

Novel *SLC5A2* mutation contributes to familial renal glucosuria: Abnormal expression in renal tissues

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Abstract. Familial renal glucosuria (FRG) is characterized by persistent glucosuria in the presence of normal serum glucose concentrations, while other impairments of tubular function are absent. Mutations in the sodium-glucose co-transporter 2 (*SLC5A2*) gene have been found to be responsible for FRG. However, direct evidence for the presence of *SLC5A2* mutant in renal tissues is very rare. In previous studies, a non-sense mutation (c.1320 G>A;p.W440X) that would cause premature termination of the protein was found. However, the effects in the renal tissues were not reported. In the current study, a patient with FRG and a urinary glucose excretion rate of 8.3 g/day is described, for whom a novel missense mutation (c.1319G>A;p.W440X) was revealed by sequencing. Furthermore, in the immunofluorescence examination of a renal biopsy specimen, *SLC5A2* was detected in the apical side of the proximal convoluted tubule, discontinuously decreased in comparison with that in normal and disease controls. The results imply that both wild-type *SLC5A2* and mutant *SLC5A2* with abnormal distribution were expressed in the renal tissues, and that the reduction of *SLC5A2* expression and function were due to the c.1319G>A;p.W440X mutation. The current study provides valuable clues regarding the *SLC5A2* molecule from genotype to phenotype in families affected by FRG.

Introduction

Sodium-glucose co-transporter 2 (*SLC5A2*) belongs to the Na⁺-glucose cotransporter family and acts as a critical

molecule in the process of glucose re-absorption from urine in the proximal convoluted tubule (1). The *SLC5A2* gene is mapped to 16p11.2 (2) with a 7.7-kb nucleic acid, containing 14 exons, and encoding 672 amino acids. The *SLC5A2* protein is located in the early proximal convoluted tubule, segment S1, and has a Na⁺-to-glucose coupling ratio of 1:1 (3). Mutations in *SLC5A2* (OMIM: 182381) have been confirmed as being responsible for the vast majority of cases of familial renal glucosuria (4-6). However, an increased glucose excretion was not observed in all individuals heterozygous for a specific mutation, and even among family members with identical *SLC5A2* mutations, only some had mild glucosuria (4-6). In previous studies, a homozygous mutation (a G to A transition at position 1320) of p.W440X within exon 11 was found in two patients (4,7). The parents and relatives of the patients in those studies were found to be heterozygous for the mutation and exhibited almost no renal glucosuria. The present study is notable in that a patient with evident renal glucosuria (8.3 g/day) was found to have a heterozygous mutation of c.1319G>A;p.W440X. As direct evidence for *SLC5A2* heterozygous mutation of p.W440X in renal tissues has not yet been obtained, the present study reports the identification of a novel heterozygous mutation in *SLC5A2* and further investigated the effect of the mutant *SLC5A2* gene on protein expression in renal tissues.

Materials and methods

Patient and control individuals. The patient was a 36-year-old woman who had persistent glucosuria with normal serum glucose and no other evidence of renal disease. Glucosuria was quantified by 24 h urine collection. A total of 100 healthy Chinese individuals (200 chromosomes) were included as controls. In the immunofluorescence analysis of renal biopsy specimens, normal renal tissue from tumor patients following partial nephrectomy and biopsy tissue from patients with minimal change disease and diabetic nephropathy were included as normal and disease controls, respectively.

The study protocol was approved by the Medical Ethics Committee of Inner Mongolia People's Hospital (Hohhot, China). Informed written consent was obtained from all participants prior to participation in the study.

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Abbreviations: *SLC5A2*, sodium-glucose co-transporter 2; FRG, familial renal glucosuria

Key words: expression, familial renal glucosuria, function, mutation, *SLC5A2*

Mutation screening. Genomic DNA was extracted by salting out from peripheral white blood cells. The entire coding region and adjacent intronic segments of the *SLC5A2* gene were screened for mutation by direct sequencing of polymerase chain reaction (PCR) products, generated with primers described previously (8). The genomic DNA reference sequences of *SLC5A2* (NC_000016.10, OMIM: 182381, Gene ID: 6524) were obtained from the Entrez gene database. The 200 control chromosomes were tested by direct sequencing of PCR products to ensure that these mutations do not represent common polymorphisms.

Immunofluorescence studies on renal biopsy specimen. Renal specimens were evaluated by routine methods using indirect immunofluorescence, and light and electron microscopy. *SLC5A2* expression in the renal biopsy specimens was detected by immunofluorescence staining as described in our previous study (9). In brief, renal biopsy specimens were incubated overnight at 4°C with a goat anti-human polyclonal antibody targeting *SLC5A2* (1:50; sc-47404; Santa Cruz Biotechnology, Inc., Dallas, TX, USA), and then a fluorescein isothiocyanate-labeled donkey anti-goat IgG antibody (1:200; sc-2024; Santa Cruz Biotechnology, Inc.) for 30 min at 37°C. Normal renal tissue from tumor patients following partial nephrectomy was included as a normal control. Biopsy tissue from patients with minimal change disease and diabetic nephropathy was included as a disease control. Specific binding was defined by replacing primary antibody with 1% bovine serum albumin (Sigma-Aldrich, St. Louis, MO, USA). Sections were observed using a confocal microscope (Olympus FV1000; Olympus Corporation, Tokyo, Japan).

Results

Clinical characterization of the patient. The patient was a 36-year-old woman who was referred to the renal division because of repeated glucosuria. The patient had no polyuria, polydipsia or weight loss. A review of systems and the physical examination were completely normal. The patient's blood pressure was 110/80 mmHg and body weight was 50 kg. Fasting plasma glucose (4.84 mmol/l), albumin (46.7 g/l), creatinine (71.00 μ mol/l), sodium (142.00 mmol/l), chloride (106.0 mmol/l), potassium (4.40 mmol/l), calcium (2.34 mmol/l), phosphate (0.96 mmol/l), magnesium (0.82 mmol/l), bicarbonate (26.30 mmol/l), uric acid (225 μ mol/l) and glycated hemoglobin (5.7%) levels were all within the normal range. Routine urinary analysis revealed 2⁺ to 4⁺ results for glucose with no other abnormalities. A quantitative test for urine glucose provided a result of 8.3 g/24 h. Urinary glucose excretion in family members was confirmed by qualitative test. The father and a uncle of patient had increased urinary glucose excretions of 1-2⁺. No other marked abnormality was detected in the urine and blood biochemistry of family members (Fig. 1A). Secondly, the patient was characterized by persistent glucosuria despite normal serum glucose and the absence of overt tubular dysfunction. Routine analysis of renal specimens using indirect immunofluorescence, and light and electron microscopy revealed no apparent glomerular or tubulointerstitial lesions. Furthermore, a novel mutation was identified in the patient. From above all, the patient could be diagnosis of familial renal glucosuria (FRG) securely.

Identification of a novel mutation in the patient. Using mutation screening of genomic DNA, a novel missense mutation was identified in the patient (c.1319G>A: p.W440X, Fig. 1). This identified mutation was not detected in any of the 200 chromosomes derived from 100 healthy, unrelated individuals, indicating that the mutation does not represent a common polymorphism.

Identified mutation alters *SLC5A2* expression in the kidney. Renal biopsy in the patient with familial renal glucosuria (FRG) revealed no apparent glomerular or tubulointerstitial lesions when examined by immunofluorescence, light and electron microscopy. However, the expression of *SLC5A2* in the apical side of the proximal convoluted tubule was discontinuously decreased in comparison with that in both normal and disease controls (patients with minimal change disease or diabetic nephropathy; Fig. 2).

Discussion

Glucose is the fuel that provides energy for normal activity in humans, and the major source of glucose is carbohydrates in food. The kidneys help to keep blood glucose levels normal by reabsorbing ~180 g glucose per day through filtration in the proximal tubules (1). *SLC5A2* 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 (1). Studies report that *SLC5A2* mutations are involved in FRG (9-12). The long-term outcome of patients with FRG is very good, and so *SLC5A2* inhibitors have been the subject of particular attention in the search for potential new drug targets for the treatment of diabetes (13,14). However, safety issues have hindered the development of *SLC5A2* inhibitors (15). Research on patients with FRG may bring about a breakthrough in the study of the treatment of diabetes. In previous studies, although a homozygous mutation (G to A transition at position 1320) within exon 11 was found in two patients, the effect of the mutation, especially the heterozygous mutation of p.W440X, on the expression of *SLC5A2* in the kidney is not yet fully known (4,7). In the present study, to exclude tubular impairments or other renal diseases that could cause glucosuria, the patient agreed to undergo a renal biopsy. This revealed no apparent glomerular or tubulointerstitial lesions under immunofluorescence, light and electron microscopy.

A novel *SLC5A2* mutation of c.1319G>A:p.W440X was found in the present patient with FRG. The data reveal that a patient with a heterozygous mutation had clinical manifestations (urinary glucose excretion rate, 8.3 g/day), which implies that the mutation causes clinically relevant *SLC5A2* dysfunction. In the immunofluorescence examination of *SLC5A2* in the renal biopsy specimen, *SLC5A2* was detected in the apical side of the proximal convoluted tubule, discontinuously decreased in comparison with both normal and disease controls. Therefore, the wild-type *SLC5A2* exhibits normal expression and the mutation appears to decrease the expression of *SLC5A2* in the apical side of the proximal convoluted tubule. Therefore, the mutation may affect transport activity by altering protein processing and impairing protein insertion into the plasma membrane. Furthermore, it implies that

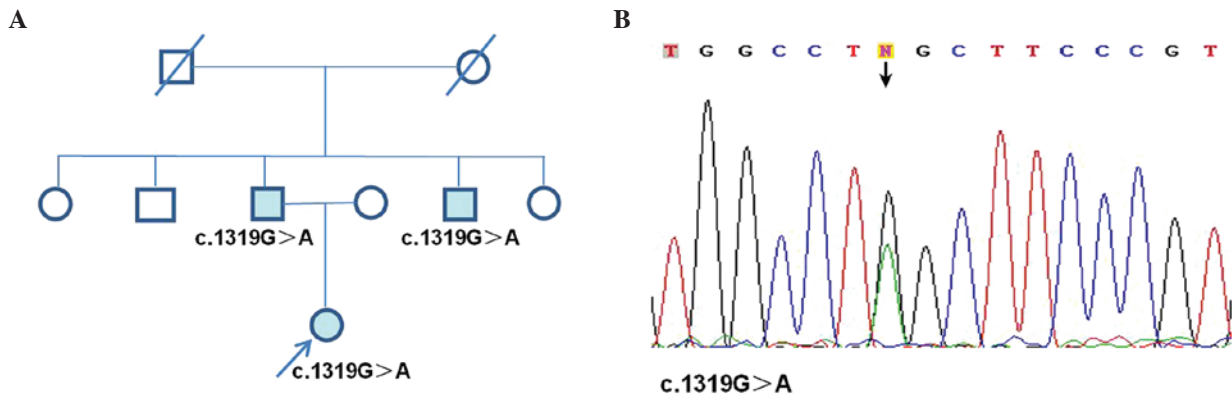


Figure 1. Familial renal glucosuria patient carried a novel mutation (c.1319G>A;p.W440X). Nucleotides are numbered according to sodium–glucose o-transporter 2 (SLC5A2) cDNA sequence. (A) Familial renal glucosuria pedigree carries the novel mutation. Circles: Females; squares: males; blue coloring: With increased urinary glucose excretion; strike through: death. (B) Mutation screening of genomic DNA and a novel missense mutation was identified in the patient.

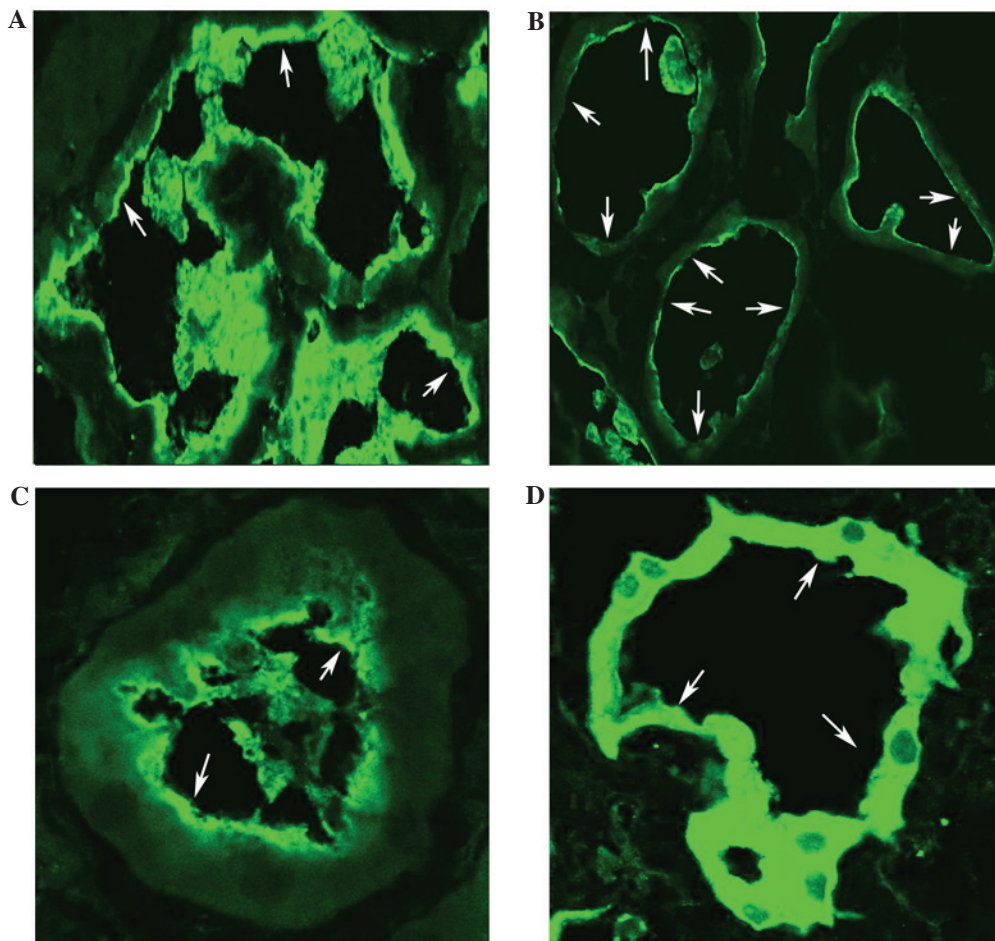


Figure 2. Immunofluorescence of SLC5A2 in kidney specimens. sodium-glucose co-transporter 2 (SLC5A2 in (A) normal kidney, (B) the patient with familial renal glucosuria (FRG), (C) a patient with minimal change disease and (D) a patient with diabetic nephropathy (magnification, x300). SLC5A2 was distributed continuously and mainly in the apical side of the proximal convoluted tubule in normal and disease controls. In the FRG patient, SLC5A2 in the apical side of the proximal convoluted tubule was discontinuously decreased in comparison with both normal and disease controls.

abnormal distribution of mutant SLC5A2 might play a key role in affecting transport activity. From the results, the inheritance of renal glucosuria can be described as a codominant trait as a whole, and it may be inferred that variable penetrance may be associated with the compensatory capacity of wild-type SLC5A2. To the best of our knowledge, the altered SLC5A2

expression caused by heterozygous mutation of p.W440X has not been reported previously in human subjects. This observation may shed light on the function of SLC5A2; however, further analysis is required.

In summary, a novel SLC5A2 mutation was identified in a Chinese patient with FRG, and SLC5A2 was observed to be

discontinuously decreased in the apical side of the proximal convoluted tubule in the patient on renal biopsy. The mutant SLC5A2 proteins may have significantly lowered SLC5A2 expression in the apical side of the proximal convoluted tubule. This study provides valuable information concerning the role of the SLC5A2 molecule from genotype to phenotype.

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