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Gitelman Syndrome

Mattias Roser, Nermin Eibl, Birgit Eisenhaber, Jasmin Seringer, Mato Nagel, Sylvia Nagorka, Friedrich C. Luft, Ulrich Frei, Maik Gollasch

Gitelman syndrome (GS) is an autosomal-recessive renal tubular disorder characterized by hypokalemia, hypomagnesemia, hypocalciuria, metabolic alkalosis, secondary hyperreninemic aldosteronism, and low blood pressure.¹⁻³ GS patients are usually diagnosed relatively late, because malaise, low blood pressure, hypokalemia, hypocalciuria, and hypomagnesemia are difficult to categorize clinically. Inactivating mutations in the *SLC12A3* gene encoding the thiazide-sensitive sodium chloride cotransporter (NCCT) cause GS.² The investigators used the criteria of Bettinelli et al⁴ to identify patients with GS. More than 100 *SLC12A3* mutations have been described.³ Most are missense mutations substituting conserved amino acid residues within putative functional domains of NCCT, whereas nonsense, frameshift, and splice site defects and gene rearrangements are less frequent. GS is clinically variable (men are more severely affected than women), and the combination of mutations present in each allele may determine phenotype variability.³ A heterozygous carrier state for 30 different inactivating mutations in NCCT, as well as genes responsible for Bartter syndrome, is associated with reduced blood pressure risk of hypertension in the general population.⁵ Approximately 80% of the mutation carriers had systolic blood pressure values below the mean of the entire 5124-subject cohort of the Framingham Heart Study. The mean blood pressure reduction in carriers averaged -6.3 mm Hg for the systolic and -3.4 mm Hg for the diastolic blood pressures, similar to values obtained with chronic thiazide treatment. There was a 60% reduction in the risk of developing hypertension by age 60 years. Thus, rare alleles that affect renal salt handling and blood pressure in the general population could account for a substantial fraction of blood pressure variation.⁵ We encountered 2 patients and then found others in our database who illustrate the importance of NCCT on blood pressure variability and, therefore, hypertension.

The Patients

A 29-year-old man presented with a 14-year history of generalized weakness, severe muscle cramps with tetany, dyspnea, and anxiety. He had visited emergency departments

on earlier occasions and received potassium and/or magnesium infusions for hypokalemia and hypomagnesemia. His symptoms had progressed to the point that he could no longer drive a car or use public transport. The patient denied nausea, vomiting, diarrhea, heat intolerance, excessive perspiration, and changes in bowel habits. He ingested no laxatives or diuretics, nor did he abuse alcohol or street drugs. His other clinical symptoms included fatigue, heart palpitations, dizziness, nocturia, polydipsia, polyuria, and thirst.

The blood pressure was 100/60 mm Hg, and heart rate was 90 bpm. There were no neurological findings or proximal muscle weaknesses. Imaging studies were unremarkable, as was the ECG. Laboratory tests showed hypokalemia (3.0 mmol/L) and hypomagnesemia (0.69 mmol/L), whereas serum calcium, sodium, and chloride levels were normal. The creatinine clearance was 134 mL/min per 1.73 m². Urinary calcium excretion (1.0 mmol/24 hours) was decreased, whereas the urinary potassium and magnesium excretion were elevated (106 mmol/24 hours and 6.82 mmol/24 hours) in the face of his low serum values. The plasma renin concentration was elevated (172 ng/mL) while supine and increased (302 ng/mL) with standing. The corresponding plasma aldosterone levels were normal to mildly increased (120 ng/mL supine and 217 ng/mL standing). The 24-hour urinary sodium excretion was 141, 176, and 350 mmol/24 hours on 3 consecutive days on an ad libitum intake. Arterial blood gases were pH 7.49, Pco₂ 41.6 mm Hg, and HCO₃⁻ 31.6 mmol/L.

The parents and an older brother were asymptomatic, whereas his younger brother had similar, albeit less severe, symptoms. We sequenced the exons and flanking regions of *SLC12A3* after written informed consent was given and found 3 mutations in exons 10, 13, and 16. The patient and his brother were compound heterozygous, having inherited Phe548Leu and Pro643Leu from their mother and Gly439Ser from their father (please see Table S1 in the online data supplement at <http://hyper.ahajournals.org>). The Phe548Leu mutation has not been reported earlier and is localized in a transmembrane region (Figure). The highly conserved phenylalanine is exchanged for a leucine (Figure S1). Gly439Ser

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From the Division of Nephrology and Intensive Care Medicine (M.R., N.E., J.S., U.F., M.G.), Department of Medicine, Charité Campus Virchow, Berlin, Germany; Experimental Therapeutics Centre and Bioinformatics Institute, Agency for Science, Technology and Research (B.E.), Singapore; Experimental and Clinical Research Center and HELIOS Klinikum-Berlin (F.C.L., M.G.), Berlin, Germany; and the Center for Nephrology and Metabolic Disorders (M.N., S.N.), Weißwasser, Germany.

Correspondence to Maik Gollasch, Charité-Universitätsmedizin Berlin, Campus Virchow-Klinikum, Nephrology/Intensive Care Medicine, Augustenburger Platz 1, D-13353 Berlin, Germany. E-mail maik.gollasch@charite.de

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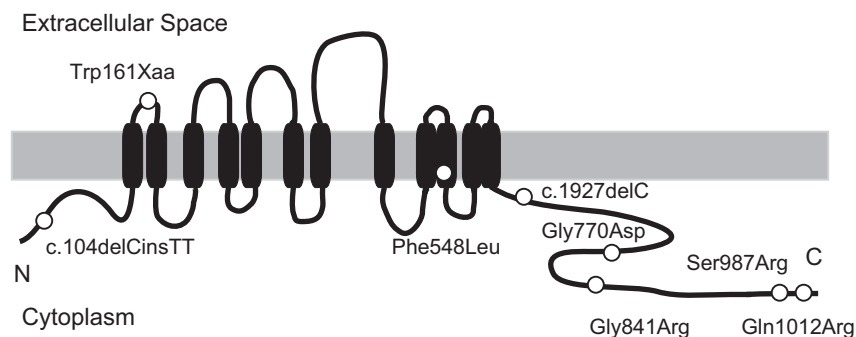


Figure. Domain architecture and positions of mutations in *SLC12A3* detected in the present study.

and Pro643Leu have been reported previously in heterozygous GS patients⁶ but not in this allelic combination.⁷ Although Gly439Ser and Pro643Leu would be sufficient to account for the symptoms in our patient, the presence of 3 mutations (Gly439Ser and Pro643Leu/Phe548Leu) is the possible reason that his symptoms were remarkably severe.

The patient was given oral magnesium and potassium supplementation (18 and 84 mmol/d, respectively). His serum potassium and magnesium levels improved to low-normal values, and his clinical symptoms improved. Magnesium and potassium supplementations in combination with antialdosterone medications, prostaglandin inhibitors, or angiotensin-converting enzyme inhibitors have been advocated.^{8–10} Aldosterone antagonists or epithelial sodium channel blockers can be considered if symptomatic hypokalemia is not corrected by MgCl₂ administration.¹¹ A potential concern of using these drugs in patients with GS is that the salt wasting might worsen, especially if dietary salt intake is reduced or when salt is lost from the body through a nonrenal mechanism. Even high doses of the epithelial sodium channel blocker amiloride may fail to curtail the excessive kaliuresis in patients with GS.¹ Low concentrations of these drugs in the lumen of the cortical collecting duct and modulation of the distal tubular potassium channel (renal outer medullary potassium channel) conductance could be responsible.¹² Amiloride is, as such, problematic, because it seems to increase aldosterone production accompanied by insufficient stimulation of plasma renin activity. The authors invoke a rise in serum potassium as an explanation.¹³

Spirolactone and angiotensin-converting enzyme inhibitors had been given empirically to our patient earlier by his family doctors. Both drugs were stopped after 1 month each because of dizziness, probably as result of low blood pressure episodes. Amiloride was also tried. However, the patient discontinued this medication because of unexplained neck pain. We tried a course of aliskiren 150 mg/d to inhibit angiotensin-mediated aldosterone release; however, the aldosterone values did not decrease, and the metabolic alkalosis did not improve. Currently, the patient can tolerate his symptoms, in part because he now has a greater understanding of his disease.

A 21-year-old woman presented with blurred vision, cramps, tongue discomfort, and fatigue. At age 11 years, she observed that eating bananas made her feel better. She had never been brought to an emergency department, was physically active, and was employed. Her general practitioner was

concerned about severe hypokalemia and hypomagnesemia. She denied nausea, vomiting, diarrhea, heat intolerance, excessive perspiration, and changes in bowel habits. She had no history of laxatives or diuretic abuse, nor did she abuse alcohol or street drugs. She denied nocturia, polydipsia, polyuria, and thirst.

The blood pressure was 115/80 mm Hg; heart rate was 85 bpm. The physical examination was normal. She had hypokalemia (2.8 mmol/L) and hypomagnesemia (0.48 mmol/L), whereas serum calcium, sodium, chloride, creatinine levels were normal. The creatinine clearance was 122 mL/min per 1.73 m². Urinary excretion of calcium (0.37 mmol/24 hours) was decreased, whereas urinary excretion of potassium and magnesium (67.00 and 2.83 mmol/24 hours, respectively) were elevated in the face of her serum values. The urinary 24-hour excretion of sodium was 145 mmol with an ad libitum intake. She also had a compensated metabolic alkalosis (pH 7.46, Pco₂ 44.8 mm Hg, and HCO₃⁻ 31.0 mmol/L). Her parents were clinically unremarkable, whereas her 19-year-old brother described similar general weakness and muscle cramps, although less frequent.

With her approval, we again sequenced the exons and flanking introns of *SLC12A3* and found a mutation in exon 26 (Gln1012Arg), as well as a deletion of exon 1, codon 35 (c.104delCinsTT). Our patient is compound heterozygous, having inherited c.104delCinsTT from her mother and Gln1012Arg from her father (please see Table S1). Gln1012Arg is a novel missense mutation (Figure) that affects a sequence position at which the glutamine is conserved throughout all vertebrates (Table S1). Because *SLC12A3* codes for 1021 amino acids, Gln1012Arg is located in close proximity to the C-terminal end of the protein sequence. This variant is located in the most distal part of the C-terminal *SCL12A3* cytoplasmic domain known to date.

Analysis of our clinically suspected GS patient databank revealed no other subjects with Gln1012Arg. However, we did find 6 additional new *SLC12A3* gene mutations in other subjects (Gly841Arg, Ser987Arg, c.1927delC, Trp161Xaa, Gly770Asp, and IVS25+1G>T; Figure and Table S1). In these subjects, the referring nephrologists or geneticists had suspected GS on the basis of clinical examination and laboratory tests. Family histories and medical charts were reviewed and showed clinical features consistent with GS.

We treated this patient with oral magnesium and potassium supplementation (2.8 and 40.0 mmol/d). In addition, her local nephrologists prescribed ramipril 2.5 mg/d. Serum potassium

and magnesium levels reached low-normal values, and clinical symptoms improved significantly but did not resolve completely. Oral magnesium supplementation was, therefore, increased, and clinical symptoms improved. However, restoration of normal magnesium and potassium values was difficult and was accompanied by nausea and diarrhea. Magnesium aspartate was replaced with magnesium chloride, which was more tolerable.

Discussion

Nephrologists are generally confronted with hypertension. However, a thorough knowledge of hypotensive syndromes, particularly GS, is important not only for clinical reasons but also to understand the complex genetics of blood pressure regulation in the general population. During a routine nephrology ward rotation, we discovered novel *SLC12A3* mutations in 2 symptomatic GS patients (Phe548Leu, Gln1012Arg, and c.104delCinsTT). These results caused us to work up our databank of "suspicious" hypokalemic hypomagnesemic patients, and, again, we were successful. We found 6 other new *SLC12A3* gene mutations (Gly841Arg, Ser987Arg, c.1927delC, Trp161Xaa, Gly770Asp, and IVS25+1G>T). We elected to move genetic analysis from the research laboratory to the routine clinical arena for several reasons. First, a precise diagnosis permits a more exact, goal-directed clinical care. Second, we learned that our patients wanted desperately to know specifically what was wrong and what the relevance could be for their current and future families. Our patients did not raise concerns about fear of privacy violations, higher insurance premiums, or similar concerns.

Decreased reabsorption of sodium at the NCCT and subsequent increased potassium losses via the renal outer medullary potassium channel, largely driven by secondary aldosteronism, explain the hypokalemia and reportedly increased salt appetite in GS patients. The hypocalciuria and hypermagnesuria are more difficult to understand. Nijenhuis et al¹⁴ argued that enhanced passive calcium transport in the proximal tubule, rather than active calcium transport in distal convoluted tubule, explains thiazide-induced hypocalciuria. Their micropuncture experiments in mice demonstrated increased reabsorption of sodium and calcium in the proximal tubule during chronic thiazide treatment, whereas calcium reabsorption in the distal convoluted tubule appeared unaffected. Furthermore, thiazide administration still induced hypocalciuria in transient receptor potential channel subfamily V, member 5 gene-deleted mice, in which active distal calcium reabsorption was abolished because of inactivation of the epithelial calcium channel TRPV5. Next, Nijenhuis et al¹⁴ found that thiazide upregulated the sodium/proton exchanger, responsible for the majority of sodium and, consequently, calcium reabsorption in the proximal tubule, whereas the expression of proteins involved in active calcium transport was unaltered. The authors then performed experiments addressing the time-dependent effect of a single thiazide dose and showed that the development of hypocalciuria paralleled a compensatory increase in sodium reabsorption secondary to an initial natriuresis. Finally, hypomagnesemia developed during chronic thiazide administration and in NCCT gene-deleted

mice, accompanied by downregulation of the epithelial magnesium channel transient receptor potential channel subfamily M, member 6. Transient receptor potential channel subfamily M member 6 downregulation could represent a general mechanism involved in the pathogenesis of hypomagnesemia accompanying NCCT inhibition or inactivation.¹⁴

Human studies have also been performed. Cheng et al¹⁵ investigated 8 GS patients and 8 control subjects. Isotonic saline (3 L) over 3 hours was given. Baseline sodium excretion and creatinine clearance rates were similar in GS patients and controls, although calcium excretion rate was lower in GS patients. In GS patients, saline infusion caused a significantly greater sodium excretion rate than in controls, but there was only a small increase in the calcium excretion rate in both the first 6 hours and the subsequent 18 hours. The authors concluded that hypovolemia is not the sole cause of hypocalciuria in patients with GS. However, their clinical study had several deficits. Long-term sodium balance was not controlled in the subjects, and the GS patients likely had a reduced extracellular circulating volume under both control and expansion conditions compared with controls.

Our patients illustrate the difficulties in managing GS. The tubular defect in GS itself cannot be corrected so that adequate supplementation of magnesium and potassium remains the cornerstone of treatment. However, restoration of normal magnesium and potassium values is difficult to achieve, because high doses of magnesium cause diarrhea. The bioavailability of magnesium preparations is variable. Magnesium oxide and magnesium sulfate have a significantly lower bioavailability compared with magnesium chloride, magnesium lactate, and magnesium aspartate.¹¹ We recommend administration of magnesium chloride orally to compensate for renal magnesium and chloride losses. Although antialdosterone therapy has been reported to increase plasma potassium and magnesium levels and to reduce the fractional excretion of potassium and magnesium in GS patients,¹⁰ the role of inhibiting the renin-angiotensin-aldosterone system in GS patients is not clear. Estrogens seem to play a role because drospirenone, a novel progestogen developed for use as hormone therapy in postmenopausal women, in combination with 17 β -estradiol, has a potassium-sparing effect that counteracts thiazide-induced potassium loss.¹⁶ Future studies on NCCT-deficient mice might be helpful to understand the role of these systems in GS. NCCT-deficient mice on a mixed background have been shown to exhibit hypocalciuria and hypomagnesemia but no potassium and acid-base homeostasis disturbances.¹⁷ NCCT-deficient mice backcrossed onto the C57BL/6 background showed mild compensated alkalosis with increased levels of plasma aldosterone¹⁸ and an increased sensitivity to develop hypokalemia when exposed to reductions in dietary potassium.¹⁹ These studies underscore the importance of the genetic background for the NCCT-deficient phenotype, which is also potentially relevant for understanding phenotypic variability and development of pharmacological treatments in GS. To date, there are no data about the effectiveness of renin and/or angiotensin-converting enzyme inhibition in GS available. Future studies on NCCT mice might help to clarify this issue.

Finally, we learned in the course of our study that mutations such as these have a distinct bearing on the complex genetics of hypertension in the general population. We could not conduct a study on GS prevalence. Our patient population is heterogeneous and generally of German, Turkish, or Middle Eastern background. The mutations reported here were all in German white subjects. Hsu et al²⁰ studied 500 unrelated children from Taipei. They found 15 *SLC12A3* mutations in 10 of the children. In a 1852-subject Japanese study, GS mutations were encountered in 3.2% of subjects.²¹ The literature would suggest that GS is not rare and GS heterozygosity certainly not that uncommon. Knowledge of novel mutations might also be important for determining alleles with health benefits that are nonetheless under purifying selection and for defining the genetic architecture of hypertension.⁵ In this respect, combined effects of rare independent mutations have been suggested to account for a substantial fraction of blood pressure variation in the population on the basis of analyzing the impact of the heterozygous carrier state for a limited number of inactivating mutations in the NCCT, sodium-potassium 2-chloride cotransporter, and renal outer medullary potassium channel.²² Recently, members of the Framingham Heart Study were screened for variation in 3 genes, namely, *SLC12A3*, *SLC12A1*, and *KCNJ1*. The investigators used comparative genomics, genetics, and biochemistry to identify subjects with mutations proven or inferred to be functional. These mutations were all heterozygous and rare but were found in $\approx 1\%$ to 2% of the Framingham population.⁵ They produced clinically significant blood pressure reductions and presumably protected the individuals from development of hypertension. These findings and the mutations we report here have implications for the genetic studies on hypertension and other common complex traits. Carriers of mutations causing GS compensate for renal salt wasting by increasing their salt intake, as shown in a large Amish kindred.²³ In agreement, we found that urinary 24-hour sodium excretion was high in the first patient probably because of increased salt appetite. However, the increased urinary sodium excretion in carriers of the mutations causing GS versus wild-type relatives was not reflected by significant blood pressure changes in adults of the above mentioned large Amish kindred with GS.²³ In contrast, another study showed that carriers of mutations causing GS had lower blood pressure values than controls, however, with similar urinary sodium excretion.²⁴ Notably, our second patient showed normal urinary 24-hour sodium excretion; however, she was a small, slight woman. For her size, her salt intake was generous. NCCT-deficient mice have no salt-losing phenotype on a regular diet,¹⁷ and compensatory mechanisms for the loss of NCCT have been documented in various mouse models.²⁵ Finally, NCCT stands out as a target of with-no-lysine kinase (WNK) regulation, as discussed recently in a scholarly review.²⁶ The mirror image of GS is pseudohypoadosteronism type II (PHAI), which is also called Gordon syndrome. PHAI features high blood pressure, hyperkalemia, and metabolic acidosis. All of the metabolic aberrancies in PHAI are corrected by thiazide diuretics, which recapitulate the features of GS. WNK4 and WNK1 mutations cause PHAI. WNK4 inhibits NCCT, an

effect that is lost when WNK4 is mutated. WNK1 phosphorylates WNK4, which diminishes the WNK4 inhibition of NCCT. A gain-of-function WNK1 mutation causes PHAI. The WNKs also influence the renal outer medullary potassium channel and epithelial sodium channel. The findings that altered levels of WNK1 and WNK4 function influence blood pressure in humans are relevant because WNK4 lies only 1 Mb from locus D17S1299, the site showing the strongest linkage to blood pressure variation in the Framingham Heart Study population. Variants that alter WNK function would of course also influence the GS phenotype in persons harboring NCCT mutations.

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Disclosures

None.

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