
**Alternating hypoglycemia and hyperglycemia in a toddler with a homozygous p.R1419H **ABCC8** mutation: an unusual clinical picture**

**Abstract**

**Background:** Inheritance of two pathogenic **ABCC8** alleles typically causes severe congenital hyperinsulinism. We describe a girl and her father, both homozygous for the same **ABCC8** mutation, who presented with unusual phenotypes.

**Methods:** Single nucleotide polymorphism microarray and Sanger sequencing were performed. Western blot, rubidium efflux, and patch clamp recordings interrogated the expression and activity of the mutant protein.

**Results:** A 16-month-old girl of consanguineous descent manifested hypoglycemia. She had dysregulation of insulin secretion, with postprandial hyperglycemia followed by hypoglycemia. Microarray revealed homozygosity for the regions encompassing **KCNJ11** and **ABCC8**. Her father had developed diabetes at 28 years of age. Sequencing of **ABCC8** identified a homozygous missense mutation, p.R1419H, in both individuals. Functional studies showed absence of working KATP channels.

**Conclusion:** This is the first description of a homozygous p.R1419H mutation. Our findings highlight that homozygous loss-of-function mutations of **ABCC8** do not necessarily translate into early-onset severe hyperinsulinemia.

**Keywords:** **ABCC8**; diabetes; hyperglycemia; hyperinsulinism; hypoglycemia.

**Introduction**

Autosomal recessive inheritance of two abnormal **ABCC8** or **KCNJ11** alleles causes severe forms of hyperinsulinism (HI) (1–4). Encoded by two adjacent genes on chromosome 11p, the sulfonylurea receptor (SUR1) modulates channel activity, whereas the inwardly rectifying potassium channel (Kir6.2) makes up the ion-conducting pore of the hetero-octameric ATP-sensitive potassium channel (KATP) (5, 6). The most common outcome of recessively inherited **ABCC8** gene mutations is to prevent expression of functional channels, leading to severe HI that presents early in life and is unresponsive to diazoxide. Most patients require subtotal pancreatectomy (7, 8).
cases, as reported for a splice site mutation of ABCC8, there can be variability in the age and severity of presentation in homozygous individuals (9, 10).

The natural history of patients who do not require pancreatectomy can include remission of the hypoglycemia, with progression to diabetes in early adulthood (11). However, long-term data is limited. These patients have been observed to have both hypoglycemic and hyperglycemic episodes a few years after presentation and pathologic responses to the oral glucose tolerance test have been described after 10 years of age (11). A blunted insulin response to intravenous dextrose was reported, suggesting reduced glucose sensitivity of the SUR-deficient β-cell (12), a condition referred to colloquially as "glucose-blindness".

In this report, we describe the unusual clinical course and workup of a patient who is homozygous for a p.R1419H mutation in ABCC8, a seemingly severe coding mutation that prevents maturation of K$_{ATP}$ channels to the cell surface. The patient’s presentation is a rare example of a weak phenotype with relatively late onset, inconsistent with the expected severity of the mutation at the cellular level.

**Materials and methods**

**Clinical data**

Clinical information was obtained by chart review, with approval from the Clinical Research Ethics Board that covers British Columbia Children’s Hospital.

**DNA mutation analysis**

Affymetrix CytoScanHD (Affymetrix, Inc., Santa Clara, CA, USA) single nucleotide polymorphism (SNP) array analysis was done according to the manufacturer’s protocol at the Cytogenetics Laboratory at British Columbia Children’s Hospital. SNP marker results were mapped to the February 2009 build of the human genome (GRCh37/hg19). Sanger sequencing of the KCNJ11 and ABCC8 genes was performed as previously described (13).

**Non-radioactive rubidium efflux assay**

Rb$^+$ efflux assays were carried out on CosM6 cells transfected with Kir6.2 and SUR1 (WT or SUR1[R1419H] mutant) as previously described (14). Rb$^+$ concentrations were determined by flame atomic absorption spectroscopy using an Aurora ICR8000 instrument (Aurora, Vancouver, Canada).

**Patch clamp electrophysiology**

Patch-clamp experiments of CosM6 cells transfected with Kir6.2 and SUR1 were carried out at room temperature, using a perfusion chamber equipped for rapid solution exchange apparatus, as previously described (15).

**Western blots**

CosM6 cells transfected with combinations of Kir6.2, SUR1, and SUR1[R1419H] were lysed with RIPA buffer at 4°C, and lysates were clarified by centrifugation. Protein concentration of lysates was determined with a BCA assay kit (Pierce, Rockford, IL, USA) and equal amounts of lysate were separated by SDS-PAGE (75% gel). Blots were probed with a monoclonal anti-SUR1 antibody (UC Davis/NIH NeuroMab Facility, Davis, CA, USA) and visualized with a Femto ECL reagent (Pierce) using a FluorChemSP (Alpha Innotech, San Leandro, CA, USA). Spot densitometry was carried out using AlphaEase software (Alpha Innotech).

**Results**

A previously healthy 16-month-old girl presented with hypoglycemia of 1.2 mmol/L (22 mg/dL) in the context of lethargy during a gastrointestinal infection. Retrospectively, the parents recalled episodes of irritability over the previous 4 months, including episodes of medial eye deviation that could represent hypoglycemic seizures. She had been born at term, weighing 3490 g, after a pregnancy complicated by gestational diabetes, with no documented history of neonatal hypoglycemia. She grew along the 50th percentiles for length and weight. She was still breastfeed-

The patient’s father reported having seizures as an infant in Pakistan. He could not recall whether they were febrile seizures or otherwise associated with intercurrent illness. He had normal intellect and he was diagnosed with diabetes at 28 years of age. His body mass index (BMI) was 22.4 kg/m$^2$ (–0.21 standard deviation) at that time. Medical records from the time of diagnosis were unavailable. He was managed by his family physician according to common guidelines with glyburide and metformin, but had not previously been tested genetically. HbA1c at 45 years of age was 8.1% (normal 4.5–6). The patient’s mother had diet-controlled gestational diabetes. Her BMI was 21.6 kg/m$^2$ (–0.04 standard deviation). The parents were first cousins through their mothers and second cousins through their fathers, and each of their pairs of parents were also related to each other (Figure 1).
The patient had recurrent hypoglycemia in hospital and critical samples demonstrated alternating ketotic and non-ketotic hypoglycemia, with detectable insulin levels. An extensive workup was otherwise normal (Tables 1 and 2). Aberrant insulin processing was ruled out as a cause (Supplementary Table 1). Finally, a diagnosis of HI was entertained based on the insulin levels, associated with relatively low β-hydroxybutyrate, as well as a glycemic response to glucagon [increase of 3.4 mmol/L (61 mg/dL)]. Monitoring after a 2 g/kg carbohydrate meal showed postprandial hyperglycemia, 10.8 mmol/L (193 mg/dL) with relative hypoinsulinemia, 98 pmol/L (16.3 μIU/mL), followed by hypoglycemia, 2.1 mmol/L (38 mg/dL) with relative hyperinsulinemia, 34 pmol/L (5.6 μIU/mL) after 4.5 h (Figure 2), confirming inappropriate regulation of insulin secretion.

The patient did not respond to diazoxide and it was stopped at a dose of 12 mg/kg/day due to severe side effects. She also did not respond to boluses of up to 5 μg/kg of octreotide. She was eventually placed on a continuous subcutaneous glucagon infusion (0.7 mg/day) with a significant improvement in blood glucose readings. She had a gastrostomy tube placed with daytime bolus feeds and continuous night-time feeding of Pediasure (Abbott Laboratories, Abbott Park, IL, USA). The glucagon infusion was decreased gradually and stopped altogether after 5 months. She remains on continuous night-time feeding and eats orally frequently during the day. With this regimen, she experiences mild hypoglycemias, as well as postprandial hyperglycemias up to 12.5 mmol/L (225 mg/dL) every 2–3 days.

Microarray studies (Supplementary Table 2) revealed homozygosity for multiple genomic regions, consistent with the family history of consanguinity. The region surrounding KCNJ11 and ABCC8 was identified as the best candidate region for harboring rare variants with major effects on the phenotype. KCNJ11 and ABCC8 mutation analysis was performed at the University of Exeter Medical School, Exeter, UK, and identified a homozygous missense coding mutation, p.R1419H (c.A256G>A) in the ABCC8 gene. Sanger sequencing of the patient and her parents (Supplementary Figure 1A–C) found that the mother was heterozygous and the father homozygous for the same mutation.

### Table 1 Critical samples during hypoglycemia.

<table>
<thead>
<tr>
<th>Test</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mmol/L</td>
<td>2.0</td>
<td>2.3</td>
<td>1.7</td>
<td>2.0</td>
</tr>
<tr>
<td>β-Hydroxybutyrate, mmol/L</td>
<td>0.93</td>
<td>0.81</td>
<td>&lt;0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Insulin, pmol/L</td>
<td>20</td>
<td>10</td>
<td>30</td>
<td>24</td>
</tr>
<tr>
<td>Cortisol, nmol/L</td>
<td>216*</td>
<td>10</td>
<td>30</td>
<td>24</td>
</tr>
<tr>
<td>Growth hormone, μg/L</td>
<td>0.42*</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In the context of hypoglycemia, normal β-hydroxybutyrate >0.3 mmol/L, insulin <10 pmol/L, cortisol >500 nmol/L, and growth hormone >5.6 μg/L. *Following these initial results, stimulation tests for cortisol and growth hormone were performed with normal results, as per Table 2.
Previous functional data in the literature has suggested that the SUR1[R1419H] mutation prevents channels from reaching the cell surface (16). However, the relatively mild phenotypes of the patient and her father contrasted with this “classical” clinical picture of homozygous recessive \( ABCC8 \) mutations, which are typically very severe and present early in life (7, 17, 18). Therefore, we tested the effects of this mutation in a reconstituted system, using \( Rb^+ \) efflux assays of CosM6 cells transfected with Kir6.2/KCNJ11 and WT or SUR1[R1419H] to measure functional \( K_{\text{ATP}} \) channel expression (Figure 3). Under conditions of metabolic inhibition (1 mM deoxyglucose+100 \( \mu \)M oligomycin), to maximally activate \( K_{\text{ATP}} \) channels, cells transfected with the SUR1[R1419H] variant (+Kir6.2) exhibited no \( Rb^+ \) efflux above untransfected cells, consistent with an absence of functional \( K_{\text{ATP}} \) channels at the cell surface (Figure 3A). Cells transfected with a 1:1 mixture of WT: SUR1[R1419H] to simulate a heterozygous state exhibited \( Rb^+ \) efflux activity intermediate between the WT-only and R1419H-only SUR1 cells. We examined the effects of SUR1[R1419H] subunits on cell surface maturation of \( K_{\text{ATP}} \) using the differential migration of mature and immature glycosylated forms of SUR1 on SDS-PAGE gels (19–21) (Figure 3B and C). Cells transfected with SUR1 alone exhibit a single molecular

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**Table 2** Metabolic workup of the proband.

<table>
<thead>
<tr>
<th>Test</th>
<th>Value</th>
<th>Normal range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate, mmol/L</td>
<td>1.2</td>
<td>0.5–2.2</td>
</tr>
<tr>
<td>Ammonia, ( \mu )mol/L</td>
<td>&lt;9</td>
<td>9–33</td>
</tr>
<tr>
<td>Cortisol secretion in response to ACTH test*, ( \mu )mol/L</td>
<td>891</td>
<td>&gt;500</td>
</tr>
<tr>
<td>Growth hormone secretion in response to glucagon test*, ( \mu )g/L</td>
<td>5.95</td>
<td>&gt;5.6</td>
</tr>
<tr>
<td>ALT, U/L</td>
<td>27</td>
<td>5–45</td>
</tr>
<tr>
<td>Uric acid, ( \mu )mol/L</td>
<td>175</td>
<td>105–300</td>
</tr>
<tr>
<td>CPK, U/L</td>
<td>71</td>
<td>60–305</td>
</tr>
<tr>
<td>Serum free fatty acids (glucose: 2.3 mmol/L), ( \mu )mol/L</td>
<td>709</td>
<td>100–900</td>
</tr>
<tr>
<td>Lipid profile</td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>Plasma amino acid profile</td>
<td>Unremarkable</td>
<td></td>
</tr>
<tr>
<td>Bloodspot and serum acylcarnitine profile</td>
<td>Unremarkable</td>
<td></td>
</tr>
<tr>
<td>Urine organic acid profile</td>
<td>Unremarkable</td>
<td></td>
</tr>
<tr>
<td>Iso-electric focusing transferrins</td>
<td>Unremarkable</td>
<td></td>
</tr>
<tr>
<td>Newborn metabolic screening†</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Urine sulfonylurea level</td>
<td>Undetectable</td>
<td></td>
</tr>
<tr>
<td>Glycogen storage disorders panel† (GSD0, 1, 2, 3, 4, 6, 9)</td>
<td>Normal</td>
<td></td>
</tr>
</tbody>
</table>

*Standard protocol low dose adrenocorticotropin hormone (ACTH) test 1 \( \mu \)g. †Standard protocol growth hormone stimulation test with glucagon, 0.03 mg/kg. ‡Newborn metabolic screening in British Columbia targets amino acid disorders, fatty acid oxidation disorders, organic acid disorders, galactosemia, congenital hypothyroidism, hemoglobinopathies, and cystic fibrosis. §Performed by massively parallel sequencing (BCM-MitomeNGS™).
weight band attributed to an immature form of the protein that fails to reach the plasma membrane. When co-transfected with Kir6.2 channels, a mature glycosylated band is apparent. However, co-transfection of SUR1[R1419H] with WT Kir6.2 channel subunits yields little or none of the mature glycosylated form (not statistically different from SUR1 alone; Figure 3C), consistent with a severe trafficking defect of this channel mutant. We also considered the possibility that the SUR1[R1419H] mutation could increase channel activity and partly counteract the trafficking defects, this might account for the relatively mild phenotype of the patient and her father if small numbers of channels containing the SUR1[R1419H] subunit could reach the cell surface. However, patch clamp recordings showed no significant change in nucleotide sensitivity of K_{ATP} channels containing the SUR1[R1419H] mutant (Supplementary Figure 2). Overall, the weak Rb⁺ efflux and lack of protein maturation are consistent with trafficking defects that are apparent in many recessively inherited SUR1-mediated hyperinsulinemias (22–24). These data indicate that virtually no functional K_{ATP} channels are present in the plasma membrane of β-cells of the patient or her father.

**Discussion**

Compared to most reports of homozygous SUR1 mutations, our patient had late onset of symptoms and she exhibited post-prandial hyperglycemia, as well as fasting and rebound hypoglycemia already at presentation. Previous reports have suggested that the R1419H mutation causes a trafficking defect of the K_{ATP} complex (16), although the only affected patient yet reported was a G70E/R1419H compound heterozygote. That patient had a severe presentation at birth, requiring subtotal pancreatectomy. These authors concluded that the R1419H mutation was consistent with a “channel absent” molecular subclass common to recessive forms of HI (16, 23). Our patient and her father are the first humans to be described with this mutation in a homozygous state, and our in vitro studies confirmed the trafficking defect caused by the R1419H mutation in transfected CosM6 cells.

Mutations that prevent cell surface maturation of K_{ATP} channels typically lead to a very severe hyperinsulinemic phenotype in homozygotes. The relatively late and unusual clinical presentations of our patient and her father diverge significantly from this prediction. We uncovered one report of extreme phenotypic variability in a family with a homozygous ABCC8 mutation (10), although it should be noted that this report involves a splicing site with unclear consequences on the generation of functional channels. In contrast, the R1419H is a coding mutation that appears to completely suppress expression of functional channels.

We were led to our top candidate genes by the clinical microarray that identified loss-of-heterozygosity (LOH) in
multiple chromosomal regions, including around \textit{ABCC8} and \textit{KCNJ11}. We began with a hypothesis that this was an autosomal recessive disease, based on parental consanguinity. We then used microarray analysis as an unbiased tool to create a list of candidate genes. We selected \textit{ABCC8} and \textit{KCNJ11} as plausible candidate genes based on the fact that there was LOH in the region around these two genes, and on the fact that we suspected an insulin secretion defect on clinical grounds.

Other glucose transporters and other “diabetes genes” for which the patient had LOH included \textit{KLF11}, \textit{SLC2A1}, \textit{PCSK1}, and \textit{TCF7L2} (Supplementary Table 2). Identification and validation of potential modifier loci was deemed unfeasible at this stage, because of the small number of affected individuals with the same \textit{ABCC8} genotype available for study.

From a phenotypic perspective, the patient exhibited both a poor post-prandial insulin response and fasting hyperinsulinism, illustrating significant dysregulation of insulin secretion. Unfortunately, information on the father’s phenotype over the years was not available, however, the history of unexplained seizures in infancy, and later diabetes in adulthood, can hint to his daughter’s future phenotype. Information regarding insulin dysregulation in patients with recessive forms of SUR1 HI is sparse, mainly because most undergo subtotal pancreatectomy.

Co-existence of hypoglycemias and hyperglycemias has been reported only in long-term follow-up of non-pancreatectomized patients with diffuse SUR1 HI (11). In addition, Grimberg et al. (12) demonstrated the dysregulation of insulin secretion in patients with diffuse SUR1 HI. Lastly, the phenotype of SUR1−/− knockout mice (25) is surprisingly similar to our patient, as the mice do not have severe hypoglycemia and demonstrate a loss of first-phase and attenuated second-phase glucose-stimulated insulin secretion. Overall, the presence of \( K_{\text{ATP}} \) channel-independent pathways (26–28) may enable these patients and animal models to exhibit some sub-optimal glucose tolerance, although it remains unclear what factors underlie the relatively weak phenotype apparent in our patients. Nevertheless, our findings highlight that the inheritance of homozygous loss-of-function mutations of SUR1 does not necessarily translate into an early onset severe hyperinsulinemia. At some level, the fact that this family’s phenotype does not match previously reported phenotypes is surprising, although perhaps it should not be given, the fact that broadening of genotype-phenotype correlations is a frequent outcome for families enrolled in the newer consortia-based genomic sequencing projects (29).

In conclusion, this the first description of a homozygous p.R1419H mutation of the \textit{ABCC8} gene. We were led to our candidate gene by looking at relevant regions of homozygosity on microarray. This case is particularly interesting because it diverges from the predicted severe phenotype that typically results from this “channel absent” subclass.

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**Conflict of interest statement:** The authors have no relevant conflict of interest to disclose.

**References**


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