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Molecular Analysis of the *SGLT2* Gene in Patients with Renal Glucosuria

RENÉ SANTER,*† MARTINA KINNER,*† CHRISTOPH L. LASSEN,* REINHARD SCHNEPPENHEIM,‡ PAUL EGGERT,* MARTIN BALD,§ JOHANNES BRODEHL,‖ MARKUS DASCHNER,¶ JOCHEN H.H. EHRICH,‖ MARKUS KEMPER,†# SALVATORE LI VOLTI,** THOMAS NEUHAUS,# FLEMMING SKOVBY,†† PETER G.F. SWIFT,‡‡ JÜRGEN SCHAUB,* and DAN KLAERKE§§

**Department of Pediatrics, University of Kiel, Kiel, Germany; †Department of Pediatrics, University of Hamburg, Hamburg, Germany; ‡Department of Pediatric Hematology and Oncology, University of Hamburg, Hamburg, Germany; §Department of Pediatrics, University of Essen, Essen, Germany; ‖Department of Pediatrics, Medical School Hannover, Hanover, Germany; ¶Department of Pediatrics, University of Heidelberg, Heidelberg, Germany; #Department of Pediatrics, University of Zurich, Zurich, Switzerland; **Department of Pediatrics, University of Catania, Catania, Italy; ††Department of Clinical Genetics, University Hospital of Copenhagen, Copenhagen, Denmark; ‡‡Department of Pediatrics, University of Leicester, Leicester, United Kingdom; and §§The Panum Institute, University of Copenhagen, Copenhagen, Denmark*

Abstract. The role of *SGLT2* (the gene for a renal sodium-dependent glucose transporter) in renal glucosuria was evaluated. Therefore, its genomic sequence and its intron-exon organization were determined, and 23 families with index cases were analyzed for mutations. In 21 families, 21 different *SGLT2* mutations were detected. Most of them were private; only a splice mutation was found in 5 families of different ethnic backgrounds, and a 12-bp deletion was found in two German families. Fourteen individuals (including the original patient with 'renal glucosuria type 0') were homozygous or compound heterozygous for an *SGLT2* mutation resulting in glucosuria in the range of 14.6 to 202 g/1.73 m^2/d (81 - 1120 mmol/1.73 m^2/d). Some, but not all, of their heterozygous family members had an increased glucose excretion of up to 4.4 g/1.73 m^2/d (24 mmol/1.73 m^2/d). Likewise, in index cases with glucosuria below 10 g/1.73 m^2/d (55 mmol/1.73 m^2/d) an *SGLT2* mutation, if present, was always detected in the heterozygous state. We conclude that SGLT2 plays an important role in renal tubular glucose reabsorption. Inheritance of renal glucosuria shows characteristics of a codominant trait with variable penetrance.

The molecular bases of three congenital defects of cellular glucose transport, affecting either facilitative ("passive") or sodium-dependent ("active") transport, have been elucidated in recent years (1–3). In addition, a defect of the renal low-affinity sodium/glucose cotransporter *SGLT2* gene (also referred to as *SLC5A2*, [OMIM 182381]) has long been proposed to cause renal glucosuria (OMIM 233100) (4).

Renal glucosuria is a "nondisease"; the great majority of affected individuals do not have any complaints, and only very rarely a propensity to hypovolemia and hypoglycemia has been described. Renal glucosuria is defined by urinary glucose excretion in the presence of a normal blood glucose concentration and the absence of any signs of a general renal tubular dysfunction. Renal glucosuria may vary from few grams to >100 g (556 mmol) per day. Mild glucosuria has been known for decades; it is relatively common and often inherited as an autosomal dominant trait (5). Severe glucosuria is rare, and the first patient with virtual absence of tubular glucose reabsorption, termed renal glucosuria type 0, was not reported until 1987. The pedigree of this patient's family suggested autosomal recessive inheritance (6).

The purpose of this study was to define the molecular basis of renal glucosuria. Therefore, we first characterized the genomic structure of the human *SGLT2* gene, which we reported to GenBank in 2000 (accession no. AF307340). We then performed mutation analysis in patients with isolated, massive glucosuria. It turned out that homozygosity or compound heterozygosity for *SGLT2* mutations may account for this condition, which was reported in abstract form in the same year (7). On the basis of these reports, a single patient with

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Correspondence to Dr. René Santer, Department of Pediatrics, University Children's Hospital, Martini Strasse 52, D-20246 Hamburg, Germany. Phone: +49-40-42803-3710; Fax: +49-40-42803-5107; E-mail: r.santer@uke.uni-hamburg.de
M.B.'s current affiliation is Olgahospital, Stuttgart, Germany.
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glucosuria and homozygosity for an *SGLT2* mutation has meanwhile been published (8). Here, we summarize our results of the characterization of the *SGLT2* gene and present our data on 23 consecutive families with index patients with renal glucosuria, including the original patient with renal glucosuria type 0.

Materials and Methods

Subjects

Ninety-three individuals from 23 unrelated families with at least one index patient with renal glucosuria were investigated. In general, urinary glucose concentration had been repeatedly determined by dipstick before it was quantified by the glucose oxidase method in aliquots of 24-h urine collected on a free diet. It ranged from a minimal elevation of <1 g/1.73 m^2 per d (5.5 mmol/1.73 m^2 per d) to a maximum of 202 g/1.73 m^2 per d (1120 mmol/1.73 m^2 per d; Table 1; normal range, 0.13 to 0.32 g/1.73 m^2 per d [0.72 to 1.8 mmol/1.73 m^2 per d] (9)). None of these subjects had any signs of a generalized tubular dysfunction or of any other type of renal disease. The families originated from Germany, Switzerland, England, Italy, former Yugoslavia, Turkey, and Pakistan. Patient 01-1 was originally described by Oemar *et al.* (6) in 1987 in the first report of an individual with virtually absent renal glucose reabsorption (renal glucosuria type 0). This individual, now 32 yr old, has had entirely normal renal function parameters so far; a detailed follow-up report of this case is currently in preparation.

Molecular Genetic Studies

High molecular genomic DNA was prepared from leukocytes from individuals with renal glucosuria and their parents and siblings and control subjects according to standard protocols (10). Determination of the genomic sequence of human *SGLT2* was accomplished by primer walking starting from a previously reported human *SGLT2* cDNA (11) with the assumption of similar intron-exon boundaries when compared with the genomic structure of *SGLT1*, the homologous glucose transporter of intestinal cells (12). The 5'-untranslated region of *SGLT2* was determined by a two-step PCR technique using random primers according to the manufacturer's instructions (Dynal A.S, Nordic, Oslo, Norway). The primer pair (with the annealing temperature given in parentheses) used for the first step was *sn* 5'-CAGTTCAAGCTTGTCCAGGAATTCNNNNNNNGGCCT-3' and *asn* (biotin)-5'-GGTTTCTCCTTCAAAGATCCAA-3' (62°C); the one for the second step was *sn* (biotin)-5'-CAGTTCAAGCTTGTCCAGGAATTC-3' and *asn* 5'- CCAGCAGCCACCAGCAGCACA -3' (62°C).

For mutation analysis in renal glucosuria families, the entire coding region and adjacent intronic segments were sequenced in as many family members as possible. PCR products including exons 1 to 14 of *SGLT2* with flanking intron sequences were generated on the basis of the newly determined intronic sequences. Primer pairs used in this study are given in Table 2. PCR was carried out in a T3 Thermocycler (Biometra, Göttingen, Germany) with reagents and *Taq* polymerase (Invitrogen, Karlsruhe, Germany). Double-stranded PCR products were analyzed by nondenaturing PAGE combined with silver and ethidium bromide staining, respectively. Purified samples were then analyzed by direct cycle sequencing of double-stranded DNA according to the protocol for ^{33}P-labeled terminators provided with the Thermo Sequenase cycle sequencing kit (Amersham Buchler, Freiburg, Germany). When necessary, sequencing was performed after cloning the respective PCR product into a phagemid vector according to the PCR Script Amp Cloning kit (Stratagene, Heidelberg, Germany). All detected mutations were confirmed by restriction enzyme digest (in some cases, after the use of a mismatch primer) and/or detection of heteroduplex formation on a polyacrylamide gel. Nucleotides of the human *SGLT2* cDNA are numbered from 1 to 2019 for the first bp of the ATG initiation codon and the last bp of the TAA stop codon; amino acids are numbered from 1 to 672 for methionine and alanine, respectively.

Results

Genomic Organization of the Human SGLT2 Gene

As a first result, our studies confirmed that the human *SGLT2* coding sequence consists of 2019 bp predicting a 672–amino acid protein. This human renal glucose carrier shows 58% amino acid identity on Clustal W multiple sequence alignment (clustalw.genome.ad.jp) with the SGLT1 protein and the degree of homology is even higher in central domains of the polypeptide. Analysis of the genomic structure of human *SGLT2* revealed an 8.0-kb gene with an intron-exon organization that also shows high similarity to *SGLT1* (Figure 1). The coding sequence of *SGLT2*, however, is interrupted by only 13 introns that, with one exception, are located at homologous positions in *SGLT1*. Intron sizes in general are remarkably smaller in *SGLT2* and at the position of intron 4 of *SGLT1*, an intron is absent in *SGLT2*. All 13 introns of *SGLT2* show the consensus sequence *ag...gt*. A highly polymorphic marker that has not previously been described in the literature with a variable number of CA repeats is present in intron 1 of *SGLT2*.

Mutation Analysis in Patients with Renal Glucosuria

The degree of urinary glucose excretion and the results of *SGLT2* mutation analysis for individual family members who participated in this study are presented in Table 1. With the exception of two families (14 and 19), *SGLT2* mutations were detected in all families investigated. The 14 individuals who were found to be carriers of two mutated alleles (boldface in Table 1) showed "severe" glucosuria, here defined by a cutoff of urinary glucose of >10 g/1.73 m^2 per d (55 mmol/1.73 m^2 per d). Among them is the patient in whom glucosuria type 0 was originally described. He was found to be homozygous for a 5-bp deletion within exon 8 of the *SGLT2* gene (Figure 2).

As expected, family members of these cases of severe glucosuria were shown to be heterozygous carriers of *SGLT2* mutations. Investigations for glucose excretion showed that some but not all of them had glucosuria in a range of up to 4.4 g/1.73 m^2 per d (24 mmol/1.73 m^2 per d). Likewise, index patients who presented with "mild" renal glucosuria (here defined by a glucose excretion <10 g/1.73 m^2 per d [55 mmol/1.73 m^2 per d]), all were heterozygous for an *SGLT2* mutation, if detected.

The study detected a total of 21 different *SGLT2* mutations, which are summarized in Table 3. Nonsense mutations, missense mutations, and small deletions were scattered over the *SGLT2* coding sequence. Most of them were confined to a single pedigree. Only the Δ385–8 mutation was found in two unrelated German patients, and five individuals who originated from Pakistan, former Yugoslavia, Italy, and Switzerland carried the same intron 7 donor splice site mutation.

Table 1. Results of glucose excretion studies and mutation analysis in patients with isolated glucosuria and their family members. Index patients with severe glucosuria and *SGLT2* mutations on both chromosomes are indicated by boldface[a]

Family-member (initials)	Glucose excretion (g/1.73 m^2 per d; normal 0.13–0.32[b])	Allele 1	Allele 2
01–1 index case (MP)	**126–162.2**	**P 324 fs. . .347 X**	**P 324 fs. . .347 X**
- 2 father[c]	1.1	P 324 fs. . .347 X	wt
- 3 mother[c]	2.7	wt	P 324 fs. . .347 X
- 4 sister	0.40	n.d.	n.d.
- 5 brother	0.31	n.d.	n.d.
- 6 sister	0.05	n.d.	n.d.
02–1 index case (BMW)	**73.6**	**G 272 R**	**G 272 R**
- 2 father[c]	0.8	G 272 R	wt
- 3 mother[c]	0	wt	G 272 R
- 4 brother	0.11	G 272 R	wt
03–1 index case (AH)	**50.6–51.3**	**IVS 7 + 5 g > a**	**IVS 7 + 5 g > a**
- 2 father[c]	0.15	IVS 7 + 5 g > a	wt
- 3 mother[c]	0.02	wt	IVS 7 + 5 g > a
- 4 sibling	0.03	IVS 7 + 5 g > a	wt
- 5 sibling	0	IVS 7 + 5 g > a	wt
- 6 sibling	0.09	IVS 7 + 5 g > a	wt
04–1 index case[d] (PC)	**21.3**	**IVS 7 + 5 g > a**	**L 307 P**
- 2 index case[d] (IC)	**28.5**	**IVS 7 + 5 g > a**	**L 307 P**
- 3 father	Ø	wt	L 307 P
- 4 mother	Ø	IVS 7 + 5 g > a	wt
05–1 index case (AK)	**43.0**	**G 449 D**	**G 449 D**
- 2 mother[c]	0.24	G 449 D	wt
- 3 sibling	0.07	wt	G 449 D
06–1 index case (OY)	**68.7**	**W 440 X**	**W 440 X**
- 2 father[c]	0.21	W 440 X	wt
- 3 mother[c]	0.12	wt	W 440 X
- 4 sibling	0.19	W 440 X	wt
- 5 sibling	0.61	W 440 X	wt
- 6 sibling	0.27	W 440 X	wt
07–1 index case (KN)	**20.8**	**W 487 C, Δ488–506**	**?**
- 2 father	0.38	W 487 C, Δ488–506	wt
- 3 mother	0.29	wt	?
08–1 index case (NB)	0.6	Δ651–64	wt
- 2 father	10.4[e]	Δ651–64	wt
- 3 mother	0.03	wt	wt
- 4 sibling	0.04	wt	wt
09–1 index case (CE)	**38.8**	**R 368 W**	**R 368 W**
- 2 father[c]	0.99	R 368 W	wt
- 3 mother[c]	0.15	wt	R 368 W
- 4 sibling	0	wt	wt

Discussion

The studies reported here led to the description of *SGLT2* mutations in patients with isolated renal glucosuria (7). As a first step, we characterized the human *SGLT2* gene at the genomic level. We assumed that *SGLT2*, which has previously been reported to be located in the chromosomal region 16

Table 1. Continued

Family-member (initials)	Glucose excretion (g/1.73 m^2 per d; normal 0.13–0.32[b])	Allele 1	Allele 2
10–1 index case (SG)	2.3–4.5	Q 168 fs. . .186 X	wt
- 2 father	0.17	Q 168 fs. . .186 X	wt
- 3 mother	n.d.	wt	wt
- 4 sibling	n.d.	wt	wt
- 5 grandfather (p)	12.3[e]	Q 168 fs. . .186 X	wt
- 6 grandmother (p)	n.d.	wt	wt
11–1 index case (FdS)	0.75	IVS 7 + 5 g > a	wt
- 2 father	Ø	wt	wt
- 3 mother	0.4	IVS 7 + 5 g > a	wt
- 4 grandmother (m)	Ø	IVS 7 + 5 g > a	wt
12–1 index case (CS)	**14.6**	**IVS 7 + 5 g > a**	**K 311 R**
- 2 mother	3.7	IVS 7 + 5 g > a	wt
- 3 sibling	4.4	IVS 7 + 5 g > a	wt
- 4 sister of grandfather (m)	1.7	IVS 7 + 5 g > a	wt
13–1 index case (CP)	5.9	F 72 L[f]	wt
- 2 husband	Ø	wt	wt
- 3 mother	0.23	F 72 L[f]	wt
- 4 son	1.3	F 72 L[f]	wt
- 5 paternal cousin	3.5	F 72 L[f]	wt
14–1 index case (SP)	2.8	wt	wt
- 2 father	0.07	wt	wt
- 3 mother	0.04	wt	wt
- 4 sister	0.01	wt	wt
- 5 grandmother (p)	0.01	wt	wt
- 6 grandfather (m)	0.04	wt	wt
- 7 grandmother (m)	0.01	wt	wt
15–1 index case[g] (FS)	**202**	**R 137 H**	**Δ385–8**
- 2 index case[g] (MS)	**79.8**	**R 137 H**	**Δ385–8**
- 3 father	0.24–0.36	R 137 H	wt
- 4 mother	0.15–0.47	wt	Δ385–8
16–1 index case (GS)	1.8	T 51 P	wt
- 2 father	0.07	wt	wt
- 3 mother	1.4	T 51 P	wt
17–1 index case (UU)	**30.1–92.4**	**T 543 P**	**T 543 P**
- 2 father[c]	n.d.	T 543 P	wt
- 3 mother[c]	n.d.	wt	T 543 P
18–1 index case (AC)	1.6–5.0	n.d.	n.d.
- 2 father	4.8	V 105 M	wt
19–1 index case (SK)	8.0–16.7	wt	wt
20–1 index case (AM)	**31.7**	**Y 150 H**	**R 499 C**
- 2 father	0.16[h]	wt	R 499 C
- 3 mother	0.05	Y 150 H	wt

Table 1. Continued

Family-member (initials)	Glucose excretion (g/1.73 m^2 per d; normal 0.13–0.32[b])	Allele 1	Allele 2
21–1 index case (NZ)	1.2	F 453 L	wt[f]
- 2 father	0.13	F 453 L	wt
- 3 mother	0.04	wt	wt[f]
22–1 index case (RW)	1.9	Δ385–8	wt
- 2 father	0	Δ385–8	wt
- 3 mother	0	wt	wt
23–1 index case (LT)	0.75	IVS 7 + 5 g > a	wt
- 2 father	0.23	wt	wt
- 3 mother	0.87	IVS 7 + 5 g > a	wt
- 4 sibling	1.5	IVS 7 + 5 g > a	wt
- 5 sibling	0.82	IVS 7 + 5 g > a	wt

[a] wt, wild type; fs, frameshift; n.d., not determined; Ø, no glucosuria by dipstick method.
[b] According to ref 11; this normal range corresponds to 0.72–1.8 mmol/1.73 m^2 per d.
[c] Consanguinity of the parents.
[d] Siblings.
[e] At elevated blood glucose concentrations as a result of type 2 diabetes.
[f] Associated by N654S, which we consider to be a polymorphism because of its location in transmembrane region 14. This α-helical transmembranous domain has been shown not to be essential for proper function of SGLT. It is missing in some members of the SGLT family, and in vitro deletion does not eliminate sugar transport (40,41). When investigating anonymous blood samples, N654S was detected in 1 of 80 chromosomes.
[g] Identical twins.
[h] Repeatedly positive when tested by dipstick.

Table 2. Oligonucleotide sequences[a]

Exon	sn Primer	asn Primer	T_a	Product (bp)
1	5′-cagtgcccctgaggtaaccattaatc-3′	5′-ccagcagccaccagcagcaca-3′	77 °C	244
2	5′-ccctaaacccaggtctcccccg-3′	5′-tccaggcccctcccgttccc-3′	78–73 °C (td)	145
3	5′-ccctgctcactccctcctct-3′	5′-ccccttttcctggagtttcccc-3′	77 °C	219
4	5′-cctcagggatgagggcaaagcc-3′	5′-accccacttgagccccaccc-3′	79 °C	272
5	5′-cctggaaaaatggagggaagctttga-3′	5′-ccactccctttcctaagcccc-3′	75 °C	244
6	5′-cccacaaagacgccttattgctaaggcc-3′	5′-gaccctccttaggaccctcagacc-3′	69 °C	266
7	5′-gagggtcccctgacggccttgccc-3′	5′-ctccgcccgtctcggctccgcc-3′	75 °C	316
8	5′-cgcaagcgggcagctgaacgccc-3′	5′-gacgggcgcccggttgctggc-3′	73 °C	218
9	5′-ccagtccccacctcctgggattccc-3′	5′-cagcgagcttgcgcctgtggccc-3′	79 °C	214
10	5′-ctcgcgcagctgcagccgccc-3′	5′-cccctgcaggtgcgcaagcccc-3′	79 °C	284
11	5′-cccagggtccgggttcgatcc-3′	5′-cccagccctgccgtcaccacgc-3′	79 °C	242
12	5′-cctcagcaggctgacctgtttcc-3′	5′-cacagtgcttgcctggggcac-3′	75 °C	279
13/14[b]	5′-tgtgcaagagactttagggcca-3′	5′-ggctgtggcttatggtgtcc-3′	75 °C	537

[a] T_a, annealing temperature; td, touch down PCR.
[b] For exon 14, an additional sequencing primer, 5′-AGGCTCCTCACTCCCTGTAC-3′, was used.

p11.2 (13), not only has a high sequence similarity to the best characterized member of the *SGLT* family, human *SGLT1*, but also shows a high degree of similarity regarding its structural organization. We could demonstrate that most of the *SGLT1* intron-exon boundaries are located at corresponding positions in *SGLT2* (Figure 1). On the basis of these results and a primer walking technique, it was easy to determine the genomic sequence of *SGLT2*, which we reported to GenBank in 2000 (accession no. AF307340).

One difference between *SGLT1* and *SGLT2* is noteworthy: The absence of an intron at the position of intron 4 in *SGLT1*. This is an interesting finding in regard to the structural organization of two novel members of the *SGLT* family that have recently been reported. In one of them, *SGLT3*, the gene for another renal low-affinity glucose transporter with 659 amino acids that, like *SGLT1*, is located on chromosome 22 and probably is the product of an ancient gene duplication; this intron is present (14,15). In contrast, in *SGLT4*, a novel mem-

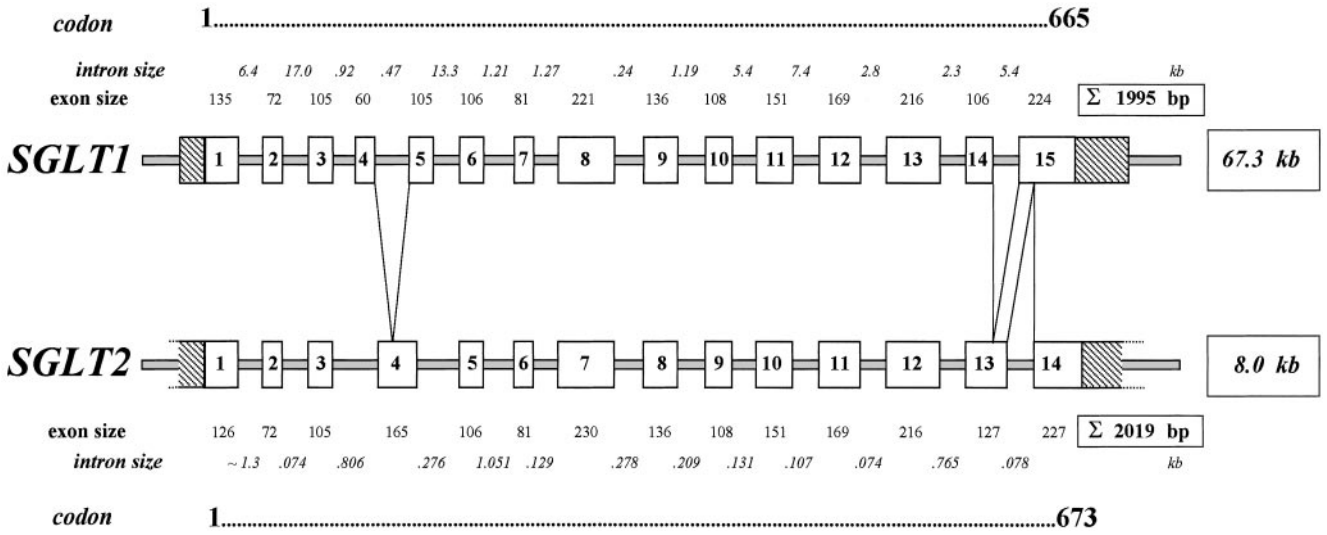

Figure 1. Comparison of the genomic structure of *SGLT1* and *SGLT2*. Exons are presented as boxes and are drawn to scale. Numbers give the different intron sizes.

ber that predicts a protein of 674 amino acids and that has been detected during systematic sequencing of the chromosomal region 1p32.1 to 33, an intron is missing at this position (15,16). Thus, both *SGLT2* and *SGLT4* have only 14 exons and 13 introns with a very similar intron-exon structure, and, possibly, *SGLT2* is more closely related to this gene than to *SGLT1*. Not surprising, *SGLT1* and *SGLT3* have the highest alignment score (69%); for comparison, the score for *SGLT2* is 53% when aligned to *SGLT4* and 58% when aligned to *SGLT1*. *SGLT1* and *SGLT3* have the same sodium-to-sugar stoichiometry (2:1) (17), whereas there is a hint that sodium-glucose coupling in SGLT2 is 1:1 (4,11,18). It will be interesting to know these and other transport properties for *SGLT4*.

With the knowledge of the genomic structure and sequence of human *SGLT2*, we were able to screen patients with isolated renal glucosuria for *SGLT2* mutations. That we detected *SGLT2* mutations in 21 of 23 consecutively investigated families clearly shows the importance of the SGLT2 protein in renal tubular glucose reabsorption. After some ambiguity about the identity of the major renal sodium-glucose co-transporter, our results are in line with the accepted dogma that the bulk of filtered glucose is reabsorbed in segments S1 and S2 of the proximal convoluted tube by the low-affinity, high-capacity carrier SGLT2 (15). They further demonstrate that other sodium-dependent transporters cannot be upregulated to compensate for a nonfunctional SGLT2 protein.

All cases with *SGLT2* mutations on both chromosomes showed "severe" glucosuria; none of these individuals had a glucose excretion <10 g/1.73 m^2 per d (55 mmol/1.73 m^2 per d). The well-documented case of glucosuria type 0 (patient 01-1 in Table 1 (6)) with a daily glucose excretion in excess of 100 g/1.73 m^2 per d demonstrates that a truncating mutation on both chromosomes may result in virtual absence of glucose reabsorption. If transferred to the brush border membrane at all, then the protein predicted by this mutation would have only the seven N-terminally located transmembranous domains, whereas domains 8 to 14 would be missing. Such a mutation has been shown to result in a nonfunctional glucose transporter because it lacks the transmembranous domains 10 to 13 that have been demonstrated to be essential for sugar binding and sugar translocation (15,19,20). It is interesting, however, that one patient (patient 06-1) homozygous for a truncating mutation showed a considerably lower glucose excretion, suggesting some glucose reabsorption by an unknown alternative mechanism.

Cases of "severe" glucosuria show the characteristics of autosomal recessive inheritance. The parents were consanguineous in a significant proportion of the families (seven of 12), suggesting that *SGLT2* mutations are rare in the populations from which our cases originated. Some of these cases had homozygous or compound heterozygous mutations that might have resulted in some residual activity of the transporter: patients with two mutated alleles and glucosuria in the range of 10 to 50 g/1.73 m^2 per d (55 to 280 mmol/1.73 m^2 per d) carried at least one mutation that could explain some residual activity (Table 1), *i.e.*, these patients carried at least one missense or a splice site mutation. An example is the relatively common *SGLT2* IVS7 +5 g>a mutation, a donor splice site mutation that was found to be associated with some residual glucose reabsorption and comparably low glucose excretion. It is well known that this type of mutation results in both abnormally and normally spliced mRNA with the effect of a decreased amount of the gene product with normal kinetic properties (21–23).

Among the patients with "mild" glucosuria, here defined by a glucose excretion <10 g/1.73 m^2 per d (55 mmol/1.73 m^2 per d), *SGLT2* mutations, if present at all, were found in the heterozygous state. However, not in all individuals heterozygous for a specific mutation was an increased glucose excretion observed, and even among family members with identical *SGLT2* mutations, only some had mild glucosuria. This suggests that other genetic or nongenetic factors have to be present

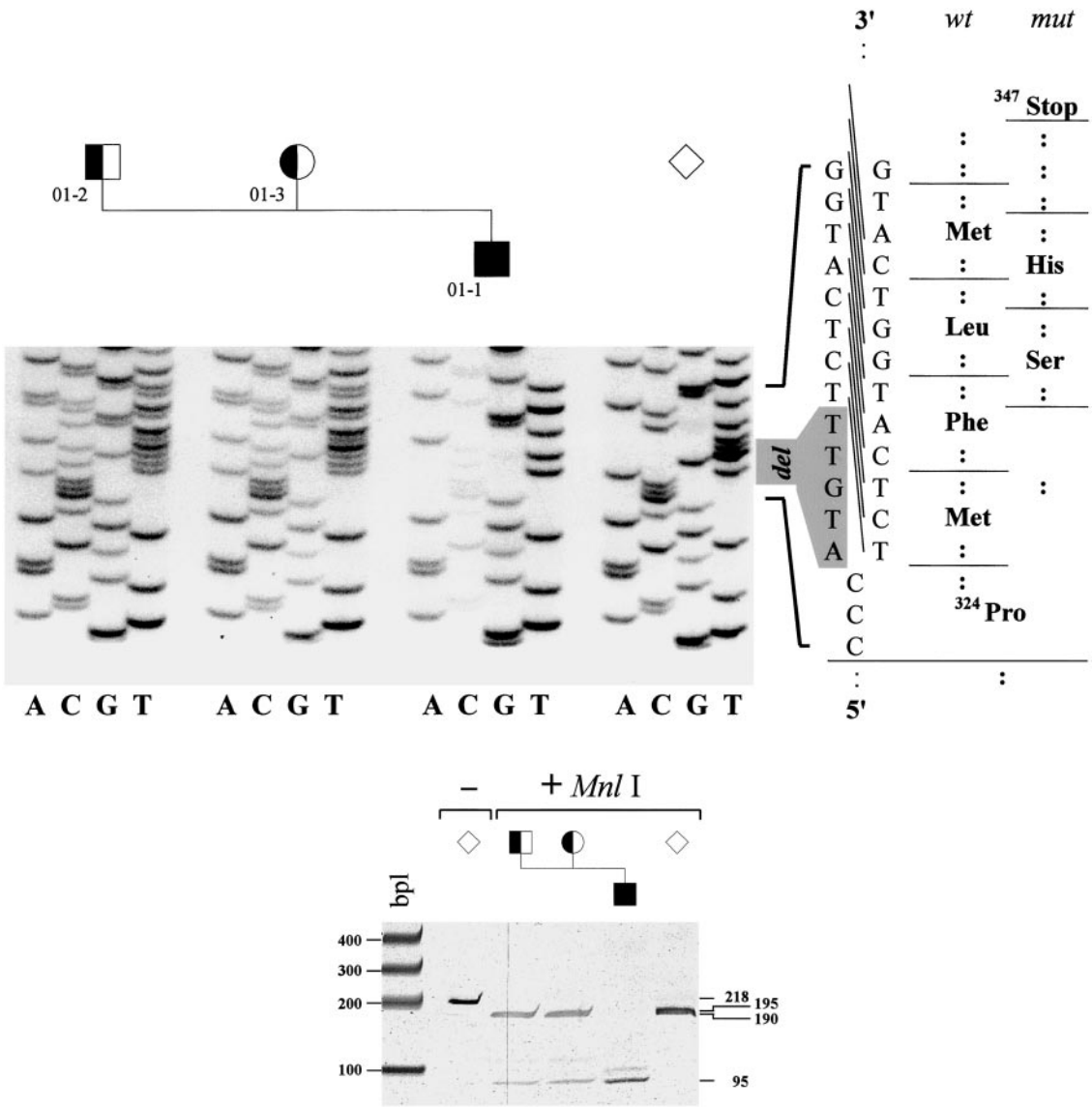

Figure 2. Detection of an *SGLT2* mutation in family 01. (A) DNA sequence in the region of codon 324. The patient shows a homozygous 5-bp deletion (c.973-7 *del* ATGTT) causing a frameshift and the introduction of a premature stop codon at position 347. In both parents, a complex banding pattern starts at the position of the deletion, which is due to sequencing of both the wild-type (*wt*) and the deletion allele (*mut*) in a heterozygous carrier. (B) Confirmation of the c.973-7 *del* ATGTT mutation by *Mnl* I digestion. Both the wild-type (218 bp) and the mutated (213 bp) PCR product of exon 8 carry an *Mnl* I restriction site that results in the cleavage of 23 bp and predicts the generation of digestion products of 195 and 190 bp, respectively. As a result of the 5-bp deletion, however, an additional *Mnl* I site is introduced into the 190-bp fragment, which results in the formation of two 95-bp fragments. *bpl*, bp ladder.

before heterozygosity for an *SGLT2* mutation results in mild glucosuria. Therefore, inheritance of renal glucosuria can best be described to follow a codominant trait with variable penetrance.

These results of our molecular genetic study are in accordance with earlier clinical observations. Before quantification of glucose excretion in urine became a clinical routine, renal glucosuria was generally considered to follow a dominant trait (5). Further studies have shown that mild glucosuria is relatively frequent and that cases of "heavy glucosuria" are extremely rare (9,24). Furthermore, it has already been reported that parents of patients with severe glucosuria may or may not show an abnormality of renal glucose transport (9).

Our results also provide an explanation for titration studies performed in the 1950s defining the classical types of renal glucosuria (25,26). A diminished amount of the normal SGLT2 protein should result in type A glucosuria, characterized by a lowered renal transport maximum for glucose (T_mG). However, patients with certain *SGLT2* missense mutations resulting in a diminished affinity of the transporter for its substrate may belong to type B for which a normal T_mG and an exaggerated splay is characteristic. T_mG and renal threshold for glucose will have to be determined for patients characterized at the molecular genetic level to determine whether these assumptions are correct. Compound heterozygosity for *SGLT2* mutations, however, is probably the reason that many cases of severe glucos-

Table 3. Twenty-one different *SGLT2* mutations detected in 23 families with renal glucosuria

	Sequence aberation	Predicted effect[a]	Confirmation[b]	Family[c]
ex 02	151 A > C	T 51 P	(+) *Bgl* I	16
ex 03	216 C > A	F 72 L	(+) *Dde* I	13
ex 04	313 G > A	V 105 M	(+) *Nla* III	18
	410 G > A	R 137 H	mm (−) *Not* I	15
	448 T > C	Y 150 H	(−) *Rsa* I	20
ex 05	500 *del* A	I 166 fs. . . 186 X	HD	2
	506 *del* C	Q 168 fs. . . 186 X	(−) *Alw* N I	10
ex 07	814 G > A	G 272 R	(+) *Bst* N I	2
IVS 7	+5 g > a	abnormal splicing variants	mm (−) *Bst* U I	**3**,4,11,12,23
ex 08	920 T > C	L 307 P	(+) *Ban* II	4
	932 A > G	K 311 R	mm (−) *Mse* I	12
	973–7 *del* ATGTT	P 324 fs. . . 347 X	mm (+) *Mnl* I	**1**
ex 09	1102 C > T	R 368 W	(+) *Fnu* 4 H I	**9**
ex 10	1152–63 *del* 12	Δ385–8	HD	15, 22
ex 11	1320 G > A	W 440 X	(−) *Bst* NI	**6**[d]
	1346 G > A	G 449 D	mm (−) *Nae* I	**5**
	1359 C > A	F 453 L	(−) *Taq* I	21
ex 12	1495 C > T	R 499 C	(−) *Sph* I	20
	1461–517 *del* 57	W 487, Δ488–506	HD	7
	1627 A > C	T 543 P	(+) *Msp* I	**17**
ex 14	1951–92 *del* 42	Δ651–64	HD	8

[a] fs. . ., frameshift mutation described by the last regular amino acid before the frameshift and the position of the predicted premature termination (X) of translation of the SGLT2 protein.

[b] mm denotes the use of a mismatch primer for DNA amplification (sequences available upon request), (+) stands for the gain and (−) for the loss of a restriction site for the given enzyme in the presence of the mutation. HD; heteroduplex formation on PAGE.

[c] Family numbers refer to Table 2 Numbers in boldface symbolize homozygosity; numbers in standard style symbolize heterozygosity for a given mutation.

[d] In addition to this Turkish family, the same mutation was detected in another Turkish family reported by van den Heuvel *et al.* (8); glucose excretion of the other case was 61.6 g/L; parents and sibilings were reported not to have glucosuria.

uria could not be clearly classified into type A or B in the past and that a broad spectrum of impairment of tubular glucose transport has been found in such patients (27).

Heterozygosity for an *SGLT2* mutation can result in mild glucosuria, and both nonsense and missense mutations may have this effect. It is interesting that this is in contrast to recent observations in individuals heterozygous for a mutation of *GLUT2*, the gene of the basolateral glucose carrier of tubular cells. Whereas *GLUT2* missense mutations were associated with mild glucosuria, nonsense mutations were reported not to result in an increased glucose excretion (28). This was explained by a dominant negative effect of the missense mutation on the GLUT2 hexamer. Such an effect might not play a role in sodium-dependent transporters because freeze-fracture electron microscopic studies have suggested that they function as monomers (29).

The 21 *SGLT2* mutations detected during the course of this study are summarized in Table 3. All of the nonsense and frameshift mutations predict proteins that lack the previously mentioned transmembranous domains 10 to 13 essential for sugar binding and sugar transport of SGLT proteins. Many of the missense mutations (T51P, R137H, G272R, K311R, and R499C) affect residues that are highly conserved across the human *SGLT* family and across *SGLT* of other species (*Mus musculus* [mouse], *Rattus norvegicus* [rat], *Oryctolagus cuniculus* [rabbit]), which underlines their pathogenic role. Other missense mutations (G449D and F453L) lie in very close vicinity to Q457, the residue interacting with O1 and O5 of the pyranose in SGLT1 (30). *SGLT2* mutations seem to be randomly distributed along the coding sequence of the gene, but, interestingly, no *SGLT2* mutation was detected in regions 289 to 304 and 369 to 405,[1] considered to be mutational hot spots in *SGLT1* (31). The only locations where naturally occurring mutations have been observed both in *SGLT1* and *SGLT2* are R137 and R499.[2] A patient with glucose-galactose malabsorption and compound heterozygosity for *SGLT1* R499H (together with R379X) was severely affected clinically, and the functional expression of this mutant in oocytes has been shown to result in a significant decrease of sugar affinity and trafficking to the membrane. It has been reported, however, that replacing this residue by cysteine restored trafficking and resulted in a functional sugar transporter (32). This stands in contradiction

[1]Note that from *SGLT2* C255 to S605, numbering is identical for corresponding codons in *SGLT1* and *SGLT2*.

[2]Homozygosity for *SGLT1* R140Q (corresponding to R137 in *SGLT2*) has recently been found in an unpublished case of glucose-galactose malabsorption in our laboratory.

to our observation of an individual with compound heterozygosity for *SGLT2* R499C (together with Y150H) and significant renal glucosuria, which cannot be solely explained by the other mutated allele (see patients 20-1 and 20-3, Table 1).

How *SGLT2* missense mutations result in an impairment of glucose transport is still unclear. However, as a result of the great sequence and structure homology of *SGLT*, it is tempting to suppose that disturbed trafficking shown to play a major role for *SGLT1* mutations also is an important factor in *SGLT2* (31). Unequivocally, it is a drawback of our study that we were not able to express *SGLT2* mutants in heterologous expression systems to investigate the effect of mutations in comparison with the wild-type glucose carrier. Although we spent particular efforts in expression studies both in *Xenopus laevis* oocytes and in isolated vesicles, techniques that have worked perfectly in our hands when expressing *SGLT1* and other transporters (33,34), we were not successful expressing *SGLT2* (data not shown). However, it has previously been noticed that human *SGLT2*, unlike *SGLT1* and *SGLT3*, expresses very poorly in oocytes and COS-7 cells (15,35). The sodium-dependent uptake of sugar and the sodium-sugar currents in *SGLT2* oocytes were, at most, 3.5 times greater than those in controls (4), whereas expression of *SGLT1* or *SGLT3* in oocytes resulted in sugar fluxes and currents up to 2000 times greater than controls. Whether additional posttranslational modification is required before *SGLT2* expresses in oocytes is uncertain. An important role of protein kinases for sodium-glucose co-transporters has been demonstrated for *SGLT1*, and it has been suggested that despite that *SGLT* show high sequence homology, minor differences in amino acid sequence may dramatically change the regulation of trafficking by protein kinases (36). The ultimate reason for poor expression of *SGLT2* in heterologous systems, however, has remained unclear.

Two of our index patients with renal glucosuria (patients 14-1 and 19-1 in Table 1) did not have any detectable *SGLT2* mutation, and in one of the cases with relatively severe glucosuria, we would have expected to find a second *SGLT2* mutation (patient 07-1). This can of course be the result of technical problems. Some *SGLT2* mutations may not be detectable with the PCR-based technique applied in this study, and *SGLT2* mutations outside the coding region, *e.g.*, within the promotor region, would have escaped our analysis. In that context, it is interesting that the transcription of mouse *SGLT2* is regulated by hepatic nuclear factor (HNF)-1α (37), and, at least for human *SGLT1*, the binding domain for this transcription factor has recently been characterized (38). Only limited information on transcriptional regulation is available for human *SGLT2*.

Despite the important role of *SGLT2*, other genes may be involved in renal glucose transport to varying degrees and may be mutated in patients with renal glucosuria. Intestinal glucose-galactose malabsorption, a congenital defect of *SGLT1*, is well known to be accompanied by glucosuria, but there were no intestinal symptoms in our patients. Other members of the *SGLT* family (*SGLT3*, *SGLT4*, and *SMIT2*), however, are candidates that should be investigated. Furthermore, a defect of HNF-1α, known to cause maturity-onset diabetes in the young type 3, has been reported to be accompanied by a diminished renal threshold for glucose. Such a defect is unlikely in our patients because other signs of maturity-onset diabetes in the young were absent. The possibility of a heterozygous *GLUT2* missense mutation has been discussed above; therefore, we will have to investigate glucosuria patients for *GLUT2* mutations even though we have not observed glucosuria in patients heterozygous for a *GLUT2* missense mutation (unpublished data). Finally, mutations in other genes, such as a hypothetical locus termed *GLYS1* detected by linkage studies on chromosome 6 (39), cannot be ruled out. However, because both of our index patients without an *SGLT2* mutation showed only mild to moderate glucosuria, this does not challenge the major result of our study. The detection of *SGLT2* mutations in all patients with severe renal glucosuria and that virtual absence of glucose reabsorption in some of the patients was associated with a mutation predicting a nonfunctioning SGLT2 protein are evidence that SGLT2 plays a very important role in renal tubular glucose transport.

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