Clinical and functional characterization of the Pro1198Leu ABCC8 gene mutation associated with permanent neonatal diabetes mellitus

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ABSTRACT

Aims/Introduction: The adenosine triphosphate (ATP)-sensitive potassium (K\text{ATP}) channel is a key component of insulin secretion in pancreatic \(\beta\)-cells. Activating mutations in ABCC8 encoding for the sulfonylurea receptor subunit of the K\text{ATP} channel have been associated with the development of neonatal diabetes mellitus (NDM). The aim was to investigate clinical and functional characterization of the Pro1198Leu ABCC8 gene mutation associated with permanent NDM (PNDM).

Materials and Methods: The coding regions and conserved splice sites of KCNJ11, ABCC8 and INS were screened for mutations in a 12-year-old girl diagnosed with PNDM. The functional property of the mutant channel identified was examined with patch-clamp experiments in COS-1 cells. We also investigated the difference of effectiveness between two groups of oral sulfonylureas in vitro and in the patient.

Results: We identified a heterozygous missense mutation (c.3593 C>T, Pro1198Leu) in ABCC8. The mutated residue (P1198) is located within a putative binding site of sulfonylureas, such as tolbutamide or gliclazide. In patch-clamp experiments, the mutant channel was less ATP sensitive than the wild type. Furthermore, the sensitivity to tolbutamide was also reduced in the mutant channel. In addition to the tolbutamide/gliclazide binding site, glibenclamide is thought to also bind to another site. Glibenclamide was more effective than other sulfonylureas in vitro and in the patient. The treatment of the patient was finally able to be switched from insulin injection to oral glibenclamide.

Conclusions: We identified the Pro1198Leu ABCC8 mutation in a PNDM patient, and clarified the functional and clinical characterization. The present findings provide new information for understanding PNDM. (J Diabetes Invest, doi: 10.1111/jdi.12049, 2013)

KEY WORDS: ABCC8, Neonatal diabetes, Sulfonylurea receptor

INTRODUCTION

Neonatal diabetes mellitus (NDM) is a specific form of diabetes\(^1\). It has been defined as diabetes with onset before 6 months-of-age and autoantibody negative for type 1 diabetes in general. NDM is classified into two categories clinically. One is transient NDM (TNDM), in which diabetes develops within the first few weeks of life and resolves by a few months of age, although it might frequently relapse in adolescence or young adulthood. The other is permanent NDM (PNDM), in which diabetes does not remit and the patients usually require insulin treatment for life. Approximately 50% of NDM is transient and 50% is permanent\(^2\). The majority (~70%) of cases of TNDM have abnormalities in the imprinted region of chromosome 6q24, such as paternal uniparental isodisomy, paternally inherited duplication and maternal methylation defects, leading to overexpression of paternally expressed genes\(^3\). The adenosine triphosphate (ATP)-sensitive potassium (K\text{ATP}) channel is a key component of insulin secretion in pancreatic \(\beta\)-cells. The channel is comprised of two proteins, an inwardly rectifying potassium ion pore-forming subunit (Kir6.2; encoded by KCNJ11) and a high-affinity \(\beta\)-cell sulfonylurea receptor (SUR1; encoded by ABCC8)\(^4\). Activating mutations in KCNJ11 and ABCC8 account for 12% and 13% of cases of TNDM, respectively\(^5\). They also account for 31% and 10% of cases of PNDM, respectively\(^6\). Furthermore, 12% of PNDM is caused by mutations in the insulin (INS) gene itself\(^6\). It has also been reported that a few cases of PNDM are attributed to genetic abnormalities in other genes, such as GCK, FOXP3, EIF2AK3, PDX1, PTF1A, GLIS3, NEUROD1 and HNF1B, which are important to pancreatic \(\beta\)-cell function and development\(^7\).

The K\text{ATP} channel plays a key role in glucose-dependent insulin secretion from pancreatic \(\beta\)-cells. After entry of glucose into
the β-cells, glucose is metabolized and ATP is produced. The elevated intracellular ATP levels cause closure of K\textsubscript{ATP} channels on the cell surface, which then depolarize the cell membrane leading to opening of the voltage-dependent calcium channels. The resultant influx of calcium triggers a cascade of events that result in the secretion of insulin. Overactive mutant channels as a result of activating mutations in \textit{KCNJ11} or \textit{ABCC8}, therefore, hyperpolarize the cell membrane, reduce calcium influx and decrease insulin secretion. Sulfonylureas bind to the SUR1 subunit of the K\textsubscript{ATP} channel, and close the channel in an ATP-independent manner. It has been reported that oral sulfonylurea administration increases insulin secretion and improves metabolic control in patients with PNDM as a result of activating mutations in \textit{KCNJ11} and \textit{ABCC8}\textsuperscript{7,8}. In the present study, we investigated genetic abnormalities in a patient with PNDM, and found the Pro1198Leu mutation in \textit{ABCC8}. We examined the functional property of a mutant channel with the patch-clamp experiments, and the clinical response to oral sulfonylurea administration.

**MATERIALS AND METHODS**

**Mutation Screening**

The proband is a 12-year-old girl. She was born after 38 weeks of an uneventful pregnancy with a birthweight of 2778 g (just under the 50th percentile). At 28 weeks-of-age, she presented with severe hyperglycemia (blood glucose 915 mg/dL, glycated hemoglobin [HbA\textsubscript{1c}] 12.8%) with ketoacidosis. The HbA\textsubscript{1c} value was estimated as a National Glycohemoglobin Standardization Program (NGSP) equivalent value calculated by the formula $HbA_{1c} = HbA_{1c} (JDS) + 0.5\%$, considering the relational expression of HbA\textsubscript{1c} (JDS) measured by the previous Japanese standard substance and measurement methods, and HbA\textsubscript{1c} (NGSP)\textsuperscript{9}. Fasting serum C-peptide level was undetectable (<0.5 ng/mL) at first, but it was recovered to 0.9 ng/mL after 2 months of treatment with insulin. NDM is defined as diabetes with onset before 6 months-of-age in general. As her HbA\textsubscript{1c} at the time of diagnosis was notably high, it could be speculated that her plasma glucose had been elevated before 6 months-of-age. Her medical record of bodyweight also suggested that failure to thrive had started from 5 months-of-age. No neurological abnormality was observed, and anti-glutamic acid decarboxylase (GAD) antibody was undetectable. Abdominal ultrasound examination at the age of 12 years showed a normally-developed pancreas. She had been treated with insulin from the onset of diabetes. The pedigree of the family is shown in Figure 1.

After obtaining written informed consent, genomic DNA was isolated from peripheral blood leukocytes. The coding regions and conserved splice sites of \textit{KCNJ11}, \textit{ABCC8} and \textit{INS} were amplified from genomic DNA by polymerase chain reaction using specific primers (Supporting Information Table S1). The products were sequenced using Dye Terminator chemistry on an ABI 3100 (Applied Biosystems, Warrington, UK). The study protocol was approved by the institutional review board.

**Functional Analysis of Mutant K\textsubscript{ATP} Channel**

The mammalian expression plasmids containing the whole coding region of the human Kir6.2 and SUR1 have been described previously\textsuperscript{4,10}. We generated the expression plasmid of SUR1

![Figure 1](image-url) | Pedigree of the family. The allele status is indicated under the symbols. Directly below the genotype is the age of diagnosis of diabetes and treatment. INS, insulin; NA, not available for testing; NM, one normal and one mutated allele; NN, two normal alleles; OHA, oral hypoglycemic agent.
containing P1198L with QuickChange™ site-directed mutagenesis system (Stratagene, La Jolla, CA, USA). The presence of P1198L and the absence of other mutations were confirmed by sequencing the whole insert.

COS-1 cells were plated on 35-mm dishes containing cover slips. The cells were then transiently transfected with wild-type or mutated human SUR1 cDNA (1.5 µg per dish) plus human Kir6.2 (1.5 µg per dish) using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). Recordings were made 24–72 h after transfection. The ATP sensitivity of the wild-type and mutant channels were determined basically as described previously4,10 with a patch-clamp amplifier, Axopatch 200B (Axon Instruments, Foster City, CA, USA). Sulfonylurea sensitivity was assessed as the ratio between the amplitudes of the KATP channel currents before and after tolbutamide or glibenclamide application. Data were analyzed with pCLAMP (version 9.0; Axon Instruments), XLfit (CTC Laboratory System Corporation, Tokyo, Japan) and in-house software. Unpaired Student’s t-test was used to test for statistical significances, and the results were expressed as mean ± SE.

RESULTS

Mutation Screening

We identified a heterozygous missense mutation in ABCC8. The mutation results in the substitution of leucine for proline at residue 1,198 in exon 29 (c.3593 C>T, p.P1198L, GenBank NM_000352). It was not found in the databases, such as dbSNP (http://www.ncbi.nlm.nih.gov/snp/) or 1,000 genome project (http://browser.1000genomes.org/index.html), and also in 150 unrelated Japanese subjects. The mutated residue is located within the short cytosolic loop that links transmembrane domains 15 and 16. The proline (P1198) is conserved across a range of species, from mammals (human and rat) to zebrafish, and is also found at a similar position in human SUR2. The in silico prediction programs, SIFT (http://sift.jcvi.org/) and PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/index.shtml), both predicted that the substitution would affect protein function. No mutations associated with diabetes were found in KCNJ11 and INS.

Functional Analysis of Mutant KATP Channel

We tested the sensitivity of ATP to block the wild-type and the mutant KATP channels in inside-out membrane patches (Figure 2a). The concentration of ATP required to half-maximally inhibit the channel (IC50) increased ≈sevenfold from 23.4 ± 2.5 µmol/L for wild-type channels to 164.7 ± 19.3 µmol/L for mutant channels. This result shows that the mutant channel is less ATP sensitive than the wild type.

We next tested the response to sulfonylureas for the wild-type and the mutant KATP channels in inside-out membrane patches in nucleotide-free condition (Figure 2b). A total of 100 µmol/L tolbutamide inhibited the current by 65.6 ± 4.8% for the mutant channel, whereas 19.3 ± 5.2% for the wild type. In contrast, 30 nmol/L glibenclamide inhibited the current by 44.5 ± 6.5% for the mutant channel, whereas 10.8 ± 2.7% for the wild type. The residual current in the presence of glibenclamide for the mutant channel was significantly smaller than that of tolbutamide (P = 0.02). These results show that the sensitivity to sulfonylureas is reduced in the mutant channel and glibenclamide is more effective than tolbutamide.

Clinical Effectiveness of Oral Sulfonylurea Therapy

After identification of an ABCC8 gene mutation, the patient’s treatment was switched from insulin injection (40 units a day)
to oral sulfonylureas under intensive monitoring in the hospital. Daily urinary C-peptide excretion, which was 27.8 µg/day before initiating sulfonylureas, increased to 58.1 µg/day at high-dose gliclazide (a daily dose of 160 mg) alone. Furthermore, daily urinary C-peptide excretion was further increased to 83 µg/day at low-dose glibenclamide (a daily dose of 0.625 mg, which is 0.017 mg/kg/day; Figure 3a). Simultaneously, daily urinary sugar excretion was decreased in inverse proportion to urinary C-peptide excretion (20.0, 8.0 and 4.0 g/day, respectively). Thus, oral glibenclamide treatment was selected for the patient’s therapy and insulin injection was completely discontinued. Her blood glucose level was finally controlled with 0.17 mg/kg/day of glibenclamide.

**DISCUSSION**

In the present study, we identified the Pro1198Leu mutation in a patient with PNDM. The mutation was not found in the databases, such as dbSNP or 1,000 genome project, and also in 150 unrelated Japanese subjects. Furthermore, two in silico prediction programs, SIFT and PolyPhen-2, predicted that the mutation would affect protein function. We further confirmed that the mutant channel was less ATP sensitive than the wild type with a patch-clamp experiment. The degree of ATP insensitivity was comparable with that in a previous report for PNDM as a result of activating mutations in ABCC8. These findings suggest that the P1198L mutation is strongly associated with the development of PNDM in the patient. Furthermore, it has only recently been reported from Turkey that the same mutation was identified in a girl with NDM. In that report, the patient presented with severe hyperglycemia with ketoacidosis at 1 month-of-age and was initially treated with insulin. After genetic diagnosis, her treatment was successfully converted from insulin to glibenclamide (0.2 mg/kg/day). In addition, no neurological abnormality and no family history of diabetes were observed in the patient. These clinical characteristics were similar to the present case, except there was no family history of diabetes. In contrast, in vitro functional analysis of the mutation was not carried out in the report.

The mutated residue P1198 is located within the eighth cytosolic loop (CL8) that links transmembrane domains 15 and 16 in the SUR1 (Figure 3b). This loop has been reported to be a binding site of tolbutamide, and our functional analysis also showed that the sensitivity to tolbutamide was reduced in the mutant channel. Based on the drug structure, gliclazide is also thought to bind to the CL8. In contrast, in addition to the CL8, glibenclamide is able to bind to another site in the SUR1, which is located within the third cytosolic loop (CL3). This could explain the reason why glibenclamide was more effective both in patch-clamp experiments and in the patient.
In the family of the patient, her elder brother carried the P1198L mutation in the heterozygous state, whereas her mother did not (Figure 1). This suggests a possibility that her father might have the same mutation. The elder brother was diagnosed with diabetes at 11 years-of-age on medical examination at his school, but his diabetes was very mild and has been treated with diet alone. Furthermore, the proband’s father, paternal aunt and paternal grandfather have diabetes. However, there is little information on these members, because her parents have divorced and members of her father’s family did not agree to cooperate in the present study. The phenotypic variability of diabetes within families has been reported in some families with ABCC8 gene mutations. The precise reason for the variability is currently unknown. It might be explained by the influence of unknown other genetic and/or epigenetic factors.

Many patients with PNDM caused by ABCC8 or KCNJ11 gene mutations have been successfully treated with sulfonylureas. In the present study, the treatment of our patient was also able to be switched from insulin injection to oral sulfonylurea therapy. In contrast, response to sulfonylureas has not been seen in PNDM caused by other gene mutations, such as INS. This suggests that genetic diagnosis can provide clinical benefits to the patients with PNDM. It has been reported that genetic testing for KCNJ11 and ABCC8 in all children diagnosed before 6 months-of-age results not only in improved quality of life, but also in cost savings. However, a large number of patients with PNDM have still been misdiagnosed as a very early onset form of type 1 diabetes and treated with insulin. The genetic diagnosis of PNDM will take on a growing importance in clinical practice.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1 | Primer sequences for amplification of ABCC8 gene