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ORIGINAL INVESTIGATION

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Autosomal recessive renal glucosuria attributable to a mutation in the sodium glucose cotransporter (SGLT2)

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Abstract Patients with primary renal glucosuria have normal blood glucose levels, normal oral glucose tolerance test results, and isolated persistant glucosuria. Congenital renal glucosuria is postulated to be attributable to defects in the *SGLT2* gene. The Na^+/glucose cotransporter gene *SGLT2* (=*SLC5A2*) was analyzed in a Turkish patient with congenital isolated renal glucosuria. Genomic DNA was used as a template for amplification by the polymerase chain reaction of each of the 14 exons of the *SGLT2* gene. The amplification products were sequenced. DNA sequence analysis revealed a homozygous nonsense mutation in exon 11 of the *SGLT2* gene leading to the formation of a truncated cotransporter. Both parents and a younger brother, all three without renal glucosuria, are heterozygous for the nonsense mutation. Our data provide the first direct evidence of an etiologic role for the sodium/glucose cotransporter type 2 in the pathogenesis of renal glucosuria.

Introduction

Glucose transport is of fundamental importance in energy metabolism. The maintenance of a relatively constant blood glucose concentration to sustain cerebral metabolism and the delivery of glucose to peripheral tissues for storage and utilization are key metabolic processes and, in many situations, the transport of glucose across cell membranes plays a crucial role in their regulation and control. Cell membranes are effectively impermeable to glucose, and so movement of glucose into and out of cells must be mediated by protein transporters. This mediated glucose transport can be divided into two forms, viz., secondary active transport and facilitated transport, each involving a different class of glucose transporter. The secondary active transport mechanism is responsible for the uptake of glucose into the intestinal mucosa and the proximal tubules of the kidney (Brown 2000).

In the kidney, glucose is reabsorbed from the urinary filtrate by the action of several types of glucose transporter arranged in series along the proximal tubule. It is generally accepted that two Na^+ glucose cotransporters (SGLT) exist in the apical membrane of the convoluted tubule (Brown 2000; Wright 2001). SGLT2, a low-affinity high-capacity transporter localized in the early proximal tubule as shown by in situ hybridization, is responsible for reabsorption of the bulk of the filtered glucose (Kanai et al. 1994; You et al. 1995). SGLT1 is located in the late proximal tubule, where it reabsorbs the remainder of the filtered glucose not reabsorbed in the early proximal tubule (Lee et al. 1994). In the early proximal tubule, glucose exits the cell via a high-capacity facilitative glucose transporter, GLUT2 (Dominguez et al. 1994). GLUT1 is expressed along the length of the proximal tubule (Dominguez et al. 1994; Lee et al. 1994). SGLT and GLUT transporters function together in polarized epithelial cells to mediate the transepithelial transport of glucose. SGLT transporters in the apical membrane catalyze active glucose uptake driven by a Na^+ gradient, and glucose diffuses passively out of the cell via basolateral GLUT facilitative transporters.

SGLT1 is present in both the intestine and the renal proximal tubule (mainly in the pars recta). A defect in SGLT1 results in glucose-galactose malabsorption (Kasahara et al. 2001). A genetic defect in GLUT2 is responsible for Fanconi-Bickel syndrome (Santer et al. 1998). SGLT2 is only expressed in the first part of the of the renal proximal tubule (Wells et al. 1992; You et al. 1995). As the major portion of the filtered glucose is reabsorbed in the early proximal tubule by SGLT2, a low-affinity high-capacity transporter, a defect in this transporter would

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be an excellent explanation for hereditary isolated renal glucosuria. In this report, a family is presented, for the first time, with a mutation in the *SGLT2* gene being responsible for autosomal recessive isolated renal glucosuria.

Materials and methods

Nomenclature

Gene mutation nomenclature used in this article follows the recommendations of den Dunnen and Antonarakis (2001). Gene symbols used in this article follow the recommendations of the HUGO Gene Nomenclature Committee (Povey et al. 2001).

Table 1 Relevant renal tubular function in the index patient

Function	Value
Aminoaciduria	Absent
Metabolic acidosis	Absent
Ratio alpha-1-microglobulin/ creatinine (mg/g)	60 (normal)[a]
Phosphate reabsorption	86% (normal 86%–98%)[b]
Glucosuria	342 mmol/l or 61.6 g/l accompanied by normal blood glucose

[a]Control value: median 55 (97.5 percentile: 149; Lehrnbecher et al. 1998)
[b]Control value from Kruse et al. (1982)

Case report

The proband is the first child of consanguineous Turkish parents. The parents are cousins. At the age of 2 years, this boy was referred because of developmental delay and movement disorder. He had mild mental retardation. As clinical manifestations, cerebellar ataxia, non-epileptic myoclonic jerks of the neck and limbs, and mild kyphoscoliosis were observed. His clinical manifestations remained stable. Both parents and his younger brother are healthy. No neurologic abnormalities are known in other family members. Extensive laboratory, radiologic, and neurophysiologic investigations were performed. Laboratory data revealed no abnormalities with the exception of glucosuria (routine chemistry, amino acids, acylcarnitines, very long chain fatty acids in serum/plasma, and lysosomal enzymes in leucocytes and organic acids, amino acids, purines and pyrimidines, and other sugars including mono- and oligosacharides and polyols in urine were all normal). Cells, protein, lactate, and pyruvate were normal in the cerebrospinal fluid (CSF). Glucose was measured in CSF and blood at the same time on two occasions with a normal result: CSF glucose 3.0 and 3.3 mmol/l with a ratio CSF/blood of 0.80 and 0.53, respectively. Cerebral magnetic resonance imaging showed normal anatomical structures of the brain and normal myelination. Electroencephalography, nerve conduction velocity, and electromyography were normal. Additional laboratory studies were performed in both parents. Serum sodium, potassium, chloride, bicarbonate, calcium, phosphate, magnesium, uric acid, and blood glucose were normal. Both parents and the younger brother had no glucosuria. The relevant renal tubular functions of the proband are presented in Table 1.

Mutation analysis

Genomic DNA was extracted by a salting out procedure from peripheral blood lymphocytes (Miller et al. 1988) and used for polymerase chain reaction (PCR) amplification of individual exons of the *SGLT2* (=*SLC5A2*) gene. Thirteen pairs of oligonucleotide

Table 2 SGLT2 primer sequences used for mutation detection at the genomic DNA level. The exons are situated in the middle of the fragments. The size of the fragments and exons is given in base pairs

Exon	Forward/ Reverse	Sequence 5′–3′	Size of fragment	Size of exon
1	Forward	aaa tct ggg ctg ggt agg	734	126
1	Reverse	ttc tcc cac cta ggg tcc		
2+3	Forward	cgt taa tct tca gcc aga aac a	408	72+105
2+3	Reverse	ccc ttc cag gtg ttc tca ct		
4	Forward	gcc act tgc ttg gag tag c	349	165
4	Reverse	ttc tcc tag gtc tca cgc c		
5	Forward	ggg aag ctt tga ggc tag tag	280	106
5	Reverse	ctc tgg cag ctc cct aaa c		
6	Forward	ttc aaa ttc cca caa aga cgc	265	81
6	Reverse	tag gac cct cag acc gga ga		
7	Forward	aca cgg tac aga cct tcg tca	519	230
7	Reverse	ctc aga ctg cgc ctt aga cc		
8	Forward	gac aga agg ctc cat cta ctc caa	229	136
8	Reverse	atg ggc gtc agc ttc agg ta		
9	Forward	cgc ctc ctc tgc tag gat t	356	108
9	Reverse	atg acc gcc agc atg agt		
10	Forward	tca tgc cca acg gta agg	421	151
10	Reverse	cga tga aca cca ccc aga g		
11	Forward	ctt cac cat gga cat cta cac g	416	169
11	Reverse	cga agg aaa cag gtc agc c		
12	Forward	cgt taa tga gca ggt gag cg	366	216
12	Reverse	tcc agg aag cag agg gta gg		
13	Forward	gac gag ctg gtg tgc aag a	280	121
13	Reverse	aaa ttg agg ccc tgc agt tag		
14	Forward	aga gtg cca tgg aga tga atg	633	224
14	Reverse	tct tgt gag cag ccc aga c		

primers (Table 2) were generated to amplify all 14 exons according to the data obtained from Genbank (accession no. AF307340). Designed primers flanking the coding regions of exons of the *SGLT2* gene were used for PCR amplification, and the amplified exons were subjected to standard dye-terminator sequencing on an ABI 377 (PE Applied Biosystems, Foster City, Calif.). The complete *SGLT2* coding region in the index patient was sequenced. The results were then visually inspected and aligned to the sequence of the wild-type gene. The segregation of the mutation in the members of the family of the index patient has also been studied by DNA sequencing.

Results

Analysis of the *SGLT2* genomic DNA sequence of the index patient identified a homozygous mutation (a G to A transition at position 1320) within exon 11 (Fig. 1). Both parents and an unaffected brother of the patient were found to be heterozygous for the mutation. A repeated PCR and DNA sequence analysis confirmed this mutation in exon 11. The mutation (G to A transition at position 1320) was not identified in 100 control chromosomes.

The identified mutation is a nonsense mutation (TGG→TGA; W440X) that would cause premature termination of translation. This would result in a truncated SGLT2 protein lacking 232 amino acids of the C-terminal part of the protein.

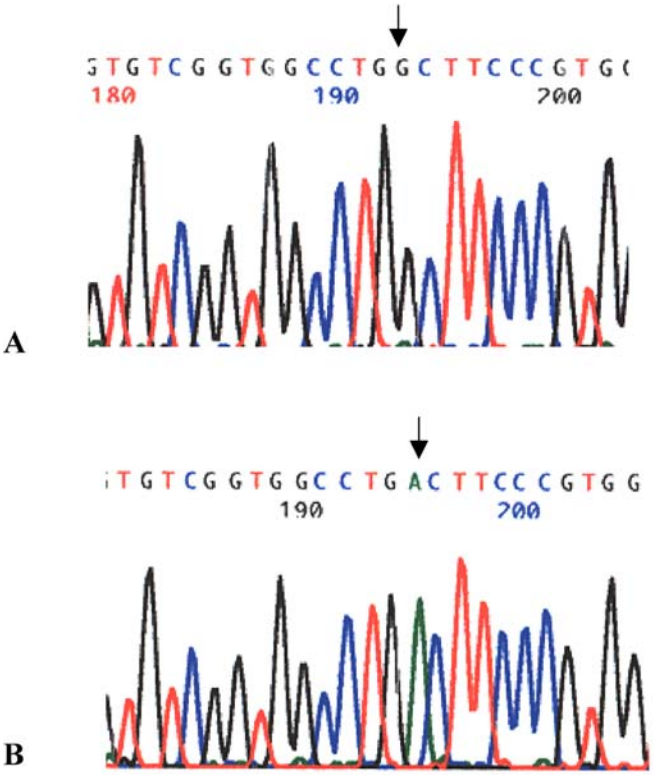

Fig. 1A, B Genomic DNA sequence of the region of the coding strand of exon 11 in the *SGLT2* (=*SLC5A2*) gene in which the mutation is found (*arrow* position of mutation). **A** Wild-type sequence. **B** Sequence of the index patient. The mutation (G1320A) in the patient results in a premature stop codon at amino acid 440

Discussion

This patient has an isolated form of renal glucosuria as demonstrated by normal values for amino acids, organic acids, low molecular proteins, and phosphate in the urine, without a metabolic acidosis. Overexcretion of arginine, carnosine, and taurine, as reported in some patients with renal glucosuria, is absent (Sankarasubbaiyan et al. 2001).

We have no explanation for the neurologic symptoms of our patient. A defect in the transport of glucose into the brain could be excluded on the basis of a normal glucose concentration in the CSF (Duelli and Kuschinsky 2001). Glucose is transported into the brain by facilitated diffusion. Two types of glucose transporters (GLUT1 and GLUT3) are localized in the membranes of brain endothelial cells, viz., astrocytes and neurons, and these are distinct from SGLT2. In patients with isolated renal glucosuria, normal brain function can be expected.

In this family, autosomal recessive inheritance is present. Renal glucosuria can also be inherited as an autosomal dominant trait. A heterozygous mutation in HNF1alpha (hepatocyte nuclear factor) can induce renal glucosuria preceding the phase of diabetes mellitus. In mice, a decrease in HNF1alpha activity results in a reduction of SGLT2 expression in the proximal tubule (Pontoglio et al. 2000). The mutation in exon 11 in our patient predicts the formation of a truncated protein. The structure and function of SGLT1 has been defined by Wright (2001): Na^+ binding to the NH_2 terminal domain causes conformational changes in the protein to permit sugar binding and the transmembrane helices 10–13 form the translocation pathway. The truncated protein in our patient lacks part of transmembrane helix 10 and transmembrane helices 11–14 and, hence, would be incapable of glucose transport. SGLTs other than the type 2 could be responsible for other forms of renal glucosuria (Diez-Sampedro et al. 2001). Wright (2001) was therefore correct when he anticipated that the gene coding for renal brush-border Na^+ glucose cotransporter would soon be positively identified.

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