JDI Journal of Diabetes Investigation Open access
Official Journal of the Asian Association for the Study of Diabetes
CASE REPORT

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

Sakiho Imaki[1], Katsumi Iizuka[2]*, Yukio Horikawa[2], Megumi Yasuda[2], Sodai Kubota[2,3], Takehiro Kato[2], Yanyan Liu[2], Ken Takao[2], Masami Mizuno[2], Takuo Hirota[2], Tetsuya Suwa[2], Kazuyoshi Hosomichi[4], Atsushi Tajima[4], Yuuka Fujiwara[3], Yuji Yamazaki[3,5], Hitoshi Kuwata[3,5], Yutaka Seino[3,5], Daisuke Yabe[2,3,6]

[1]Faculty of Medicine, Gifu University, Gifu, Japan, [2]Department of Diabetes and Endocrinology, Gifu University Graduate School of Medicine, Gifu, Japan, [3]Yutaka Seino Distinguished Center for Diabetes Research, Kansai Electric Power Medical Research Institution, Kobe, Japan, [4]Department of Bioinformatics and Genomics, Graduate School of Advanced Preventive Medical Sciences, Kanazawa University, Kanazawa, Japan, [5]Center for Diabetes, Metabolism and Endocrinology, Kansai Electric Power Hospital, Osaka, Japan, and [6]Division of Molecular and Metabolic Medicine, Department of Physiology and Cell Biology, Kobe University Graduate School of Medicine, Kobe, Japan

Keywords
GIP, GLP-1, RFX6

***Correspondence**
Katsumi Iizuka
Tel.: +81-58-230-6377
Fax: +81-58-230-6376
E-mail address:
kiizuka@gifu-u.ac.jp

J Diabetes Investig 2021; 12: 1914–1918

doi: 10.1111/jdi.13545

ABSTRACT

Heterozygous *RFX6* mutation has emerged as a potential cause of maturity-onset diabetes mellitus of the young (MODY). A 16-year-old female was diagnosed with diabetes by her family doctor and was referred to our institution for genetic examination. Genetic testing revealed a novel *RFX6* heterozygous mutation (NM_173560: exon17: c.1954C>T: p.R652X) in the patient and in her mother and brother. 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.

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 dysfunction[1]. 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 involved[1].

Regulatory factor X6 (*RFX6*) is a member of the regulatory factor X (RFX) family of transcription factors[2,3]. *RFX6* regulates the differentiation and function of insulin-producing cells[2,3]. 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 agenesis[4]. Recently, heterozygous *RFX6* gene mutations have been noted in MODY cases[5]. *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 ingestion[5]. 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. Autoantibodies against glutamic acid decarboxylase and insulin and islet antibody-2 were negative, and basal levels of pituitary, thyroid, and adrenal hormones were normal (Table 1). She had no diabetic neuropathy or retinopathy, while her urinary albumin level was 43.1 mg/g Cre. Her abdominal ultrasound and computer tomography (CT) scan revealed no pancreas, intestine, or gall bladder anomalies except for fatty liver. After 7 days of intensive insulin therapy (a total of 10 units per day), 500 mg metformin was started instead of insulin therapy. Her fasting glucose levels fell to 90–100 mg/dL and she was discharged 16 days after

Received 17 November 2020; revised 1 March 2021; accepted 8 March 2021

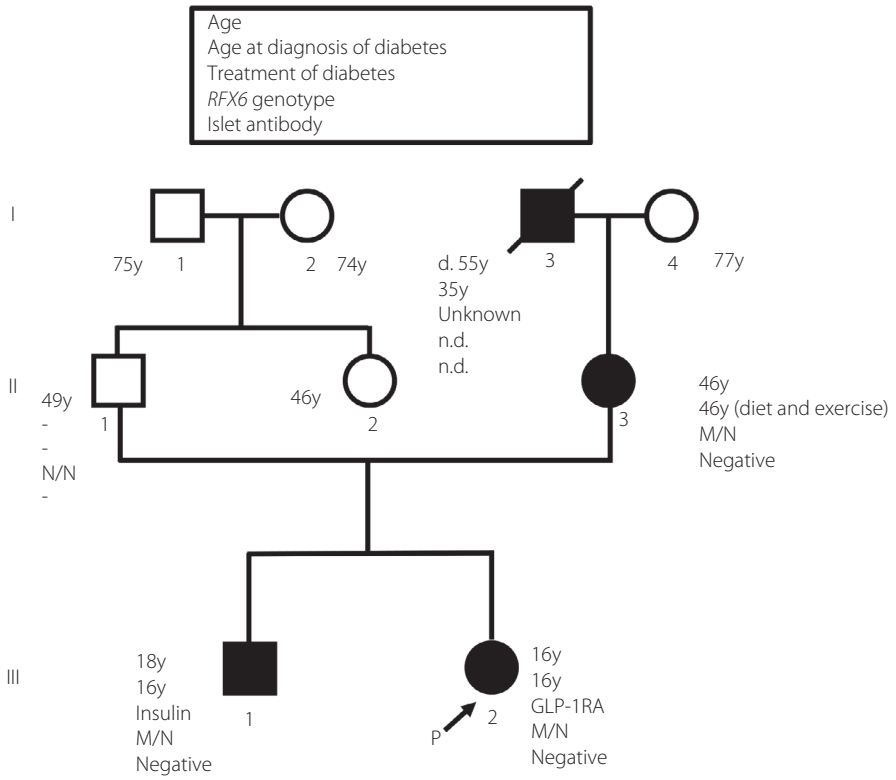


Figure 1 | A family tree of the patient. Squares, circles, and arrows indicate males, females, and proband, respectively. Family members, including her brother, mother, and maternal grandfather had a history of diabetes. d. Deceased. M and N, wild type and p.R652X mutation alleles, respectively. Roman numerals on the left of the pedigrees indicate generation number, and the numbers below the symbols indicate the subject's number within each pedigree. Arrow shows the proband. The proband's family members generally have short stature regardless of the presence (M/N) or absence (N/N) of the identified heterozygous *RFX6* mutation (p.R652X): III-2 (M/N) 142 cm, II-1 (N/N) 155 cm, II-3 (M/N) 152 cm, III-1 (M/N) 156 cm, I-1 148 cm, I-2 155.5 cm and II-2 156 cm, respectively. Thus, it is likely that the short stature of the proband is unrelated to the heterozygous *RFX6* mutation, p.R652X.

admission. While her HbA1c remained >7% for 4 months after discharge, it improved substantially after initiation of the glucagon-like peptide-1 (GLP-1) receptor agonist liraglutide (Figure 2a).

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 *RFX6* mutation (*RFX6*: NM_173560: 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 *RFX6* mutation p.R652X. Our patient had no islet autoantibodies and reduced insulin and GIP response, which is consistent with the characteristics of *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 *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 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-

Table 1 | Laboratory data of the patient

Urinalysis		AST (U/L)	23 (13–30)	Hormones	
Specific gravity	>1.040	ALT (U/L)	29 (7–23)	IGF1 (ng/mL)	210
Protein	-	LDH (U/L)	131 (124–222)	GH (ng/mL)	1.51
Glucose	3+	ALP (U/L)	258 (106–322)	FT3 (pg/mL)	3.03 (2.39–4.06)
RBC	-	γGTP (U/L)	20 (9–32)	FT4 (ng/dL)	0.97 (0.76–1.65)
Ketone	-	AMY (U/L)	40 (44–132)	TSH (μIU/mL)	0.24 (0.54–4.26)
Blood counts		CRE (mg/dL)	0.45 (0.46–0.79)	Cortisol (μg/dL)	15.5 (6.24–18.0)
WBC ($\times 10^3/\mu L$)	7.32 (3.3–8.6)	UA (mg/dL)	4.7 (2.6–7.0)	ACTH (pg/mL)	31.9 (7.2–63.3)
RBC ($\times 10^6/\mu L$)	4.35 (3.86–4.92)	Na^+ (mEq/L)	136 (138–145)	Insulin (mU/L)	5.10 (1.84–12.2)
Hb (g/dL)	12.8 (13.7–16.8)	K^+ (mEq/L)	4.0 (3.6–4.8)	CPR (ng/mL)	1.49 (1.84–12.2)
Ht (%)	37.5 (40.7–50.1)	Cl^- (mEq/L)	106 (101–108)	Others	
MCV (fL)	86.2 (83.6–98.2)	Ca^{2+} (mg/dL)	9.6 (8.8–10.1)	Anti-GAD Ab (U/mL)	<5.0 (<5.0)
MCHC (%)	29.4 (31.7–35.3)	Pi^{2-} (mg/dL)	3.1 (2.7–4.6)	IA2 (U/mL)	0.7 (<0.6)
Plt ($\times 10^4/\mu L$)	30.4 (15.8–34.8)	T-cho (mg/dL)	187 (142–220)	Anti-Insulin Ab (U/mL)	<0.4 (<0.4)
Biochemistry		TG (mg/dL)	127 (30–150)		
TP (g/dL)	6.8 (6.6–8.1)	HDL-c (mg/dL)	51 (30–150)		
Albumin (g/dL)	4.5 (4.1–5.1)	Glucose (mg/dL)	329		

ACTH, adrenocorticotropic hormone; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AMY, amylase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; ChE, choline esterase; CPR, C peptide immunoreactivity; CRE, creatinine; FT3, free tri-iodo-thyronine; FT4, free thyroxine; GAD, glutamate decarboxylase; GH, growth hormone; Hb, hemoglobin; Ht, hematocrit; IA-2, islet antigen 2; IGF-1, insulin-like growth factor; LDH, lactate dehydrogenase; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; Plt, platelet; RBC, red blood cell; T-Bil, total bilirubin; T-cho, total cholesterol; TG, triglyceride; TP, total protein; UA, uric acid; WBC, white blood cell; γGTP, γ-glutamyltransferase.

1 receptor agonists and DPP-4 inhibitors in *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 β-cell line EndoC-βH2 cells[3]. Thus, it is likely that the novel heterozygous *RFX6* mutation p.R652X directly impairs β-cell function in humans.

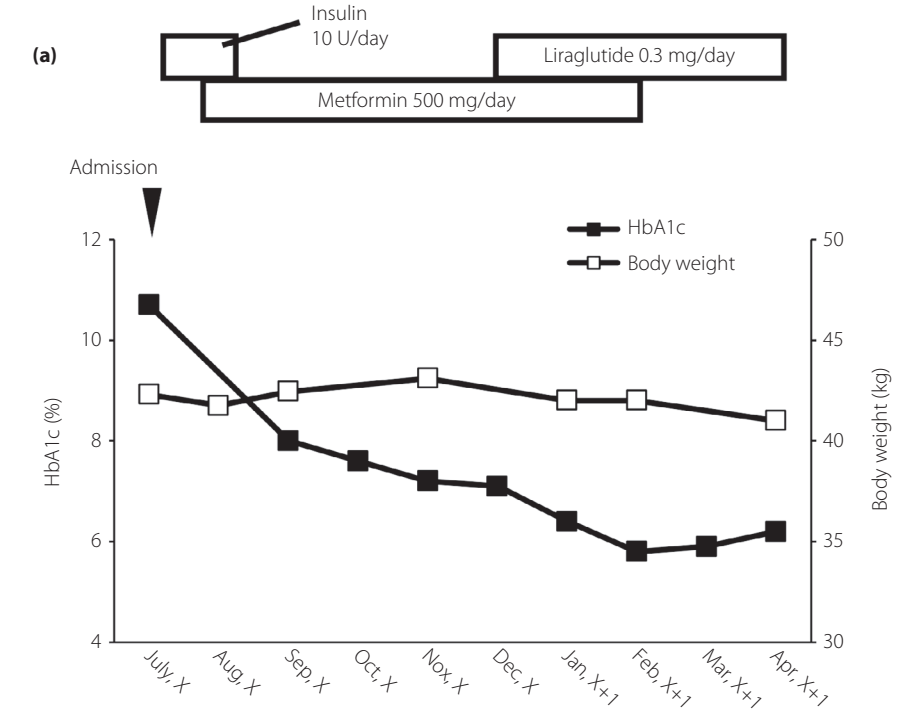
RFX6 directly binds to an X-box motif located at −288 to −269 bp from the transcription initiation site of the human insulin gene and activates insulin gene transcription[3]. We found that RFX6(R652X) not only failed to activate the human insulin promoter but also suppressed RFX6-induced activation of 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 β-cells, thereby impairing insulin secretion.

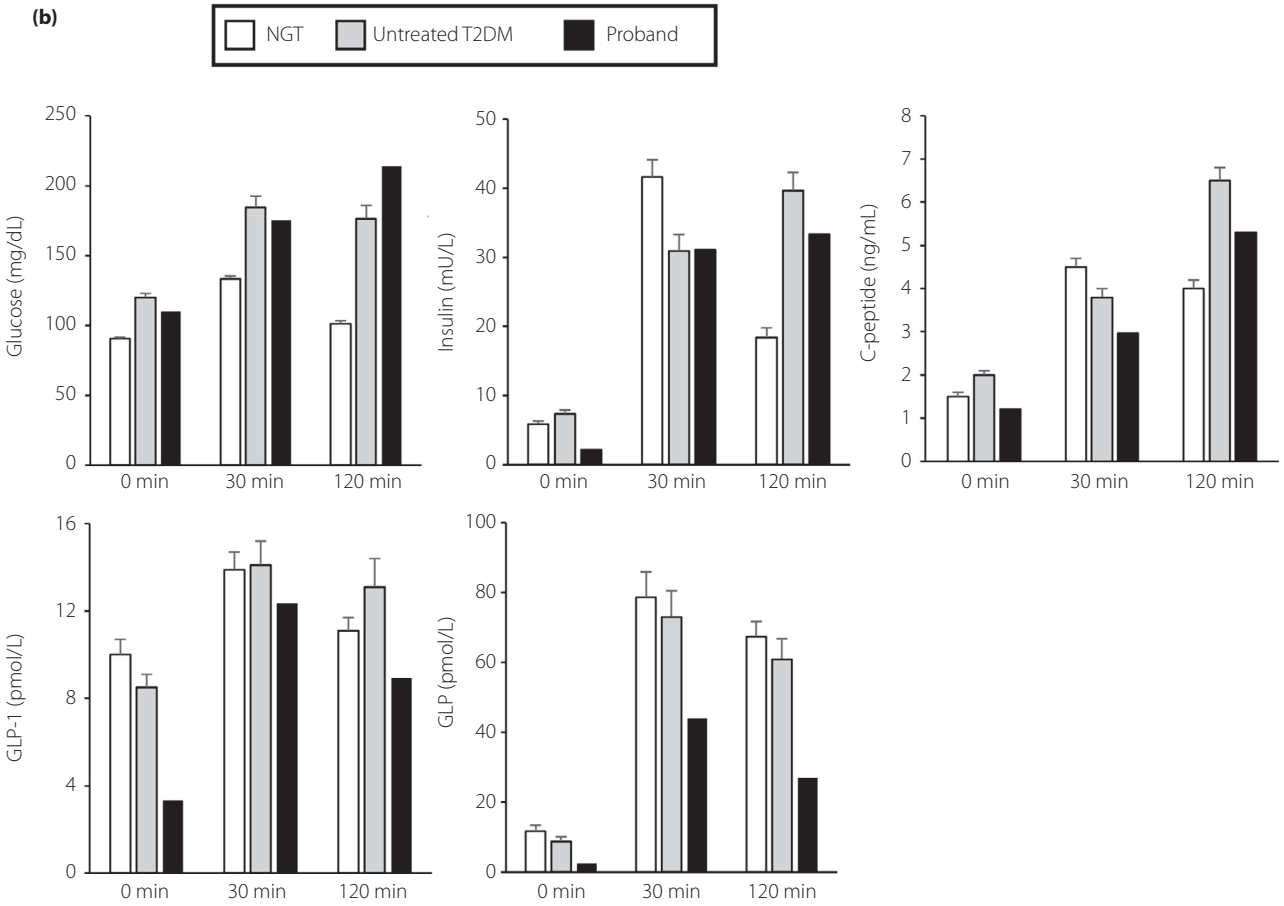
In conclusion, we report a case of MODY due to the novel heterozygous mutation p.R652X.

ACKNOWLEDGMENTS

This work was supported by grants from the Japan Society for the Promotion of Sciences [17K09825 and 26111004 (DY), 20K19673 (LY), 20K11645 (KI), 18H02779 (YH)], the Japan

Figure 2 | Clinical course and the response of insulin and incretins to meal ingestion in the patient. (a) HbA1c (closed square) and body weight (open square). (b) Meal tolerance test (480 kcal; carbohydrate 58.4%, protein 15.9% and fat 25.7%) was performed 9 months after her discharge. Glucagon-like peptide-1 (GLP-1) liraglutide was stopped 7 days before the test. We compared the proband's data with our previous results of 18 drug-naïve individuals with type 2 diabetes (T2DM) and 17 individuals with normal glucose tolerance (NGT)[7]. Values of glucose, insulin, GLP-1, and glucose-dependent insulinotropic polypeptide (GIP) at the indicated times are represented as mean ± SEM for NGT and type 2 diabetes.


(a)
Insulin
10 U/day
Liraglutide 0.3 mg/day
Metformin 500 mg/day
Admission
HbA1c
Body weight
HbA1c (%)
Body weight (kg)
July, X
Aug, X
Sep, X
Oct, X
Nox, X
Dec, X
Jan, X+1
Feb, X+1
Mar, X+1
Apr, X+1


(b)
NGT
Untreated T2DM
Proband
Glucose (mg/dL)
Insulin (mU/L)
C-peptide (ng/mL)
GLP-1 (pmol/L)
GLP (pmol/L)
0 min
30 min
120 min

Agency for medical research and development [JP20ek0109352, JP20ek0210111h0003, JP20ek0210123s0602, and JP20ek0210 101h0003 (DY)]; Suzuken Memorial Foundation Grant (DY) and Japan Association for Diabetes Education and Care (TK).

DISCLOSURE

The authors declare no conflict of interest.

REFERENCES

1. Horikawa Y. Maturity-onset diabetes of the young as a model for elucidating the multifactorial origin of type 2 diabetes mellitus. *J Diabetes Investig* 2018; 9: 704–712.
2. Smith SB, Qu HQ, Taleb N, *et al*. Rfx6 directs islet formation and insulin production in mice and humans. *Nature* 2010; 463: 775–780.
3. Chandra V, Albagli-Curiel O, Hastoy B, *et al*. RFX6 regulates insulin secretion by modulating Ca2+ homeostasis in human β cells. *Cell Rep* 2014; 9: 2206–2218.
4. Mitchell J, Punthakee Z, Lo B, *et al*. Neonatal diabetes, with hypoplastic pancreas, intestinal atresia and gall bladder hypoplasia: search for the aetiology of a new autosomal recessive syndrome. *Diabetologia* 2004; 47: 2160–2167.
5. Patel KA, Kettunen J, Laakso M, *et al*. Heterozygous RFX6 protein truncating variants are associated with MODY with reduced penetrance. *Nat Commun* 2017; 8: 888.
6. Yabe D, Kuroe A, Lee S, *et al*. Little enhancement of meal-induced glucagon-like peptide 1 secretion in Japanese: comparison of type 2 diabetes patients and healthy controls. *J Diabetes Investig* 2010; 1: 56–59.
7. Piccand J, Vagne C, Blot F, *et al*. Rfx6 promotes the differentiation of peptide-secreting enteroendocrine cells while repressing genetic programs controlling serotonin production. *Mol Metab* 2019; 29: 24–39.
8. Artuso R, Provenzano A, Mazzinghi B, *et al*. Therapeutic implications of novel mutations of the RFX6 gene associated with early-onset diabetes. *Pharmacogenomics J* 2015; 15: 49–54.

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.