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SHORT REPORT

Clinical and genetic analysis in a family with familial renal glucosuria: Identification of an N101K mutation in the sodium–glucose cotransporter 2 encoded by a solute carrier family 5 member 2 gene

Kentaro Sada[1], Shuji Hidaka[1]*, Nao Imaishi[1], Kohei Shibata[2], Rumi Katashima[3], Shinsuke Noso[4], Hiroshi Ikegami[4], Tetsuya Kakuma[5], Hirotaka Shibata[6]

Departments of [1]Diabetes and Metabolism, [2]Gastrointestinal Surgery, Koseiren Tsurumi Hospital, Oita, [3]Laboratory for Pediatric Genome Medicine, Department of Clinical Research, National Hospital Organization Shikoku Medical Center for Children and Adults, Kagawa, [4]Department of Endocrinology, Metabolism and Diabetes, Faculty of Medicine, Kindai University, Osaka, [5]Department of Health Support Center, and [6]Department of Endocrinology, Metabolism, Rheumatology and Nephrology, Faculty of Medicine, Oita University, Oita, Japan

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***Correspondence**
Shuji Hidaka
Tel.: +81-977-23-7111
Fax: +81-977-23-7884
E-mail address:
shuji@oita-u.ac.jp

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ABSTRACT

We report the identification of a mutation in the solute carrier family 5 member 2 (*SLC5A2*) gene, which encodes sodium–glucose cotransporter 2, in a family with familial renal glucosuria. The proband was a 26-year-old Japanese man referred to the diabetes division with repeated glucosuria without hyperglycemia. His mother, uncle and grandfather also had a history of glucosuria. A heterozygous missense mutation (c.303T>A: p.N101K) in *SLC5A2* was identified in the patient and his mother, but not in 200 chromosomes from 100 healthy and unrelated individuals, or in 3,408 Japanese individuals in the Tohoku Medical Megabank. Furthermore, bioinformatics software predicted that this lesion would be pathogenic. We infer that the mutation led to clinically relevant sodium–glucose cotransporter 2 dysfunction. The patient showed no symptoms of hypoglycemia, but continuous glucose monitoring confirmed asymptomatic hypoglycemia.

INTRODUCTION

Familial renal glucosuria (FRG) is a rare renal tubular disorder characterized by isolated persistent glucosuria without hyperglycemia[1]. Mutations in the solute carrier family 5 member 2 (*SLC5A2*) gene, which encodes sodium–glucose cotransporter 2 (SGLT2), are responsible for the vast majority of FRG cases[2,3]. Most individuals with heterozygous mutations have mild glucosuria; those with homozygous or compound heterozygous mutations usually have severe glucosuria. In pedigrees with mild glucosuria, FRG is often inherited as an autosomal dominant trait[4]; pedigrees with severe glucosuria suggest autosomal recessive inheritance[5]. However, recent studies have shown that inheritance of FRG might best be described as a codominant trait with variable penetrance[2].

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SGLT2 inhibitors have been proposed as a novel approach for the treatment of diabetes; such inhibitors are a new category of glucose-lowering drugs with insulin-independent action. Thus, FRG patients might provide an ideal model for evaluating the long-term safety of these agents.

FRG has been suggested to have a prevalence of 0.007% in Japanese schoolchildren[6]. However, the genetic basis of FRG in Japan is largely unknown. In the present study, clinical and genetic findings are described in a family with FRG associated with a *SLC5A2* gene mutation.

METHODS

Participants

The pedigree of a family with FRG is shown in Figure 1. The proband was a 26-year-old Japanese man who was referred to the diabetes division because of repeated glucosuria. The patient was first noted to have glucosuria without hyperglycemia and

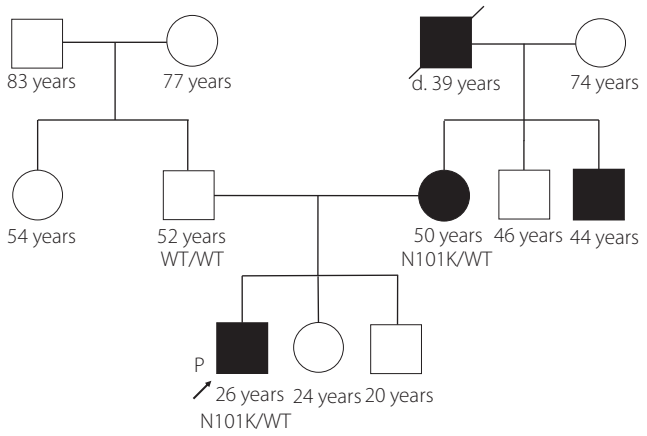

Figure 1 | Pedigree of a family with familial renal glucosuria. The proband (P) is indicated by the arrow. His mother, uncle and grandfather (deceased [d.]) also have or had a history of glucosuria. His mother was first noted to have glucosuria without hyperglycemia at the age of 11 years. His uncle was first noted to have glucosuria without hyperglycemia at the age of 22 years. His grandfather was first noted to have glucosuria at the age of 27 years, when he was prescribed a medicine, the details of which are unknown. After taking the prescribed medicine, he became comatose and was transported to an emergency room. At that time, he was diagnosed with renal glucosuria. N101K, asparagine-to-lysine substitution at position 101 of the sodium–glucose cotransporter 2 protein; WT, wild type.

any other symptoms at the age of 3 years. His growth rate and intellectual development were normal.

Genetic analysis

After obtaining informed consent from the patient and his parents, blood samples were collected. Genomic DNA was extracted from peripheral white blood cells using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA). We sequenced 14 coding exons in *SLC5A2* together with their flanking intronic regions (Appendix S1; Table S1).

Pathogenicity prediction

The functional consequences of mutations were predicted using three online tools: Polyphen 2 (http://genetics.bwh.harvard.edu/pph2/), SIFT (http://sift.jcvi.org) and MutationTaster (http://www.mutationtaster.org). These *in silico* tools predict the effect of an amino acid substitution on protein function based on the degree of the conservation of the amino acid residues and the changes of protein structure[7–9].

Continuous glucose monitoring

To evaluate the possible pathogenic effect of the *SLC5A2* mutation on glycemic excursion, daily glucose profiles and low-glucose events were monitored for 14 consecutive days with a continuous glucose monitoring (CGM) system (FreeStyle Libre; Abbott Japan Co., Ltd., Chiba, Japan).

Ethical considerations

Ethical approval for this study was obtained from the ethics committee of Koseiren Tsurumi Hospital (approval No. 19-006), and the study conforms to the provisions of the Declaration of Helsinki.

RESULTS

Clinical characteristics of the participants are shown in Table 1. Laboratory tests, such as blood glucose and fasting counter-regulatory hormones, detected no abnormalities, except for glucosuria. The proband's glucose tolerance was normal during a 3-h, 75-g oral glucose tolerance test.

A T-to-A transition at complementary deoxyribonucleic acid (cDNA) position 303 of *SLC5A2* (c.303T>A) was identified in the patient and his mother; this mutation corresponded to an asparagine-to-lysine substitution at position 101 of the SGLT2 protein (N101K) (Figure 2). The proband and his mother were heterozygous for the mutation. The mutation was not detected in the proband's father, nor in 200 chromosomes derived from 100 healthy, unrelated individuals.

Online analysis using Polyphen 2, SIFT and MutationTaster suggested that the N101K mutation would be deleterious and could be associated with FRG (Figure S1).

Although the proband did not complain of symptoms of hypoglycemia, two weeks of CGM showed both nocturnal and diurnal asymptomatic hypoglycemia (<70 mg/dL) for a mean of 236 min/day (Figure 3).

DISCUSSION

In healthy individuals, the kidneys filter approximately 180 g of glucose per day; nearly 100% of the glucose is reabsorbed in the proximal convoluted tubule through SGLTs to maintain glucose homeostasis[10]. SGLT2 is responsible for the active transport of glucose across the brush border membrane, and is expressed almost exclusively in the kidney, accounting for the bulk of glucose reabsorption[10]. Mutations in the *SLC5A2* gene, which encodes SGLT2, have been reported to be responsible for FRG[2,3].

In the present study, a heterozygous missense mutation (c.303T>A:p.N101K) in *SLC5A2* was identified in a Japanese family with FRG. This mutation was not detected in 200 chromosomes from 100 healthy and unrelated individuals, or in 3,408 Japanese individuals in the Tohoku Medical Megabank, which is the largest genome database based on whole-genome sequences of the Japanese general population (https://jmorp.megabank.tohoku.ac.jp/201905/variants/by-gene/SLC5A2). In the Genome Aggregation Database derived from 123,136 exome sequences and 15,496 whole-genome sequences from unrelated individuals sequenced as part of various disease-specific and population genetic studies, only one example of this variant (rs1306939757) has been reported, corresponding to a frequency of 0.0000319 (http://gnomad.broadinstitute.org/), indicating the rarity of this variant. The N101 amino acid residue was found to be highly conserved across SGLT2 homologs in

Table 1 | Laboratory findings and medication of the proband and parents

	Proband	Father	Mother		Proband	Father	Mother
Age (years)	26	52	50	Glucose metabolism			
Height (cm)	174.0	179.3	157.9	Fasting plasma glucose (mg/dL)	91	99	89
Weight (kg)	72.0	83.2	52.9	IRI (μU/mL)	5.9		3.7
BMI (kg/m^2)	23.8	25.9	21.2	C-peptide (ng/mL)	1.75		0.99
Blood pressure (mmHg)	130/77	143/95	121/70	Hemoglobin A1c (%)	5.3	5.7	5.4
				HOMA-R	1.33		0.81
Urine testing				Insulinogenic index	1.78		
pH	6.0	5.0	5.0				
Protein	–	–	–	75-g Oral glucose tolerance test			
Glucose	4+	–	–	Plasma glucose (mg/dL)			
Ketone	–		–	0 min	91		
Blood	–	–	–	30 min	119		
Urine glucose (g/1.73 m^2/24 h)	43.0		0.2	60 min	106		
				90 min	100		
Biochemistry				120 min	103		
Total cholesterol (mg/dL)	203	241	240	180 min	83		
Triglyceride (mg/dL)	80	145	41				
High-density lipoprotein (mg/dL)	64	53	94	Serum IRI (μU/mL)			
Blood urea nitrogen (mg/dL)	24.1	15.5	9.0	0 min	5.9		
Creatinine (mg/dL)	0.70	0.95	0.72	30 min	55.6		
eGFR (mL/min/1.73 m^2)	115.1	66.0	66.8	60 min	24.8		
Sodium (mEq/L)	139		142	90 min	44.7		
Potassium (mEq/L)	3.9		3.7	120 min	37.2		
Chloride (mEq/L)	102		106	180 min	5.1		
Counter-regulatory hormones				Urine glucose (mg/dL)			
Glucagon (5.4–55.0 pg/mL)	19.6		18.0	0 min	3,320		
Adrenocorticotropic hormone (7.2–63.3 pg/mL)	23.9		27.6	30 min	4,502		
Cortisol (6.24–18.0 μg/dL)	6.77		8.71	60 min	6,036		
Growth horme (Male; ≤2.47 ng/mL)	0.40			90 min	5,132		
Growth hormone (Female; 0.13–9.88 ng/mL)			1.46	120 min	5,373		
Adrenaline (≤100 pg/mL)	15		38	180 min	4,650		
Noradrenaline (100–450 pg/mL)	139		133				
Dopamine (≤20 pg/mL)	≤5		≤5	Medication	(–)	(–)	(–)

Fasting plasma glucagon levels were measured using the sandwich enzyme-linked immuno sorbent assay (Mercodia, Uppsala, Sweden). BMI, body mass index; eGFR, estimated glomerular filtration rate; HOMA-R, homeostasis model assessment for insulin resistance; IRI, immunoreactive insulin.

multiple species and among human SGLT subtypes (Figure S2). In humans, only SGLT3, which is encoded by the *SLC5A4* gene, harbors a replacement of the asparagine residue (by threonine). However, SGLT3 is not a cotransporter, but rather a glucosensor expressed in cholinergic neurons at the neuromuscular junction[11], suggesting that the N101 amino acid residue in those SLC5 members that co-transport glucose might play a critical role in the symporter function. Furthermore, all three of the bioinformatics programs consulted here predicted that the N101K mutation would be pathogenic (Figure S1). Further work will be required to prove that the N101K mutation actually results in a loss of function, thereby increasing renal glucosuria. Given the clinical manifestations shown by the patient, we infer that the mutation leads to clinically relevant SGLT2 dysfunction. Notably, in the present study, the heterozygous patient had severe glucosuria (urine glucose: 43.0 g/1.73 m^2/24 h), whereas his mother (also heterozygous for the same mutation) did not (urine glucose: 0.2 g/1.73 m^2/24 h). This result indicates that the inheritance of renal glucosuria is best described as codominant, and its variable penetrance might relate to the compensatory ability of the wild-type allele or environment factors might be associated with the glucose reabsorption[2]. Notably, although *Sglt2*–/– mice excrete much of the filtered glucose, urinary glucose concentrations do not appreciably differ between *Sglt2*+/– and wild-type mice, indicating that one intact *Sglt2* allele is sufficient to achieve normal renal glucose reabsorption[12].

To our knowledge, this pedigree represents the first case in which CGM has been carried out in a patient with FRG. Renal glucosuria is thought to be a "non-disease"; the great majority of affected individuals do not have any complaints, and only rarely have been reported to show a propensity to hypoglycemia[2,13]. However, CGM of this proband suggested the presence of asymptomatic hypoglycemia (as judged by frequency and severity; Figure 3) in an otherwise asymptomatic

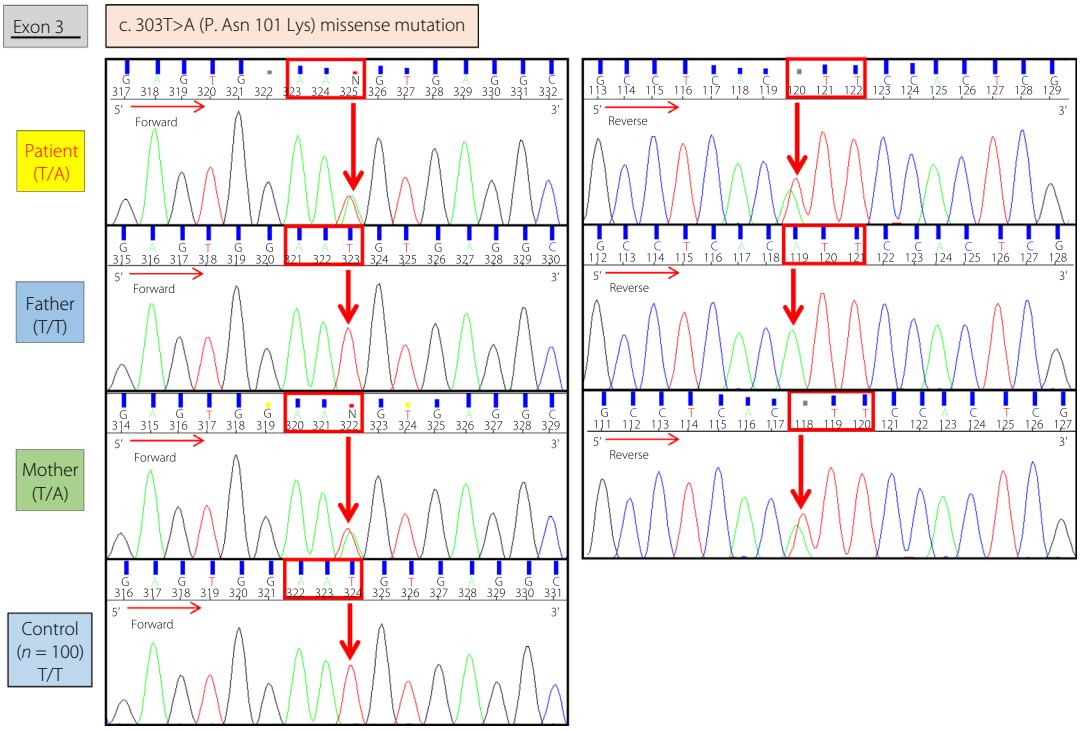

Figure 2 | Genomic deoxyribonucleic acid sequence analysis of the solute carrier family 5 member 2 gene in members of a family with familial renal glucosuria. A T-to-A transition at solute carrier family 5 member 2 complementary deoxyribonucleic acid position 303 (indicated by the red arrows) results in an asparagine-to-lysine substitution at amino acid residue 101 of the sodium–glucose cotransporter 2 protein (N101K). The proband and his mother were heterozygous for the mutation (c.303T > A). His father did not harbor this mutation, nor was the mutation detected in 200 chromosomes derived from 100 healthy, unrelated individuals. The left column shows the forward sequences, and the right column shows the reverse sequences.

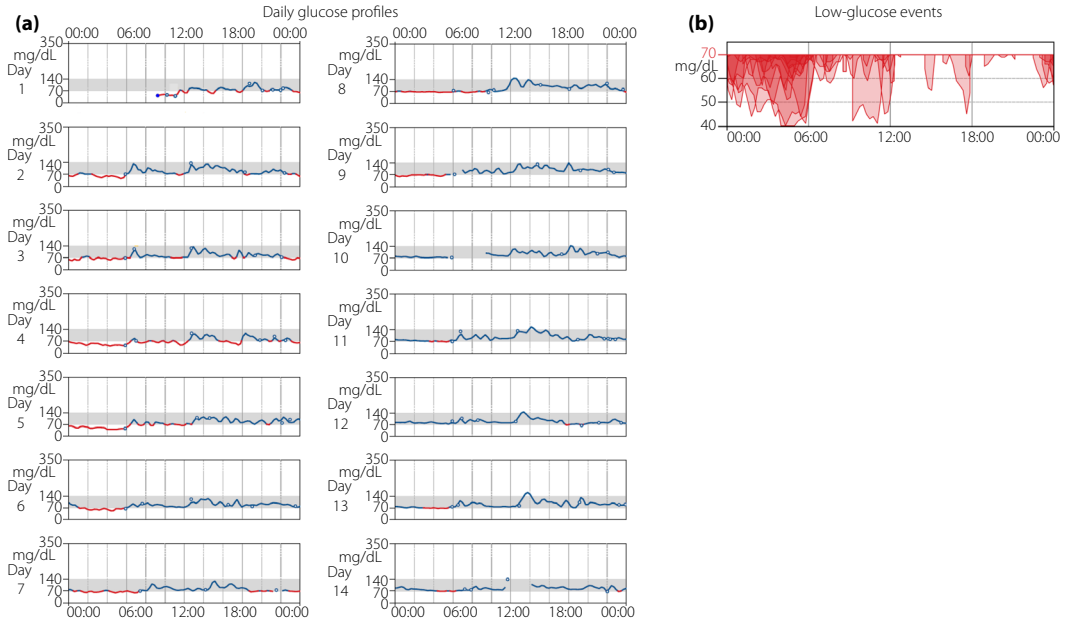

Figure 3 | (a) Daily glucose profiles and (b) low-glucose events for 14 consecutive days during monitoring with a continuous glucose monitoring system (the FreeStyle Libre). The red lines indicate hypoglycemia (<70 mg/dL), and the blue lines indicate the appropriate blood glucose level (70–140 mg/dL). Open circles indicate when the proband checked blood glucose levels. The closed circle indicates the start of continuous glucose monitoring.

FRG patient. Because of their experience with extended hypoglycemic periods since birth, patients with FRG might be accustomed to hypoglycemia.

The present study had two limitations. First, hypoglycemia was detected with CGM, but not by laboratory test or by self-monitoring of blood glucose. Conflicting data about the accuracy of CGM at low-glucose levels have been reported[14,15]. Second, details of clinical phenotypes were studied only in a proband in the present study. Urinary glucose excretion in FRG varies widely among patients, ranging from 0.6 to 202 g/1.73 m^2/24 h[2,16]. The present patient excreted large amounts of urinary glucose, despite being heterozygous for the mutation. Although FRG has been considered to be a "non-disease," the condition sometimes might be accompanied by asymptomatic hypoglycemia on an everyday basis, as shown in the present case. Further studies on CGM in patients with FRG will be required to define the pathological characteristics of this disease.

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 | Pathogenicity prediction.
Figure S2 | Multiple amino acid sequence alignments.
Table S1 | Primers used for solute carrier family 5 member 2 gene polymerase chain reaction and sequencing analysis.
Appendix S1 | Mutation analysis.