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Identification of a Novel Mutation in Carboxyl Ester Lipase Gene in a Patient with MODY-like Diabetes

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Maturity-onset diabetes of the young (MODY) is a form of diabetes mellitus characterized by autosomal dominant inheritance, early onset, and the absence of pancreatic autoimmune markers. MODY-causing mutations have been identified in 14 genes, and carboxyl ester lipase (*CEL*) has been implicated in MODY8. We report a Japanese patient with MODY who harbored a heterogeneous mutation in *CEL* exon 2 (NM_001807.4:c.146_147delCT; NP_001798.2:p.Ser49CysfsTer52). A 13-year-old girl experienced her first episode of diabetic ketoacidosis, during which her endogenous insulin secretion was poor. However, her insulin secretion had apparently recovered 2 months after the commencement of insulin treatment, and no further treatment was required for the following 2 years. Diabetic ketoacidosis recurred when the patient was 15 years old, when her insulin secretion was again poor. Since that time, the patient, who is now 18 years old, has been undergoing continuous insulin treatment. The large fluctuations in her insulin secretory capacity led us to suspect MODY. MODY8 patients that carry a mutation in the variable number of tandem repeats in the last exon of the *CEL* gene typically show pancreatic exocrine dysfunction. However, in the present case, which features premature termination, there is no involvement of exocrine dysfunction, potentially demonstrating a genotype-phenotype correlation.

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Introduction

The diagnosis of atypical non-autoimmune forms of diabetes mellitus, such as maturity-onset diabetes of the young (MODY; MIM# 606391), continues to present challenges. MODY is a monogenic form of diabetes characterized by autosomal dominant inheritance, an early age of onset (typically < 25 years), and partial preservation of pancreatic β-cell function. Molecular genetic studies have identified heterozygous causal variants in 14 MODY genes. Although MODY comprises 1%-6% of pediatric diabetes cases (Hattersley et al. 2018), it frequently goes undiagnosed or is misclassified as type 1 or type 2 diabetes, owing to overlapping clinical features. Given the opportunities, it provides for more accurate diagnosis and informed treatment, genetic testing for MODY genes has the potential to provide considerable benefits for the patient.

In Japan, glucokinase gene abnormalities (MODY2) and hepatocyte nuclear factor-1α gene abnormalities (MODY3) are the most common forms of MODY, followed by hepatocyte nuclear factor-1β (MODY5), hepatocyte nuclear factor-4α (MODY1), and neurogenic differentiation 1 (MODY6) gene abnormalities (Yorifuji et al. 2012). Several other types, including MODY8, have not been reported in Japan to date.

Here, we report a patient initially misdiagnosed as having type 2 diabetes, in whom a pathogenic mutation was eventually identified in the carboxyl ester lipase (*CEL*) gene. MODY8 involves an abnormality in *CEL*, and is characterized by both endocrine and exocrine dysfunction.

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It was first reported in two Norwegian families who had a single base-pair deletion in the variable number of tandem repeats (VNTR) region of *CEL* and pancreatic exocrine dysfunction (Raeder et al. 2006). Intracellular aggregates of the mutant protein trigger pancreatic cell death via a deleterious gain-of-function effect (Johansson et al. 2011), and indeed most of the pathogenic *CEL* variants that have been reported in association with dyslipidemia to date are located within the VNTR of the last exon (Johansen et al. 2014). Recently, a comprehensive genetic analysis of patients with atypical early-onset non-autoimmune diabetes revealed that some have mutations in *CEL* other than in the VNTR, but their symptoms are thought to relate to diabetes alone (Mohan et al. 2018; Sarmadi et al. 2020). Here, we have discussed previously reported MODY8 patients with reference to the site of the *CEL* gene mutations and the presence or absence of exocrine dysfunction.

Case Presentation

A 13-year-old girl was admitted to hospital because of epigastric pain and unconsciousness. One week beforehand, she began to experience nausea, and her body mass had decreased from 56 kg [body mass index (BMI), 24.8 kg/m^2] to 48 kg at admission. There was a history of type 2 diabetes in her maternal and paternal grandfathers (onset of diabetes at 55 and 58 years, respectively). Her older sister (15 years old) was healthy. On the basis of the laboratory test results, she was diagnosed as having diabetic ketoacidosis (DKA). Saline and continuous low-volume intravenous insulin restored her level of consciousness within 24 h. The required dose of insulin was initially 1.2 units/kg/day. Tests for serum glutamic acid decarboxylase (GAD), islet antigen-2 (IA-2), and islet cell cytoplasmic (IC) autoantibodies were negative, and the measurement of urinary C-peptide immunoreactivity (CPR) showed an extremely low level of < 0.1 μg per day. Her endogenous insulin secretion was suspected to have been insufficient in the acute phase. However, this improved gradually, and her 24-h urinary CPR had increased to within the normal range (189 μg/day) 9 weeks after the commencement of treatment.

Type 2 diabetes was diagnosed on the basis of the recovery of endogenous insulin secretion. The patient's blood glucose was subsequently well controlled using diet and exercise therapy, and her insulin dose was tapered off and ultimately discontinued. However, 2 years later, diet and exercise therapy were discontinued and she gained weight. She complained of fatigue, and polydipsia and polyuria lasting several days. Blood tests showed a recurrence of DKA, and insulin injection therapy was resumed to improve her blood glucose control. Serum GAD, IA-2, IC, IA and zinc transporter 8 antibody tests were negative. Her urine CPR had decreased to 5.8 μg/day, and slightly increased after 2 weeks of insulin therapy, but was still insufficient (22 μg/day). The required dose of insulin gradually decreased and her blood glucose was well controlled using 0.5 units/kg/day of insulin.

At the ages of 16 and 17 years, her insulin treatment was self-interrupted and her HbA1c markedly increased from 6.1% to 9.6% and from 6.7% to 11.8%, respectively. Her clinical course of insulin treatment, BMI, 24-h urinary CPR and HbA1c levels from the onset to the present are shown in Fig. 1. No abnormality was found on abdominal magnetic resonance imaging when she was 16 years old. The fecal elastase test at the age of 18 revealed her pancreatic exocrine function is normal (> 200 μg/g). A glucagon load test was performed when she was 17 years old. The basal CPR value was 1.9 ng/dL, and the CPR values measured 3, 6, 9, and 15 min after the injection of 1 mg glucagon were 4.6, 4.0, 2.9, and 2.2 ng/mL, respectively. The ΔCPR (6-min value) was 2.1 ng/mL, indicating that her insulin secretion was insufficient. Because she had an atypical non-autoimmune form of diabetes, and despite the lack of a family history of early-onset diabetes, we considered that she might have MODY.

Methods

Editorial policies and ethical considerations

Ethics approval was obtained from the Ethics Committee of Fujita Health University for the study (number HG20-054), and written informed consent was obtained from the patient and her parents.

Methods of genetic analysis

Peripheral blood samples were obtained and genomic DNA was extracted using a Gentra Puregene Kit (Qiagen, Toronto, Canada). To identify the causative mutation, we used a Trusight One Sequencing Panel on the MiSeq platform (Illumina, San Diego, CA, USA). Sequence data were analyzed using Variant Studio software (Illumina). All the identified variants were filtered on the basis of population frequencies, with a minor allele frequency cut-off of 0.5%, a presumption of an effect on function, and with reference to genes related to known forms of MODY. Candidate variants were validated using Sanger sequencing. To compare the area under the curve of Sanger sequences, GeneMapper software was used (Lee et al. 2018).

Results

In *CEL* exon 2, a heterozygous NM_001807.4:c.146_147delCT variant that caused a frameshift (NP_001798.2:p.Ser49CysfsTer52) was found (Fig. 2a). This variant was not registered in the ExACv.1.0 nor gnomAD v2.1.1 database. Sanger sequencing of *CEL* exon 2 in the parents revealed that the patient's mother was a combined somatic and germline mosaic carrier of the c.146_147delCT variant, but not her father. Genetic analysis of the maternal grandfather, who presented with type 2 diabetes when he was 55 years old, was not performed because informed consent could not be obtained. The pedigree tree of the patient is shown in Fig. 2b. Neither pathogenic nor likely pathogenic variants were identified in the other MODY genes, includ-

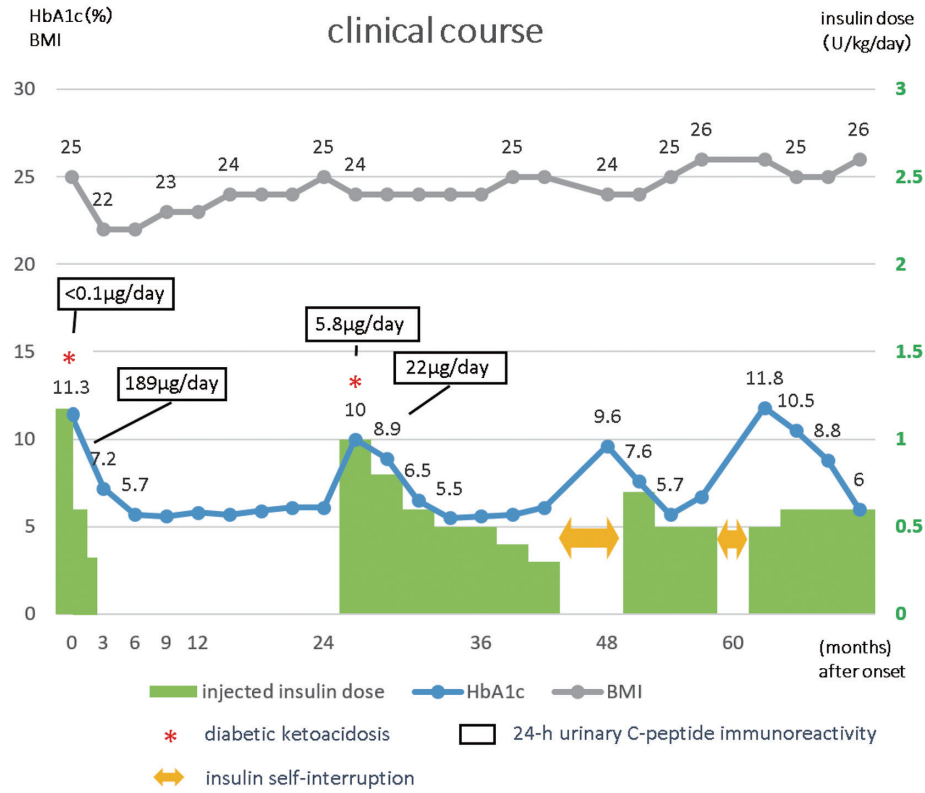

Fig. 1. The clinical course of insulin treatment, BMI and HbA1c levels from the onset at the age of 13 years to the present at the age of 18 years.
The required dosage of insulin injection was initially 1.2 units/kg/day at onset. Her insulin dose was tapered and discontinued after 9 weeks. Two years later, at the age of 15 years, she developed ketoacidosis for the second time and has been on insulin therapy to date. At the ages of 16 and 17 years, her insulin treatment was self-interrupted and her HbA1c markedly increased.

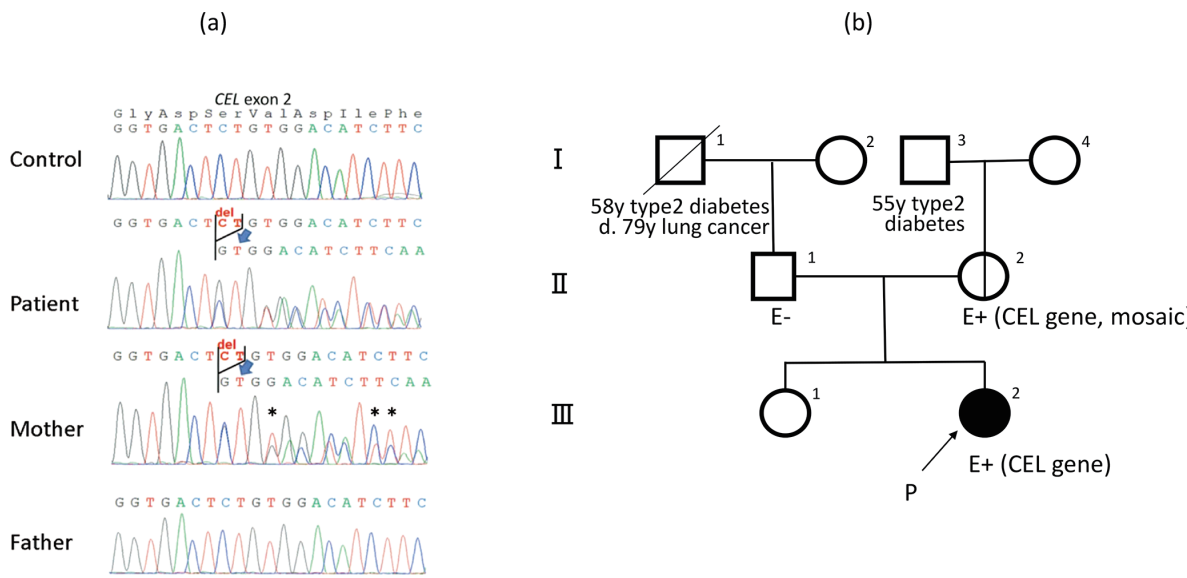

Fig. 2. Representative Sanger sequencing electropherogram in the region of the mutation in exon 2 of *CEL* (a) and the pedigree tree of the patient's family (b).
(a) The c.146_147delCT mutation is present in the patient and her mother but not in her father. The patient's mother was a mosaic carrier with a mutation rate of 0.65. *indicates the nucleotides for which the area ratios were calculated. The percentage areas of the normal and mutant sequences under the curves were 61%:39%, 67%:33%, and 66%:34% (Lee et al. 2018). Del, deletion. (b) E+, being positive for the variant; E−, being negative for the variant.

ing the genes responsible for MODY1-6.

Discussion

We have identified a patient with a non-autoimmune form of diabetes who showed large fluctuations in her insulin secretory capacity and was eventually diagnosed with MODY8 using targeted exome analysis. MODY8 is characterized by endocrine and pancreatic exocrine dysfunction caused by an abnormality in *CEL*. CEL hydrolyzes dietary fat, cholesteryl esters, and fat-soluble vitamins in the duodenum, and represents approximately 4% of the total protein mass of the pancreatic juice (Lombardo et al. 1978). CEL is mainly expressed in pancreatic acinar cells and lactating mammary glands, and is not expressed in the islets of Langerhans. The human *CEL* gene is located on chromosome band 9q34.13 and consists of 11 exons (Taylor et al. 1991; Johansson et al. 2018). The last exon of *CEL* contains a very guanine-cytosine-rich VNTR region. It has been proposed that MODY8 is a protein-misfolding disease that develops because a heterozygous single nucleotide deletion in the VNTR causes the production of a mutant CEL protein, which increases endoplasmic reticulum stress, resulting in apoptosis (Johansson et al. 2011). In addition, pancreatic ductal adenocarcinoma and pancreatitis are caused by abnormalities in *CEL*. However, the mechanism of diabetes development is unknown.

Although *CEL* c.146_147delCT has not been reported previously, a two-base deletion results in a premature stop codon because of a frameshift, which leads to the production of truncated CEL protein molecules with defective function or zero protein expression secondary to nonsense-mediated mRNA decay. Indeed, heterozygous mutations, P291fsinsC in *HNF1A* and R177X in *HNF1B*, cause MODY3 and MODY5, respectively, via haploinsufficiency rather than dominant negative effect (Yabe et al. 2019). Likewise, it is possible that the loss-of-function of one allele caused by this variant may be a cause of MODY8. However, the present patient has had no pancreatic exocrine dysfunction to date. A study of a Norwegian family with a mutation in the VNTR region of *CEL* showed that this form of MODY had low penetrance, whereas there was 100% penetrance of the pancreatic exocrine dysfunction (Raeder et al. 2006). It may take time for the present patient to show a decline in pancreatic exocrine function, but the phenotype of MODY8 may differ according to the CEL genotype, particularly whether the mutation is in the VNTR region. The patient's mother harbors the same mutation as her daughter, but has not yet developed the disease, which may be because the mother is a mosaic carrier or because of low disease penetrance. Although pathogenicity of variants other than VNTR in *CEL* has not been elucidated yet, we evaluated using the American College of Medical Genetics recommendations (Richards et al. 2015). Assuming that *CEL* c.146_147delCT might cause haploinsufficiency due to loss-of-function, PM2, PVS1 and PP4 applied, indicating that it was "likely pathogenic".

MODY8 owing to a *CEL* abnormality is extremely rare; however, it is likely that there are undiagnosed or misclassified cases. An analysis of the known MODY genes in 152 patients with atypical diabetes who were clinically suspected to have MODY revealed that one carried a nonsense variant (Tyr98*) in *CEL* that was considered to be patho-

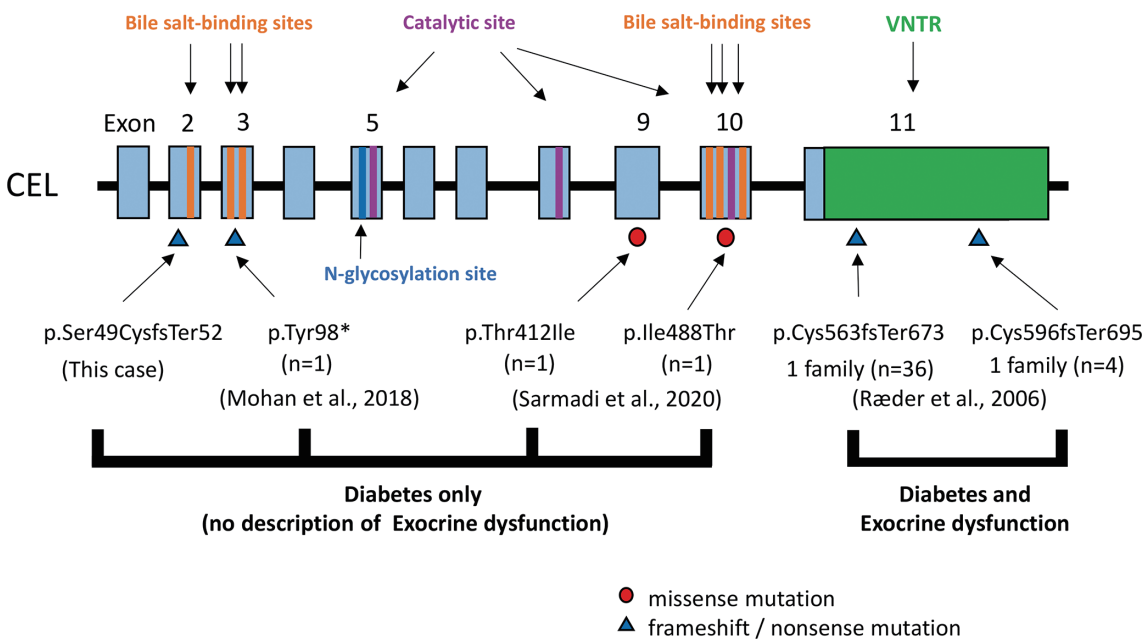

Fig. 3. Schematic representation of genomic organization the carboxyl ester lipase (*CEL*) gene.
The boxes denote exons and the known roles of specific sites are shown. The mutations thought to cause diabetes are indicated by arrows. The symbols differ according to the type of mutation. The symptoms of the reported cases are summarized below. VNTR, variable number of tandem repeats; n, number of the reported cases carrying each variant.

genic (Mohan et al. 2018). Moreover, whole exome sequencing analysis of 10 families with suspected MODY that carried no pathogenic mutations in the *GCK* or *HNF1A* genes identified two variants in *CEL* (Sarmadi et al. 2020). p.Ile488Thr was considered to be pathogenic and p.Thr412Ile was not confirmed to be pathogenic, but both families presented with a typical MODY phenotype (Sarmadi et al. 2020). None of these variants in regions other than the VNTR of *CEL* were not associated with pancreatic exocrine dysfunction; instead, the symptoms were related to diabetes alone. In a mouse experiment, the complete phenotype of human CEL-MODY was not reproduced in CEL-knockout mice. However, mild glucose intolerance was observed in female mice with systemic knockout of *CEL*, indicating not statistically significant but some effect on glycemic control in female mice (Vesterhus et al. 2010).

Fig. 3 describes the previously reported MODY patients with mutation in *CEL*. Although the number of patients is low, those with clinical features of pancreatic exocrine abnormalities had variants at the VNTR site, while those without had variants at sites other than the VNTR. It is important to determine the underlying mechanism of the diabetes caused by *CEL* abnormalities, which has not been elucidated to date.

In atypical non-autoimmune early-onset diabetes, even without a clear family history, the possibility of MODY should be considered and genetic testing should be performed. With an increase in the number of diagnoses of MODY8 and progress in research, the role of CEL protein in insulin secretion and the mechanism of diabetes onset is expected to be elucidated in the future.

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Conflict of Interest

The authors declare no conflict of interest.

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