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SHORT REPORT

Identification of a compound heterozygous inactivating *ABCC8* gene mutation responsible for young-onset diabetes with exome sequencing

Norihiko Matsutani[1], Hiroto Furuta[1]*, Shohei Matsuno[1], Yoshimasa Oku[2], Shuhei Morita[1], Shinsuke Uraki[1], Asako Doi[1], Machi Furuta[3], Hiroshi Iwakura[1], Hiroyuki Ariyasu[1], Masahiro Nishi[4], Takashi Akamizu[1]

[1]First Department of Internal Medicine, Wakayama Medical University, [2]Oku Medical Clinic, [3]Clinical Laboratory Medicine, and [4]Department of Clinical Nutrition and Metabolism, Wakayama Medical University, Wakayama, Japan

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***Correspondence**
Hiroto Furuta
Tel.: +81-73-441-0625
Fax: +81-73-445-9436
E-mail address:
hfuruta@wakayama-med.ac.jp

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ABSTRACT

Activating mutations in the *ABCC8* gene cause diabetes and inactivating mutations usually cause hyperinsulinemic hypoglycemia in infancy. Patients with hypoglycemia in infancy due to a heterozygous inactivating mutation have been reported to occasionally progress to diabetes later in life. We explored the gene responsible for diabetes in two brothers, who were suspected to have diabetes at 15 and 18 years-of-age, respectively, with whole exome sequencing, and identified a compound heterozygous *ABCC8* gene mutation (p.Arg168Cys and p.Arg1421Cys). Although their father and mother were heterozygous carriers of the p.Arg168Cys and the p.Arg1421Cys mutation, respectively, neither parent had diabetes. These mutations have been reported to be responsible for hypoglycemia in infancy and function as an inactivating mutation. Our results suggest that the inactivating *ABCC8* gene mutation is also important in the etiology of diabetes.

INTRODUCTION

The adenosine triphosphate-sensitive potassium (K_{ATP}) channel is located at the center in the glucose-stimulated insulin secretion pathway of pancreatic β-cells, and consists of the pore-forming subunit by Kir6.2 and regulatory subunit by sulfonylurea receptor 1 (SUR1)[1]. Activating mutations of the *KCNJ11* and *ABCC8* genes, which encode the Kir6.2 and SUR1, cause neonatal diabetes or maturity-onset diabetes of the young[2]. In contrast, inactivating mutations of these genes usually cause hyperinsulinemic hypoglycemia in infancy (HHI)[3].

The HHI caused by the K_{ATP} channel abnormality is subdivided into focal and diffuse forms based on the histological appearance of the pancreas. The *H19* and *CDKN1C* genes, which are a tumor suppressors, are only expressed from the maternally-inherited chromosome, and are located relatively closely to the *ABCC8* and *KCNJ11* genes on chromosome 11. The focal form is caused by the somatic loss of the maternal chromosome region, which includes the *H19*, *CDKN1C*, *ABCC8* and *KCNJ11* genes, in an individuals carrying the *ABCC8* or *KCNJ11* gene mutation on the paternal chromosome[4,5]. Conversely, the diffuse form is usually caused by homozygous or compound heterozygous mutations, and sometimes by heterozygous mutations.

In the diffuse form, homozygous or compound heterozygous mutations usually cause a severe form of HHI. In contrast, heterozygous mutations usually cause only a mild form of HHI[6], but individuals with HHI due to heterozygous mutations occasionally reportedly progress to diabetes later in life[7–9]. The present study explored the causal gene of siblings with young-onset diabetes using exome sequencing, and identified a compound heterozygous inactivating *ABCC8* gene mutation that was responsible for their diabetes.

METHODS

The proband's birthweight was 3,750 g (+1.9 standard deviation; Figure 1). Glycosuria was detected in a regular health checkup at 15 years-of-age. He was asymptomatic, however, so blood glucose levels were unchecked. At the age of 28 years, hyperglycemia (glycated hemoglobin [HbA1c] 11.6%) and low fasting serum C-peptide levels (0.8 ng/mL [0.27 pmol/L]) were

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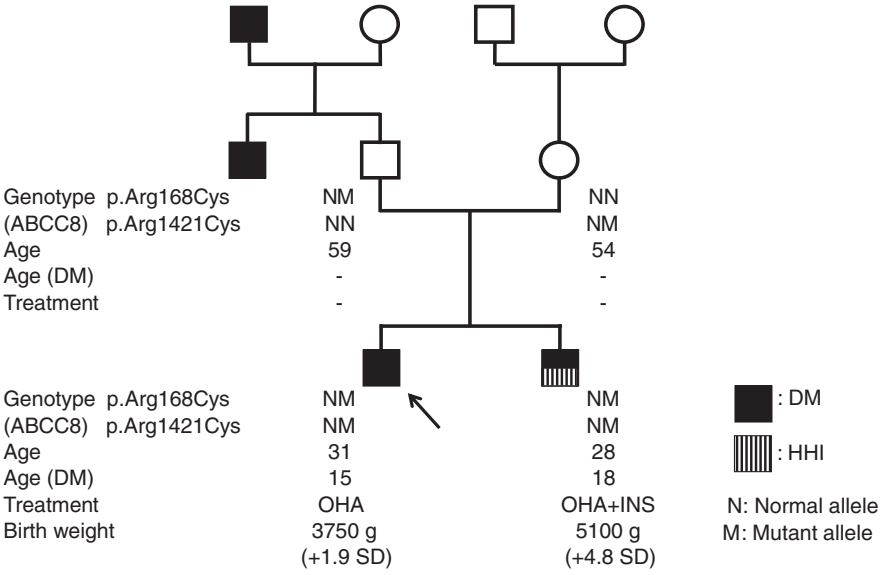

Figure 1 | Pedigree of a family with young-onset diabetes due to a compound heterozygous inactivating *ABCC8* gene mutation. Circles represent females and squares indicate males. An arrow indicates the proband. Vertical hatching denotes hyperinsulinemic hypoglycemia in infancy (HHI). Black filling denotes diabetes mellitus (DM). Individuals who progressed from hypoglycemia to diabetes are shown by half-filled and half-hatched symbols. The genotype is given below each symbol. Age (DM), age at diagnosis of diabetes mellitus; INS, insulin; OHA,oral hypoglycemic agent; M, mutant allele; N, normal allele

detected and insulin therapy was started. Glycemic control and serum C-peptide levels have improved since beginning therapy. He is currently aged 31 years (body mass index [BMI] 25.7 kg/m^2), and is treated only with dipeptidyl peptidase-4 inhibitor (HbA1c 6.7%, fasting serum C-peptide levels 2.3 ng/mL [0.76 pmol/L]).

The proband's younger brother was born with macrosomia (birthweight 5,100 g, +4.8 standard deviation). He was treated for mild hypoglycemia until 2 months after birth, but special drug treatment, such as by diazoxide, was unnecessary. Elevated blood glucose levels were detected in regular health checkups at the age of 18 years, but he did not undergo any treatment for diabetes. At the age of 27 years, hyperglycemia (HbA1c 9.7%) and low fasting serum C-peptide levels (1.0 ng/mL [0.33 pmol/L]) were detected, and insulin therapy was started. He is currently aged 28 years (BMI 22.1 kg/m^2), and is treated with insulin (0.31 IU/kg/day) and sodium–glucose cotransporter 2 inhibitor (HbA1c 7.8%, fasting serum C-peptide levels 1.0 ng/mL [0.33 pmol/L]). The autoantibody to glutamic acid decarboxylase was negative in both individuals. The brothers' father is currently aged 59 years (BMI 23.0 kg/m^2), and their mother is aged 54 years (BMI 27.1 kg/m^2). Their HbA1c levels were within the normal range (5.2% and 5.4%, respectively).

Whole exome sequencing was used to explore the proband's causal gene, as described in detail in our previous report[10]. This research was approved by the Wakayama Medical University Ethics Committee (approval number: 83), and written informed consent was obtained from all participants.

RESULTS

We checked the whole exome sequencing result for 26 genes, which have been reported as the causal genes of maturity-onset diabetes of the young and/or neonatal diabetes: *HNF4A*, *GCK*, *HNF1A*, *PDX1*, *HNF1B*, *NEUROD1*, *KLF11*, *CEL*, *PAX4*, *INS*, *BLK*, *ABCC8*, *KCNJ11*, *EIF2AK3*, *PTF1A*, *ZFP57*, *SLC19A2*, *GATA6*, *GATA4*, *SLC2A2*, *MNX1*, *IER3IP1*, *GLIS3*, *RFX6*, *NKX2-2* and *NEUROG3*. We found a compound heterozygous mutation, the p.Arg168Cys (p.R168C, c.502C>T, rs756823374) and p.Arg1421Cys (p.R1421C, c.4261C>T, rs28938469) mutations, in the *ABCC8* gene (accession number NM_001287174, which incorporates the alternatively spliced amino acid in exon 17) and in the heterozygous p.Leu27Phe (p.L27F, c.79C>T, rs766645933) mutation in the *NEUROD1* gene. We validated these mutations with Sanger DNA sequencing (Figure S1) and found the same mutations in his brother. Their father was a heterozygous carrier for the p.R168C mutation and their mother was a heterozygous carrier for the p.R1421C mutation. The p.L27F mutation in the *NEUROD1* gene was also found in a heterozygous state in their father.

The p.R168C and p.R1421C mutations in the *ABCC8* gene were predicted to be deleterious by multiple prediction tools (SIFT score = 0.0, PolyPhen2 HumDiv score = 1.000, PolyPhen2 HumVar score = 0.990 for the p.R168C mutation; SIFT score = 0.0, PolyPhen2 HumDiv score = 1.000, PolyPhen2 HumVar score = 0.990 for the p.R1421C mutation). Although activating mutations of the *ABCC8* genes usually causes diabetes, the p.R168C and p.R1421C mutations have been found in HHI patients[6,11–13], and have been reported to function as

an inactivating mutation[13,14]. In contrast, the p.L27F mutation in the *NEUROD1* gene was observed as a very rare variation in the database (rs766645933, MAF = 0.0001, GnomAD East Asian; MAF = 0.0008, Japanese Multi Omics Reference Panel), but the amino acid change was predicted to have no impact on the function according to multiple prediction tools (SIFT score = 0.059, PolyPhen2 HumDiv score = 0.438, PolyPhen2 HumVar score = 0.035). Based on these findings, we concluded that the compound heterozygous inactivating *ABCC8* gene mutation was mainly responsible for young-onset diabetes in the siblings.

DISCUSSION

The p.R1421C mutation was originally reported in an HHI patient with focal adenomatous hyperplasia of pancreatic β-cells[11]. Furthermore, the homozygous mutation was reported in a Japanese sibling pair with HHI[13]. A functional study showed that the Rb^+ efflux from COS-7 cells expressing the mutant K_{ATP} channels with SUR1-1421C was reduced by 50% compared with that of the wild-type channels[13]. The p.R168C mutation was also originally reported in a patient with HHI[12]. The mutation is located in an N-terminal transmembrane domain (TMD0) of SUR1 (Figure S1), which is a region known to mediate interactions between SUR1 and Kir6.2[15]. Furthermore, the region is important for surface expression of K_{ATP} channels, and the p.R168C mutation reportedly causes a K_{ATP} channel trafficking defect to the plasma membrane[14].

Several reports suggest the mechanism for the development of diabetes in people with inactivating mutations of the K_{ATP} channel genes. In human islets with inactivating mutation, basal insulin release at low glucose concentration has been shown to be elevated, but glucose-stimulated insulin secretion was impaired[16]. Furthermore, transgenic mice with inactivating K_{ATP} channels due to express dominant-negative Kir6.2 developed hypoglycemia with hyperinsulinemia in neonates and hyperglycemia with hypoinsulinemia in adults, and a decreased number of pancreatic β-cells and increased β-cell apoptosis were observed in adult mice[17]. Furthermore, insulin content and gene expression were decreased in the islets from a mouse model with an inactivating mutation of the *ABCC8* gene[18]. Unresponsiveness to glucose, increased apoptosis and/or decreased insulin gene expression in the pancreatic β-cells might be associated with the development of diabetes caused by inactivating mutations.

In the family in the present study, the phenotype was not the same between the two affected siblings. The proband's younger brother was born with macrosomia and had HHI in the neonatal period in addition to diabetes later in life. The intrauterine environment, such as the mother's blood glucose levels, might have contributed to the difference. His mother had not been aware of hyperglycemia during the period of pregnancy by her recollection, but she might have had mild hyperglycemia, which was difficult to detect by the usual screening test.

The response to a glucagon-like peptide-1 was reportedly conserved in people with HHI caused by the *ABCC8* gene mutation[19]. Furthermore, blood glucose levels were well controlled with treatment by dipeptidyl peptidase-4 inhibitor in the present proband. Treatment with incretin-related drugs might be a useful therapeutic approach for people with diabetes caused by the inactivating mutation.

Finally, it has been reported that individuals with HHI due to dominantly-inherited inactivating mutations of the K_{ATP} channel genes occasionally progress to diabetes later in life[7–9]. In the present study, we newly identified a compound heterozygous inactivating *ABCC8* gene mutation in two Japanese siblings who had been treated as young-onset type 2 diabetes.

The present results expand knowledge about the clinical characteristics of people with diabetes caused by inactivating mutations of the *ABCC8* gene and suggest that in addition to the activating mutation, the inactivating mutation is more important than previously thought in the etiology of diabetes.

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DISCLOSURE

The authors declare no conflict of interest.

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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 | The structure of *ABCC8* (sulfonylurea receptor 1) and *KCNJ11* (Kir 6.2), and the Sanger sequence images around the mutations detected in the proband.