacy of GLP-1 receptor agonists and DPP-4 inhibitors in \(\text{RFX6}\)-related MODY cases.

Insulin and C-peptide levels were reduced in the proband before and 30 min after meal ingestion compared with those of NGT, and the insulinogenic index was lower in the proband than that in NGT. These results clearly indicate impaired insulin secretion in the proband, which may be
related to the reduced GLP-1 and GIP response (Figure 2b). RFX6 upregulates the expression of the insulin gene and other genes involved in insulin secretion (e.g., glucokinase and voltage-dependent calcium channel) in human b-cell line EndoC®-BH2 cells. Thus, it is likely that the novel heterozygous RFX6 mutation p.R652X directly impairs b-cell function in humans.

RFX6 directly binds to an X-box motif located at −288 to −269 bp from the transcription initiation site of the human insulin gene and activates insulin gene transcription. We found that RFX6(R652X) not only failed to activate the human insulin promoter but also suppressed RFX6-induced activation of the human insulin gene dose-dependently (Figure S1). Subcellular localization of RFX6 (R652X) revealed that the p.R652X mutation had little effect on nuclear localization of the protein, except that some fractions of the protein caused perinuclear aggregation (Figure S2). These results together strongly suggest that RFX6(R652X) interacts with RFX6 in the nucleus, thereby suppressing RFX6-induced activation of the target genes involved in insulin secretion. However, it is also possible that perinuclear aggregation of RFX6(R652X) disturbs b-cells, thereby impairing insulin secretion.

In conclusion, we report a case of MODY due to the novel heterozygous mutation p.R652X.

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DISCLOSURE
The authors declare no conflict of interest.

Table 1 | Laboratory data of the patient

| Laboratory test | Value | Reference range |
|-----------------|-------|-----------------|
| Specific gravity | >1.040 | 1.00-1.030 |
| Protein         | 29 (7-23) | 30-85 |
| Glucose         | 131 (124-222) | 30-100 |
| RBC             | 20 (9-32) | 30-45 |
| Ketone          | 40 (44-132) | 5-20 |
| WBC (×10³/µL)   | 7.32 (3.3-8.6) | 4.0-11.0 |
| RBC (×10³/µL)   | 4.35 (3.86-4.92) | 4.0-5.5 |
| Hb (g/dL)       | 12.8 (13.7-16.8) | 11.0-16.0 |
| Ht (%)          | 37.5 (40.7-50.1) | 37.0-47.0 |
| MCV (fl)        | 86.2 (83.6-98.2) | 75.0-100.0 |
| MCHC (%)        | 29.4 (31.7-35.3) | 31.0-36.0 |
| Plt (×10³/µL)   | 30.4 (15.8-34.8) | 15.0-35.0 |
| TP (g/dL)       | 68 (66-81) | 60-75 |
| Albumin (g/dL)  | 4.5 (4.1-5.1) | 3.5-5.0 |

Hormones

| Hormone          | Value | Reference range |
|------------------|-------|-----------------|
| ACTH (pg/mL)     | 47.0 (2.6-70.0) | 4.0-60.0 |
| Insulin (mU/L)   | 5.10 (1.84-12.2) | 0.0-10.0 |
| Anti-GAD Ab (U/mL) | <0.0 (<0.6) | 0.0-1.0 |
| Anti-Insulin Ab (U/mL) | <0.4 (<0.4) | 0.0-1.0 |
| Others           |       |                 |

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CASE REPORT

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(a) Treatment regimen: Insulin 10 U/day, Metformin 500 mg/day, Liraglutide 0.3 mg/day.

(b) Graphs showing changes in HbA1c, Body weight, Glucose, Insulin, C-peptide, GLP-1 over time.

Admission
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SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1 | Transcriptional activities of RFX6 and RFX6(R652X) in the human embryonic kidney (HEK) 293 cells. (a) Schematics of RFX6 and RFX6(R652X). (b) Luciferase activities of human insulin promoter in HEK293 cells transfected with indicated amounts of pCMV6b (Empty), pCMV6b-RFX6 (RFX6) or pCMV6b-RFX6(R652X) (RFX6(R652X)). Data represent the mean ± SD of 6 wells per condition. *Indicates P < 0.05 (Tukey’s test) versus 0.4 μg pCMV6b-RFX6. Data were analyzed using Graphpad Prism versus 9 (Graphpad Software, CA, USA).

Figure S2 | Subcellular localization of RFX6 and RFX6(R652X) tagged with 3xFLAG epitope at the N-terminus in indicated cell lines. (a) Schematics of RFX6 and RFX6(R652X) tagged with 3xFLAG epitope at N-terminus. (b) Representative images of HEK293, INS-1 832/12 cells and STC-1 cells expressing RFX6 and RFX6(R652X) tagged with 3xFLAG epitope. Magnification, x40. Red arrows indicate perinuclear aggregates of 3xFLAG-RFX6 (R652X).

Supplementary Material | Supplementary methods.