A novel compound heterozygous mutation in *SLC5A2* contributes to familial renal glucosuria in a Chinese family, and a review of the relevant literature

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Abstract. Familial renal glucosuria (FRG) is a rare condition that involves isolated glucosuria despite normal blood glucose levels. Mutations in the solute carrier family 5 member 2 (*SLC5A2*) gene, which encodes sodium-glucose cotransporter 2 (SGLT2), have been reported to be responsible for the disease. Genetic testing of the *SLC5A2* gene was conducted in a Chinese family with FRG. A number of online tools were used to predict the potential effect of the identified mutations on SGLT2 function. Additionally, the *SLC5A2* mutations previously reported in PubMed were summarized. A novel compound heterozygous mutation (c.514T>C, p.W172R; c.1540C>T, p.P514S) of the *SLC5A2* gene in a Chinese child with FRG was identified. In total, 86 mutations of the *SLC5A2* gene have been reported to be associated with FRG. The novel compound heterozygous mutation (c.514T>C, p.W172R; c.1540C>T, p.P514S) of the *SLC5A2* gene may be responsible for the onset of FRG. The present study provides a starting point for further investigation of the molecular pathogenesis of the *SLC5A2* gene mutation in patients with FRG.

Introduction

Familial renal glucosuria [FRG; Online Mendelian Inheritance in Man (https://www.omim.org) no. 233100] is a hereditary kidney disease characterized by persistent glucosuria due to a reduction in the renal tubular reuptake of glucose, along with normal blood glucose levels and no other impaired tubular functions (1). In general, FRG is a benign condition that does not require any specific therapy. The ability of the kidney to reabsorb glucose principally involves the lower-affinity high-capacity sodium-glucose cotransporter 2 (SGLT2), which is located in the proximal convoluted tubule segment S1 and has a Na^+-glucose coupling ratio of 1:1 (2). SGLT2 is encoded by the solute carrier family 5 member 2 (*SLC5A2*) gene and has 672 amino acids. A large number of case reports conducted using patients of different ethnicities have confirmed that *SLC5A2* mutations are responsible for the majority of FRG cases (3-22). Variations in the *SLC5A2* gene impact the function of SGLT2, leading to isolated glucosuria. However, various different modes of inheritance have been reported for FRG. Notably, research on SGLT2 has been benefitted in recent years by its identification as a therapeutic target in type 2 diabetes mellitus. In the present study, an association between FRG and a novel compound heterozygous mutation of the *SLC5A2* gene was identified. Moreover, all the *SLC5A2* mutations in patients with FRG that have been reported to date are summarized in the present study. The present study provides additional information on the genetic mechanism of FRG.

Materials and methods

Subject. The subject of the present study was a Han Chinese girl. The patient was observed to exhibit glucosuria in the absence of hyperglycemia at the age of 1 year and 9 months, following an initial urine test. Routine urinary analysis showed glucose in the range + (100 mg/dl) to +++ (500 mg/dl), with no other abnormalities. The quantitative test for urine glucose gave a result of 15.77 g/1.73 m^2/24 h. The patient was subjected to an oral glucose tolerance test and exhibited a 2-h postprandial sugar level of 5.1 mmol/l. The patient had no polyuria, polydipsia or polyphagia, and her body weight gain was the same as that of age-matched children. The patient experienced no problems with activity, eating, sleeping or excretory function. There was no reported history of trauma or poisoning. The parents and other family members had no history of glucosuria.

Genetic testing. Following collection of 2 ml blood samples from the parents of the patient, who had no history of FRG, and healthy controls from July to August 2017, genomic DNA

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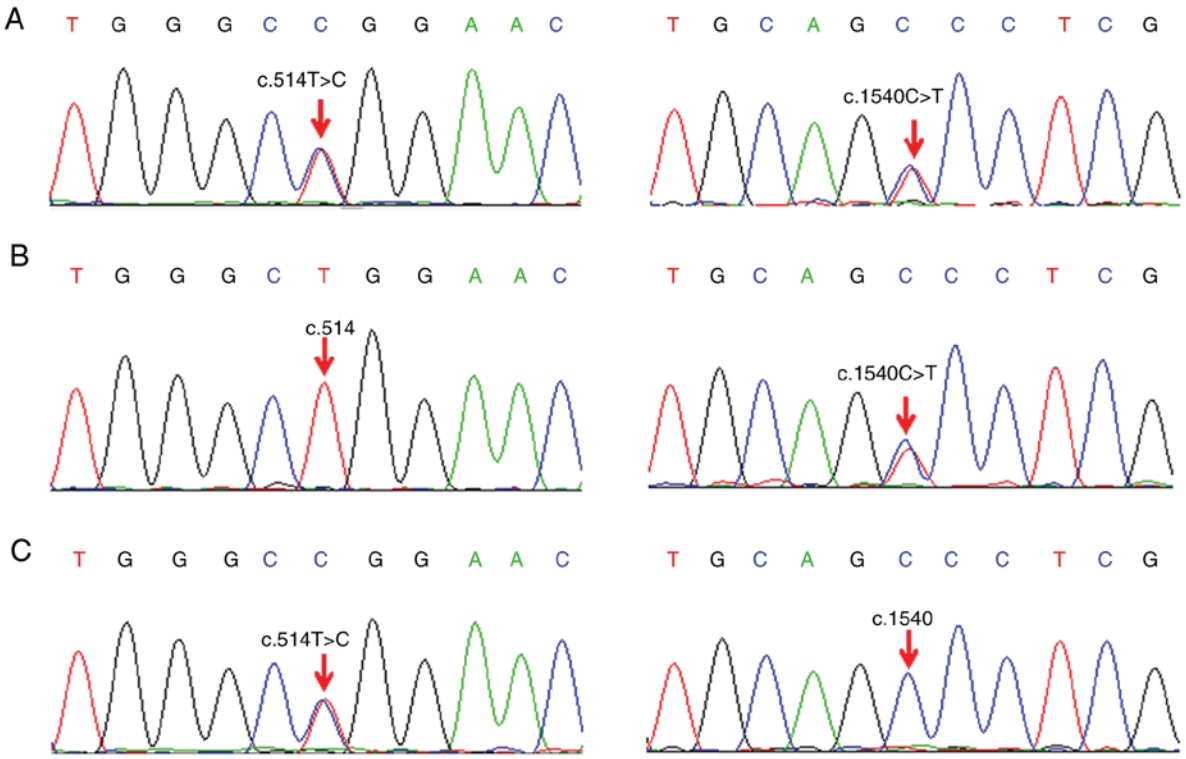

Figure 1. Mutation analysis of the solute carrier family 5 member 2 gene in family members affected with familial renal glucosuria. The positions of the mutations are indicated by the red arrows. (A) The proband harbored a compound heterozygous mutation, c.514T>C and c.1540C>T. (B) The father of the proband was an asymptomatic heterozygous mutation c.1540C>T carrier. (C) The mother of the proband was detected to have an asymptomatic heterozygous mutation c.514T>C.

was extracted from the peripheral blood leukocytes using a Wizard genomic DNA purification kit (Promega Corporation, Madison, WI, USA), according to the manufacturer's protocol. A total of fifty healthy controls (28 males and 22 females; average age 38.84±29.78 months) were recruited. Initially, 900 μl of cell lysis solution was added to a sterile 1.5 ml microcentrifuge tube with 300 μl collected blood to separate the leukocytes. All the exons and conterminal intronic regions of the *SLC5A2* gene were amplified via polymerase chain reaction (PCR) using a Thermal Cycler 9700 (Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA, USA). The primers (forward for Exon5, 5'-ACCACTGCGAGG GTTATGAT-3' and reverse for Exon5, 5'-TCCTCACTCAAG CCCAGCAT-3'; forward for Exon12, 5'-GTGTTCATCGTG GTAGTGTCGG-3' and reverse for Exon12, 5'-CCCTCAGTC GAGAAATTCAGG-3') were designed using Primer Premier 5.0 software (Premier Biosoft International, Palo Alto, CA, USA). The PCRs were conducted in a total volume of 20 μl containing 1.6 μl DNA, 10 μl 2X Taq Master Mix (CWBIO, Beijing, China), 0.8 μl forward primer (Sangon Biotech Co., Ltd., Shanghai, China), 0.8 μl reverse primer (Sangon Biotech Co., Ltd.) and ddH_2O (added to a final volume of 20 μl), with the following thermal cycling conditions: Denaturing at 94˚C for 5 min, 35 cycles of denaturing at 94˚C for 30 sec, annealing at 57˚C for 30 sec and extension at 72˚C for 30 sec, followed by extension at 72˚C for 10 min. The sequence analysis of the two coding exons of the *SLC5A2* gene was performed using an ABI Prism 3130 genetic analyzer (Applied Biosystems; Thermo Fisher Scientific, Inc.). Potential mutations were defined by their exclusion from the Human Gene Mutation Database (http://www.hgmd.cf.ac.uk) and previously reported mutations on PubMed (http://ncbi.nlm.nih.gov/PubMed/). A total of fifty healthy Chinese individuals containing 100 chromosomes were included as controls. A total of three databases, the dbSNP database of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/snp/), Exome Variant Server (http://evs.gs.washington.edu/EVS) and 1000 Genomes Project (http://www.1000genomes.org/), were used to eliminate single-nucleotide polymorphisms (SNPs). The study was approved by the Institutional Review Board of the Third Xiangya Hospital, Central South University (Changsha, China).

Homology analysis. A comparative analysis of multiple amino acid sequences of SGLT2 was performed for different species using the Basic Local Alignment Search Tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The aligned reference sequences were *Homo sapiens* (GenBank NP_003032.1), *Pan troglodytes* (XP_003315117.1), *Macaca mulatta* (XP_001113206.1), *Canis lupus* (XP_005621284.1), *Bos taurus* (NP_976236.1), *Mus musculus* (NP_573517.1), *Rattus norvegicus* (NP_072112.2), *Danio rerio* (NP_998091.1) and *Xenopus tropicalis* (XP_002940641.2).

Pathogenicity prediction. The functional effects of protein variants were predicted using three online prediction tools, PolyPhen2 (http://genetics.bwh.harvard.edu/pph2/), SIFT (http://sift.jcvi.org) and Mutation Taster (http://www.mutationtaster.org). These online tools predict the pathogenicity of an altered protein based on the number of conserved amino acids and changes in protein structure.

Table I. Predicted pathogenicity of two missense mutations in the solute carrier family 5 member 2 gene.

Site	Nucleotide changes	Amino acid change	SIFT	PolyPhen-2	Mutation taster
Exon5	c.514T>C	p.W172R	Deleterious	Probably damaging	Disease causing
Exon12	c.1540C>T	p.P514S	Tolerated	Probably damaging	Disease causing

Figure 2. Multiple amino acid sequence alignments of the solute carrier family 5 member 2 gene. The Trp172 and Pro514 residues were highly conserved across various species. The specific position is indicated with a black rectangle.

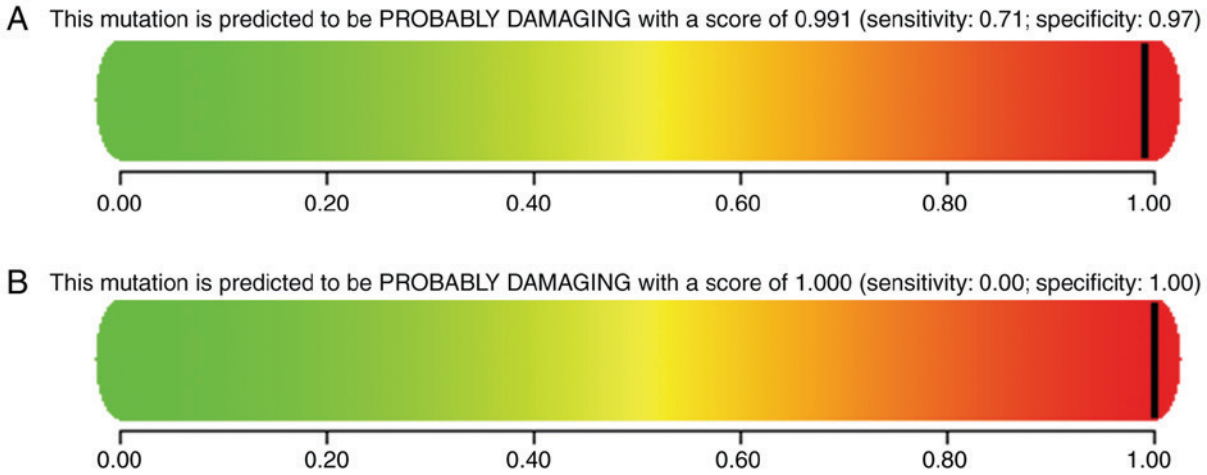

Figure 3. Pathogenicity of identified mutations in the solute carrier family 5 member 2 gene predicted by PolyPhen-2. The two missense mutations (c.514T>C and c.1540C>T) were both predicted to be 'probably damaging', with a score of (A) 0.991 and (B) 1.000, respectively.

Literature review. All of the literature previously published on the *SLC5A2* mutations (between 2002 and 2017) was retrieved from PubMed. The mutation locations and types for the *SLC5A2* gene were summarized.

Results

Genetic testing of the SLC5A2 gene. According to the direct sequencing of the *SLC5A2* gene from the patient with FRG, a novel 1 bp missense mutation in exon 5 (c.514T>C, p.W172R) and a previously reported 1 bp missense mutation in exon 12 (c.1540C>T, p.P514S) were revealed (Fig. 1A). The father of the patient carried the same p.P514S mutation, while her mother had the same p.W172R mutation (Fig. 1B and C). However, neither of the parents exhibited glycosuria or hyperglycemia, with fasting plasma glucose levels of 4.8 and 3.9 mmol/l. Screening of the *SLC5A2* gene in healthy Chinese individuals revealed no mutant alleles in exon 5 or exon 12

Table II. Literature review of the clinical characteristics and mutational analysis of the solute carrier family 5 member 2 gene in patients with familial renal glucosuria.

Author, year	Age, years[a]	Sex	Urine glucose (g/1.73 m^2/24 h)	Allele 1	Allele 2	Mutation state	(Refs.)
van den Heuvel *et al*, 2002	2	Male	61.6 g/l[b]	c.1320G>A	c.1320G>A	Homozygous	(3)
Santer *et al*, 2003	-	-	126-162.2	c.973-7 del ATGTT	c.973-7 del ATGTT	Homozygous	(4)
Santer *et al*, 2003	-	-	73.6	c.814G>A	c.814G>A	Homozygous	(4)
Santer *et al*, 2003	-	-	50.6-51.3	IVS7+5G>A	IVS7+5G>A	Homozygous	(4)
Santer *et al*, 2003	-	-	21.3	IVS7+5G>A	c.920T>C	Compound heterozygous	(4)
Santer *et al*, 2003	-	-	28.5	IVS7+5G>A	c.920T>C	Compound heterozygous	(4)
Santer *et al*, 2003	-	-	43.0	c.1346G>A	c.1346G>A	Homozygous	(4)
Santer *et al*, 2003	-	-	68.7	c.1320G>A	c.1320G>A	Homozygous	(4)
Santer *et al*, 2003	-	-	20.8	c.1461-517 del 57	-	-	(4)
Santer *et al*, 2003	-	-	0.6	c.1951-92 del 42	WT	Heterozygous	(4)
Santer *et al*, 2003	-	-	38.8	c.1102C>T	c.1102C>T	Homozygous	(4)
Santer *et al*, 2003	-	-	2.3-4.5	c.506delC	WT	Heterozygous	(4)
Santer *et al*, 2003	-	-	0.75	IVS7+5G>A	WT	Heterozygous	(4)
Santer *et al*, 2003	-	-	14.6	IVS7+5G>A	c.932A>G	Compound heterozygous	(4)
Santer *et al*, 2003	-	-	5.9	c.216C>A	WT	Heterozygous	(4)
Santer *et al*, 2003	-	-	2.8	WT	WT	-	(4)
Santer *et al*, 2003	-	-	202	c.410G>A	c.1152-63 del 12	Compound heterozygous	(4)
Santer *et al*, 2003	-	-	79.8	c.410G>A	c.1152-63 del 12	Compound heterozygous	(4)
Santer *et al*, 2003	-	-	1.8	c.151A>C	WT	Heterozygous	(4)
Santer *et al*, 2003	-	-	30.1-92.4	c.1627A>C	c.1627A>C	Homozygous	(4)
Santer *et al*, 2003	-	-	4.8	c.313G>A	WT	Heterozygous	(4)
Santer *et al*, 2003	-	-	8.0-16.7	WT	WT	-	(4)
Santer *et al*, 2003	-	-	31.7	c.448T>C	c.1495C>T	Compound heterozygous	(4)
Santer *et al*, 2003	-	-	1.2	c.1359C>A	WT	Heterozygous	(4)
Santer *et al*, 2003	-	-	1.9	c.1152-63 del 12	WT	Heterozygous	(4)
Santer *et al*, 2003	-	-	0.75	IVS7+5G>A	WT	Heterozygous	(4)
Calado *et al*, 2004	41	Male	12	c.500delA	c.1961A>G	Compound heterozygous	(5)
Kleta *et al*, 2004	19	Female	9.1	c.599C>A	c.1961A>G	Compound heterozygous	(6)
Francis *et al*, 2004	82	Female	>30	c.G910A+G911A	c.G910A+G911A	Homozygous	(7)
Magen *et al*, 2005	3	Male	83	c.962A>G	c.962A>G	Homozygous	(8)

Table II. Continued.

Author, year	Age, years[a]	Sex	Urine glucose (g/1.73 m^2/24 h)	Allele 1	Allele 2	Mutation state	(Refs.)
Magen *et al*, 2005	1.5	Female	101	c.962A>G	c.962A>G	Homozygous	(8)
Magen *et al*, 2005	0.2	Male	95	c.962A>G	c.962A>G	Homozygous	(8)
Magen *et al*, 2005	0.5	Male	114	c.962A>G	c.962A>G	Homozygous	(8)
Magen *et al*, 2005	1	Female	124	c.962A>G	c.962A>G	Homozygous	(8)
Magen *et al*, 2005	9	Male	169	c.962A>G	c.962A>G	Homozygous	(8)
Calado *et al*, 2006	50	-	7.6	c.500delA	WT	Heterozygous	(9)
Calado *et al*, 2006	40	-	11.6	IVS12+1G>A	WT	Heterozygous	(9)
Calado *et al*, 2006	8	-	6.4	IVS12+1G>A	IVS12+1G>A	Homozygous	(9)
Calado *et al*, 2006	3	-	12.2	IVS12+1G>A	IVS12+1G>A	Homozygous	(9)
Calado *et al*, 2006	1	-	6.2	c.395G>A	c.655G>A	Compound heterozygous	(9)
Calado *et al*, 2006	6	-	12.1	WT	WT	-	(9)
Calado *et al*, 2006	26	-	65.6	c.305C>T	c.305C>T	Homozygous	(9)
Calado *et al*, 2008	21	Male	3.2	c.346G>A	WT	Heterozygous	(10)
Calado *et al*, 2008	48	Male	6.1	c.1672C>T	WT	Heterozygous	(10)
Calado *et al*, 2008	28	Female	2.7	c.1961A>G	WT	Heterozygous	(10)
Calado *et al*, 2008	6	Male	62.3	IVS7+5G>A	IVS7+5G>A	Homozygous	(10)
Calado *et al*, 2008	24	Female	86.5	c.670G>C	c.670G>C	Homozygous	(10)
Calado *et al*, 2008	42	Female	30	c.131T>A	c.1145T>C	Compound heterozygous	(10)
Calado *et al*, 2008	14	Female	61.1	c.601G>A	c.1159C>A	Compound heterozygous	(10)
Calado *et al*, 2008	33	Male	n.q.	c.968C>G	c.1961A>G	Compound heterozygous	(10)
Calado *et al*, 2008	1.5	Male	35.5	c.1102C>T	c.1359C>A	Compound heterozygous	(10)
Calado *et al*, 2008	8	Female	n.q.	IVS7+5G>A	c.1428C>G	Compound heterozygous	(10)
Calado *et al*, 2008	66	Female	10	c.1446G>C	c.1961A>G	Compound heterozygous	(10)
Calado *et al*, 2008	16	Female	6.5	c.898C>T	WT	Heterozygous	(10)
Calado *et al*, 2008	8	Male	n.q.	IVS7+5G>A	IVS7+5G>A	Homozygous	(10)
Calado *et al*, 2008	5	Female	n.q.	IVS7+5G>A	IVS7+5G>A	Homozygous	(10)
Calado *et al*, 2008	12	Male	15.2	c.1616T>C	c.1616T>C	Homozygous	(10)
Calado *et al*, 2008	9	Male	23.1	c.1616T>C	c.1616T>C	Homozygous	(10)
Calado *et al*, 2008	2	Female	14.2	IVS7+5G>A	c.1405G>A	Compound heterozygous	(10)
Calado *et al*, 2008	16	Female	66.9	c.1068G>A	IVS12+1G>A	Compound heterozygous	(10)

Table II. Continued.

Author, year	Age, years[a]	Sex	Urine glucose (g/1.73 m^2/24 h)	Allele 1	Allele 2	Mutation state	(Refs.)
Calado *et al*, 2008	12	Female	72.7	c.384C>G	c.384C>G	Homozygous	(10)
Yu *et al*, 2011	36	Female	16.06	IVS11+1G>C	IVS1-16C>A	Compound heterozygous	(11)
Yu *et al*, 2011	27	Male	6.47	c.294C>A	WT	Heterozygous	(11)
Yu *et al*, 2011	41	Female	6.30	c.1388T>G	WT	Heterozygous	(11)
Yu *et al*, 2011	15	Female	27	IVS1-16C>A	c.1435C>G	Compound heterozygous	(11)
Lee *et al*, 2012	-	Male	46.6[c]	c.1435C>G,	c.1346G>A	Compound heterozygous	(12)
Lee *et al*, 2012	-	Male	18.3[c]	c.979C>T	c.1499T>G	Compound heterozygous	(12)
Lee *et al*, 2012	-	Male	25.9[c]	c.1540C>T	c.1430T>G	Compound heterozygous	(12)
Lee *et al*, 2012	-	Male	67.8[c]	c.409C>T	c.1732C>T	Compound heterozygous	(12)
Lee *et al*, 2012	-	Male	39.7[c]	c.1346G>A	c.1540C>T	Compound heterozygous	(12)
Lee *et al*, 2012	-	Female	22.0[c]	c.736C>T	c.1499T>G	Compound heterozygous	(12)
Lee *et al*, 2012	-	Male	15.3[c]	c.1346G>A	c.1346G>A	Homozygous	(12)
Lee *et al*, 2012	-	Female	76.8[c]	c.409C>T	c.1475_1476insC	Compound heterozygous	(12)
Lee *et al*, 2012	-	Male	32.7[c]	c.983T>G	c.1894_1895ins6	Compound heterozygous	(12)
Lee *et al*, 2012	-	Male	42.6[c]	c.1382G>A	c.1540C>T	Compound heterozygous	(12)
Lee *et al*, 2012	-	Male	24.9[c]	c.1346G>A	WT	Heterozygous	(12)
Lee *et al*, 2012	-	Male	12.1[c]	c.867G>C	WT	Heterozygous	(12)
Lee *et al*, 2012	-	Female	35.2[c]	c.1346G>A	WT	Heterozygous	(12)
Lee *et al*, 2012	-	Male	30.9[c]	c.1798delC	WT	Heterozygous	(12)
Lee *et al*, 2012	-	Female	8.9[c]	c.320T>C	WT	Heterozygous	(12)
Lee *et al*, 2012	-	Male	33.7[c]	c.938G>A	WT	Heterozygous	(12)
Lee *et al*, 2012	-	Male	16.2[c]	c.1346G>A	WT	Heterozygous	(12)
Lee *et al*, 2012	-	Male	6.9[c]	c.1507G>A	WT	Heterozygous	(12)
Lee *et al*, 2012	-	Female	5.1[c]	c.1540C>T	WT	Heterozygous	(12)
Lee *et al*, 2012	-	Male	25.2[c]	c.1418_1432dup15	WT	Heterozygous	(12)
Lee *et al*, 2012	-	Female	1.9[c]	c.1357T>A	WT	Heterozygous	(12)
Lee *et al*, 2012	-	Female	15.5[c]	c.1346G>A	WT	Heterozygous	(12)
Lee *et al*, 2012	-	Male	4.7[c]	c.170T>C	WT	Heterozygous	(12)
Lee, 2013	40	Male	10.8	c.1162delG	WT	Heterozygous	(13)
Yu *et al*, 2014	50	Male	4.8	c.229G>C	WT	Heterozygous	(14)

Table II. Continued.

Author, year	Age, years[a]	Sex	Urine glucose (g/1.73 m^2/24 h)	Allele 1	Allele 2	Mutation state	(Refs.)
Lee, 2013	40	Male	10.8	c.1162delG	WT	Heterozygous	(13)
Yu *et al*, 2015	36	Female	12.9	c.294C>A	WT	Heterozygous	(15)
Yu *et al*, 2015	27	Male	19.6	c.736C>T	c.1420G>C	Compound heterozygous	(15)
Yu *et al*, 2015	20	Male	5.9	c.1051T>C	WT	Heterozygous	(15)
Yu *et al*, 2015	58	Male	7.3	c.1400T>C	WT	Heterozygous	(15)
Yu *et al*, 2015	61	Male	8.1	c.1691G>A	WT	Heterozygous	(15)
Dhayat *et al*, 2016	70.3	Female	6.71	c.265G>A	WT	Heterozygous	(16)
Dhayat *et al*, 2016	65.3	Female	11.77	c.265G>A	WT	Heterozygous	(16)
Dhayat *et al*, 2016	61.8	Female	5.54	c.265G>A	WT	Heterozygous	(16)
Dhayat *et al*, 2016	40.2	Female	6.53	c.265G>A	WT	Heterozygous	(16)
Dhayat *et al*, 2016	27.1	Male	1.10	c.265G>A	WT	Heterozygous	(16)
Ottosson-Laakso *et al*, 2016	-	Female	55.2	c.300-303+2del	-	Compound heterozygous	(17)
Yu *et al*, 2016	39	Female	7.56	c.1891G>A	WT	Heterozygous	(18)
Yu *et al*, 2016	36	Female	8.3	c.1319G>A	WT	Heterozygous	(19)
Zhao *et al*, 2016	22	Male	10.56	c.1003A>G	c.1343A>G + c.1739G>A	Compound heterozygous	(20)
Zhao *et al*, 2016	26	Male	1.96	c.886(-10_-31)del	WT	Heterozygous	(20)
Zhao *et al*, 2016	30	Male	1.77	c.886(-10_-31)del	WT	Heterozygous	(20)
Zhao *et al*, 2016	32	Female	1.66	c.886(-10_-31)del	WT	Heterozygous	(20)
Zhao *et al*, 2016	25	Male	12.74	c.886(-10_-31)del	WT	Heterozygous	(20)
Zhao *et al*, 2016	52	Male	1.34	c.1420G>C	WT	Heterozygous	(20)
Zhao *et al*, 2016	38	Male	50.68	c.886(-10_-31)del	c.886(-10_-31)del	Compound heterozygous	(20)
Zhao *et al*, 2016	48	Female	1.78	c.393G>C	WT	Heterozygous	(20)
Kim *et al*, 2016	26	Male	3.7	c.395G>A	WT	Heterozygous	(21)
Wang *et al*, 2017	24	Female	8.06	c.877A>T	WT	Heterozygous	(22)
Wang *et al*, 2017	4	Female	10.96	c.229G>C	c.1540C>T	Compound heterozygous	(22)
-[d]	1.75	Female	15.77	c.514T>C	c.1540C>T	Compound heterozygous	-

[a]At the time of evaluation; [b]the level of urine glucose was only available in g/l; [c]spot urine glucose/creatinine ratio (mg/mg); [d]the present study. WT, wild type; n.q., persistent glucosuria not quantified.

among 100 screened chromosomes. The novel p.W172R mutation was not identified in the three SNP databases used in the present study.

Functional prediction of the SLC5A2 mutations. The results of a comparative analysis of multiple amino acid sequences revealed that the p.W172R and p.P514S variants occurred in highly conserved locations. In addition, the amino acid residues adjacent to the p.W172R and p.P514S variants were also highly conserved among a number of species (Fig. 2). The results of the online analysis performed using PolyPhen-2, SIFT, and Mutation Taster demonstrated that the mutations p.W172R and p.P514S may be deleterious and may be associated with FRG (Fig. 3; Table I).

Results of the literature review. To date, 115 index cases of FRG, including the proband assessed in the present study, have been retrieved in total (Table II). The age of patients upon diagnosis with FRG via an initial urine test is between 2 months and 82 years. Among the 83 cases for which the sex was identified, the male-to-female ratio was 1.18:1. The mutation states are heterozygous, homozygous and compound heterozygous. In summary, 86 mutations of the *SLC5A2* gene, including one containing the novel mutation p.W172R in the present study, throughout exons 2-14 and the flanking intronic regions, have been reported to be associated with FRG in patients of different ethnicities (Table III). The three most common mutation sites are located in exon 11 (16/86=18.60%), exon 8 (11/86=12.79%) and exon 4 (10/86=11.63%). The mutations are primarily missense (65/86=75.58%), frameshift (7/86=8.14%), splicing (5/86=5.81%), and nonsense (4/86=4.65%) mutations. Chinese and Korean patients in the East Asian region account for 44.31% (39/88) of all reported mutations.

Discussion

FRG is an isolated disorder of glucose transport in the proximal tubule with normal glucose metabolism, and may occur in all age groups. The disease has not been reported to occur at any increased frequency in either males or females. The prevalence of FRG has been suggested to be 0.29% in the general Caucasian population (23), while it is suspected to have a prevalence of <0.1% in Japanese schoolchildren (24). FRG is classified into three types (A, B and O) according to urinary glucose levels (25). Severe FRG (glucosuria ≥10 g/1.73 m^2/24 h), termed type O FRG, is a rare subtype. Patients with type A FRG are characterized by a low renal threshold for glucose and low maximum tubular glucose reabsorption. Those with type B have a low threshold but normal maximum tubular glucose reabsorption. By contrast, patients with type O have a complete absence of renal glucose transport (25). In the majority of affected individuals, the condition causes no apparent symptoms or serious effects associated with the excessive urinary excretion of glucose, such as polyuria or enuresis. However, polyuria, enuresis and a mild delay in growth are reported in patients with type O FRG (26). Various other manifestations, such as episodic dehydration and starvation ketosis, and an increased incidence of urinary tract or genital infection, have also been observed in cases of severe FRG (25). Collectively, kidney biopsies in patients with FRG indicate normal kidney tissue via light microscopy, immunofluorescence and electron microscopy (14).

As the member of the sodium glucose cotransporter family, SGLT2 is primarily expressed in the kidney and helps to maintain ~90% glomerular filtration during glucose reabsorption (27). The *SLC5A2* gene is localized in chromosome 16p11.2, with 14 exons, and encodes SGLT2, which contains 672 amino acids. Previous studies have revealed that *SLC5A2* mutations are closely associated with the occurrence of FRG (3-22). FRG is primarily caused by mutations in the *SLC5A2* gene, which are responsible for the majority of cases. Regarding inheritance patterns, FRG may be inherited in an autosomal recessive or autosomal dominant pattern. However, studies have demonstrated that the inheritance of FRG may best be described as co-dominant with incomplete penetrance (4,22). Previous studies have suggested that patients with heterozygous *SLC5A2* mutations are likely to exhibit mild glucosuria (glucosuria <10 g/1.73 m^2/24 h), while homozygous or compound heterozygous mutations tend to lead to severe glucosuria (4,8,12). Not all individuals with heterozygous *SLC5A2* variants exhibit glucosuria; this highlights the issue of penetrance (28). Penetrance is difficult to determine reliably, even for genetic diseases that are caused by a single polymorphic allele. For many hereditary diseases, the onset of symptoms is age-associated and affected by environmental factors, such as diet and climate, in addition to genetic cofactors and the epigenetic regulation of expression (29). Specifically, a diagnosis of FRG depends on the detection of urine glucose levels, thus it may be missed due to alterations in the urine glucose level. For example, the urine glucose level will be impacted by the amount of sugar consumed recently.

In the present study, two missense mutations in the *SLC5A2* gene of a Chinese patient with FRG accompanied by benign clinical symptoms were reported, one of which was a novel missense mutation (c.514T>C; p.W172R). A total of two previous studies reported that the p.P514S mutation led to FRG with single heterozygous or compound heterozygous status (p.G77R, p.P514S; p.V477G, p.P514S) (12,22). The parents of the proband in the present study carried missense mutations at different locations in terms of *SLC5A2* cDNA position, but neither of them had history of glucosuria. Nevertheless, the patient, with p.W172R and p.P514S missense mutations, exhibited severe glucosuria. It is possible that wild-type SGLT2 may serve a compensatory role during the occurrence of FRG caused by *SLC5A2* mutations. These results indicated that the inheritance patterns of FRG are best described as co-dominant. Therefore, it may be surmised that the p.W172R and p.P514S compound heterozygous mutation of the *SLC5A2* gene contributes to FRG.

SGLT2 has 14 transmembrane helices (TMHs) with the hydrophobic N- and C-terminal domains lying in the extracellular space and contains a sodium solute symporter domain (http://smart.embl-heidelberg.de/; TMHMM server V.2.0). An earlier study on the transport mechanisms of the SGLT1/SGLT2 chimera indicated that the C-terminal domain determined sugar affinity and selectivity (30). p.W172 and p.P514 are localized in the extracellular loops between TMH 4 and TMH 5, and between TMH 12 and 13, respectively (Fig. 4). p.W172 and p.P514 residues were identified to be highly conserved among numerous other species. Meanwhile,

Table III. Literature review of solute carrier family 5 member 2 gene mutations of patients with familial renal glucosuria.

Author, year	Site	Ethnicity	Nucleotide change	Amino acid change	Mutation type	(Refs.)
Yu *et al*, 2011	Intron 1	Chinese	IVS1-16C>A	-	Splicing	(11)
Calado *et al*, 2008	Exon 2	American	c.131T>A	p.M44K	Missense	(10)
Santer *et al*, 2003	Exon 2	NA	c.151A>C	p.T51P	Missense	(4)
Lee *et al*, 2012	Exon 2	Korean	c.170T>C	p.L57P	Missense	(12)
Santer *et al*, 2003	Exon 3	NA	c.216C>A	p.F72L	Missense	(4)
Yu *et al*, 2014; Wang *et al*, 2017	Exon 3	Chinese	c.229G>C	p.G77R	Missense	(14,22)
Dhayat *et al*, 2016	Exon 3	Swiss	c.265G>A	p.A89T	Missense	(16)
Yu *et al*, 2011; Yu *et al*, 2015	Exon 3	Chinese	c.294C>A	p.F98L	Missense	(11,15)
Laakso *et al*, 2016	Exon 3	Finnish	c.300-303+2del	-	Frameshift	(17)
Calado *et al*, 2006	Exon 4	Turkish	c.305C>T	p.A102V	Missense	(9)
Santer *et al*, 2003	Exon 4	NA	c.313G>A	p.V105M	Missense	(4)
Lee *et al*, 2012	Exon 4	Korean	c.320T>C	p.L107P	Missense	(12)
Calado *et al*, 2008	Exon 4	Portuguese	c.346G>A	p.V116M	Missense	(10)
Calado *et al*, 2008	Exon 4	Turkish	c.384C>G	p.Y128X	Nonsense	(10)
Zhao *et al*, 2016	Exon 4	Chinese	c.393G>C	p.K131N	Missense	(20)
Calado *et al*, 2006; Kim *et al*, 2016	Exon 4	Korean, Turkish	c.395G>A	p.R132H	Missense	(9,21)
Lee *et al*, 2012	Exon 4	Korean	c.409C>T	p.R137C	Missense	(12)
Santer *et al*, 2003	Exon 4	German	c.410G>A	p.R137H	Missense	(4)
Santer *et al*, 2003	Exon 4	NA	c.448T>C	p.Y150H	Missense	(4)
Santer *et al*, 2003; Calado *et al*, 2004; Calado *et al*, 2006	Exon 5	Portuguese, NA	c.500delA	p.Q167fsX186	Frameshift	(4,5,9)
Santer *et al*, 2003	Exon 5	NA	c.506delC	p.Q168fs…186X	Frameshift	(4)
	Exon 5	Chinese	c.514T>C	p.W172R	Missense	-[a]
Kleta *et al*, 2004	Exon 6	Russian/Cuban/Spanish ancestry	c.599C>A	p.T200K	Missense	(6)
Calado *et al*, 2008	Exon 6	Belgian	c.601G>A	p.D201N	Missense	(10)
Calado *et al*, 2006	Exon 6	Turkish	c.655G>A	p.A219T	Missense	(9)
Calado *et al*, 2008	Exon 7	Belgian	c.670G>C	p.G224R	Missense	(10)
Lee *et al*, 2012; Yu *et al*, 2015	Exon 7	Korean, Chinese	c.736C>T	p.P246S	Missense	(12,15)
Santer *et al*, 2003	Exon 7	NA	c.814G>A	p.G272R	Missense	(4)
Lee *et al*, 2012	Exon 7	Korean	c.867G>C	p.W289C	Missense	(12)
Wang *et al*, 2017	Exon 7	Chinese	c.877A>T	p.S293C	Missense	(22)

Table III. Continued.

Author, year	Site	Ethnicity	Nucleotide change	Amino acid change	Mutation type	(Refs.)
Santer *et al*, 2003; Calado *et al*, 2008	Intron 7	Pakistani, former Yugoslav, Italian, Swiss, Canadian, German, Turkish, Macedonian	IVS7+5G>A	-	Splicing	(4,10)
Zhao *et al*, 2016	Intron 7	Chinese	c.886(-10_-31)del	-	Splicing	(20)
Calado *et al*, 2008	Exon 8	German	c.898C>T	p.R300C	Missense	(10)
Francis *et al*, 2004	Exon 8	Italian	c.G910A+G911A	p.G304K	Missense	(7)
Santer *et al*, 2003	Exon 8	NA	c.920T>C	p.L307P	Missense	(4)
Santer *et al*, 2003	Exon 8	NA	c.932A>G	p.K311R	Missense	(4)
Lee *et al*, 2012	Exon 8	Korean	c.938G>A	p.G313D	Missense	(12)
Magen *et al*, 2005	Exon 8	Israeli-Arab descent	c.962A>G	p.K321R	Missense	(8)
Calado *et al*, 2008	Exon 8	Belgian	c.968C>G	p.T323R	Missense	(10)
Santer *et al*, 2003	Exon 8	German	c.973-7 del ATGTT	p.P324fs…347X	Frameshift	(4)
Lee *et al*, 2012	Exon 8	Korean	c.979C>T	p.L327F	Missense	(12)
Lee *et al*, 2012	Exon 8	Korean	c.983T>G	p.M328R	Missense	(12)
Zhao *et al*, 2016	Exon 8	Chinese	c.1003A>G	p.S335G	Missense	(20)
Laakso *et al*, 2016	Exon 9	Finnish		p.A343V	Missense	(17)
Yu *et al*, 2015	Exon 9	Chinese	c.1051T>C	p.C351R	Missense	(15)
Calado *et al*, 2008	Exon 9	Macedonian	c.1068G>A	p.G356S	Missense	(10)
Santer *et al*, 2003; Calado *et al*, 2008	Exon 9	NA, Greek	c.1102C>T	p.R368W	Missense	(4,10)
Calado *et al*, 2008	Exon 10	American	c.1145T>C	p.M382T	Missense	(10)
Calado *et al*, 2008	Exon 10	Brazilian	c.1159C>A	p.L387M	Missense	(10)
Lee *et al*, 2013	Exon 10	Korean	c.1162delG	p.A388PfsX48	Frameshift	(13)
Santer *et al*, 2003	Exon 10	German	c.1152-63 del 12	Δ385-8	Deletion	(4)
Yu *et al*, 2016	Exon 11	Chinese	c.1319G>A	p.W440X	Nonsense	(19)
van den Heuvel *et al*, 2002; Santer *et al*, 2003; Yu *et al*, 2016	Exon 11	Chinese, Turkish	c.1320G>A	p.W440X	Nonsense	(3,4,19)
Zhao *et al*, 2016	Exon 11	Chinese	c.1343A>G	p.Q448R	Missense	(20)
Santer *et al*, 2003; Lee *et al*, 2012	Exon 11	Korean, NA	c.1346G>A	p.G449D	Missense	(4,12)
Lee *et al*, 2012	Exon 11	Korean	c.1357T>A	p.F453I	Missense	(12)
Santer *et al*, 2003; Calado *et al*, 2008	Exon 11	NA, Greek	c.1359C>A	p.F453L	Missense	(4,10)
Lee *et al*, 2012	Exon 11	Korean	c.1382G>A	p.S461N	Missense	(12)

Table III. Continued.

Author, year	Site	Ethnicity	Nucleotide change	Amino acid change	Mutation type	(Refs.)
Yu *et al*, 2011	Exon 11	Chinese	c.1388T>G	p.L463R	Missense	(11)
Yu *et al*, 2015	Exon 11	Chinese	c.1400T>C	p.V467A	Missense	(15)
Calado *et al*, 2008	Exon 11	Macedonian	c.1405G>A	p.A469T	Missense	(10)
Lee *et al*, 2012	Exon 11	Korean	c.1418_1432dup15	p.473_477dupLALFV	Duplication	(12)
Yu *et al*, 2015; Zhao *et al*, 2016	Exon 11	Chinese	c.1420G>C	p.A474P	Missense	(15,20)
Calado *et al*, 2008	Exon 11	German	c.1428C>G	p.F476L	Missense	(10)
Lee *et al*, 2012	Exon 11	Korean	c.1430T>G	p.V477G	Missense	(12)
Yu *et al*, 2011; Lee *et al*, 2012	Exon 11	Chinese, Korean	c.1435C>G	p.R479G	Missense	(11,12)
Calado *et al*, 2008	Exon 11	Swiss	c.1446G>C	p.E482D	Missense	(10)
Yu *et al*, 2011	Intron 11	Chinese	IVS11+1G>C	-	Splicing	(11)
Santer *et al*, 2003	Exon 12	NA	c.1461-517 del 57	p.W487, Δ488-506	Deletion	(4)
Lee *et al*, 2012	Exon 12	Korean	c.1475_1476insC	p.L493Pfs*74	Frameshift	(12)
Santer *et al*, 2003	Exon 12	NA	c.1495C>T	p.R499C	missense	(4)
Lee *et al*, 2012	Exon 12	Korean	c.1499T>G	p.L500R	Missense	(12)
Lee *et al*, 2012	Exon 12	Korean	c.1507G>A	p.E503K	Missense	(12)
Lee *et al*, 2012; Wang *et al*, 2017	Exon 12	Korean, Chinese	c.1540C>T	p.P514S	Missense	(12,22)[a]
Calado *et al*, 2008	Exon 12	Romanian	c.1616T>C	p.L539P	Missense	(10)
Santer *et al*, 2003	Exon 12	NA	c.1627A>C	p.T543P	Missense	(4)
Calado *et al*, 2006; Calado *et al*, 2008	Intron 12	Turkish, Macedonian	IVS12+1G>A	-	Splicing	(9,10)
Calado *et al*, 2008	Exon 13	Portuguese	c.1672C>T	p.R558C	Missense	(10)
Yu *et al*, 2015	Exon 13	Chinese	c.1691G>A	p.R564Q	Missense	(15)
Lee *et al*, 2012	Exon 13	Korean	c.1732C>T	p.Q578*	Nonsense	(12)
Zhao *et al*, 2016	Exon 13	Chinese	c.1739G>A	p.G580D	Missense	(20)
Lee *et al*, 2012	Exon 14	Korean	c.1798delC	p.Q600Rfs*18	Frameshift	(12)
Yu *et al*, 2016	Exon 14	Chinese	c.1891G>A	p.E631K	Missense	(18)
Lee *et al*, 2012	Exon 14	Korean	c.1894_1895ins6	p.[A634E; 634_635ins2]	Insertion	(12)
Santer *et al*, 2003	Exon 14	NA	c.1951-92 del 42	Δ651-64	Deletion	(4)
Calado *et al*, 2004; Kleta *et al*, 2004; Calado *et al*, 2008	Exon 14	Portuguese, Belgian, Swiss, Russian/Cuban/Spanish ancestry	c.1961A>G	p.N654S	Missense	(5,6,10)

[a]The present study. NA, not available; the patients were from Germany, Switzerland, England, Italy, former Yugoslavia, Turkey or Pakistan, but their countries of origin were not indicated clearly in the article. FRG, familial renal glucosuria.

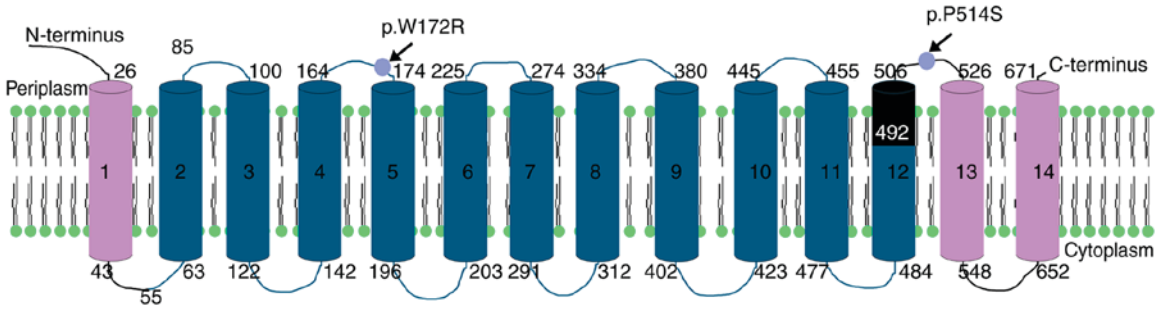

Figure 4. Schematic diagram of SGLT2. The SGLT2 protein is represented as a 14 transmembrane domain protein with extracellular amino and carboxyl termini. Transmembrane helices 1-14 are illustrated as cylinders. Arabic numerals represent the number of amino acid. The blue lines and cylinders indicate the sodium solute symporter domain. The sites of the mutations (p.W172R and p.P514S) identified in the present study are denoted by arrows. SGLT2, sodium-glucose cotransporter 2.

these two mutations were not detected in 100 chromosomes derived from 50 healthy and unrelated individuals, or in the three SNP databases retrieved for this study, indicating that this is not a common polymorphism. Moreover, the pathogenicity prediction based on three online algorithms demonstrated that the mutations p.W172R and p.P514S may be deleterious. A previous *in vitro* functional expression study of *SLC5A2* mutations demonstrated that six missense mutations (c.294C>A, c.736C>T, c.1051T>C, c.1400T>C, c.1420G>C and c.1691G>A) appeared to affect transport activity by reducing intrinsic transporter activity, impairing protein insertion into the cell membrane, suppressing protein synthesis and promoting protein removal or degradation (15). Therefore, it is thought that these two mutation sites may be of particular functional significance in the pathogenesis of FRG. Further *in vitro* research projects on kidney cells involving the construction of specific plasmids are required to confirm the pathogenic nature of these mutations.

According to a review of the literature, 86 mutations in the *SLC5A2* gene have been reported to be associated with FRG. Missense, frameshift and splicing mutations are the most common among these. It is likely that mutations of the *SLC5A2* gene may occur among different demographic groups. Among the 115 patients with FRG considered in the present study, there is no specific age at diagnosis that is most common, nor a significant sex difference. A majority of severe FRG cases exhibit mutation states that are homozygous or compound heterozygous, suggesting that the mode of inheritance may be explained as a co-dominant pattern with incomplete penetrance. It is noteworthy that three FRG patients had no mutations in the *SLC5A2* gene (4,9). In addition, not all individuals with similar or identical mutations have the same degree of increased glucose excretion, suggesting a role for non-genetic factors or other genes in glucose transport. Also, other SGLTs that are known to be expressed in the kidney and whose functions have not yet been clarified are candidate modified genes in cases of FRG (4,31). In the majority of affected individuals, the condition causes no apparent symptoms or serious effects, such as hypoglycemia, polyuria or dehydration. Therefore, patients with FRG have a good prognosis. Based on this, SGLT2 inhibitors are gradually becoming a promising antihyperglycemic medication with which to improve the prognosis of diabetic kidney disease while maintaining cardiovascular safety, according to a number of clinical trials (32). Thus, understanding the functional significance and pathogenic role of variants in the *SLC5A2* gene is essential.

However, there remain certain limitations to the present study. First, histological analysis of the kidneys in the patient was not performed to verify the expression of SGLT2. Second, since there was only one case included in the present study, it was difficult to acquire abundant information regarding the genotype-phenotype association. Third, further *in vitro* studies are required to confirm the pathogenic variants.

In conclusion, the present study identified a compound heterozygous mutation (p.W172R and p.P514S) of the *SLC5A2* gene in a Chinese patient with FRG. The mechanism whereby the p.W172R and p.P514S mutations impair SGLT2 function, in addition to the exact mechanism of abnormal glucose transport in FRG, requires further investigation.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

SL and ZY conceived and designed the experiments. SL conducted the experiments. YY, LH and MK were involved in conducting the experiments. SL collected the data and wrote the paper. ZY revised the manuscript. All authors read and approved the final paper.

Ethics approval and consent to participate

The study was approved by the Institutional Review Board of the Third Xiangya Hospital, Central South University

(Changsha, China), and written informed consent was obtained from all participants.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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