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EDITORIAL COMMENTARY

Gitelman's syndrome: towards genotype-phenotype correlations?

Eva Riveira-Munoz · Qing Chang · René J. Bindels · Olivier Devuyst

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Abstract Gitelman's syndrome (GS) is a salt-losing tubulopathy characterized by hypokalemic alkalosis with hypomagnesemia and hypocalciuria. The disease is associated with inactivating mutations in the *SLC12A3* gene that codes for the thiazide-sensitive Na^+–Cl^- cotransporter (NCCT) that is expressed in the apical membrane of the cells lining the distal convoluted tubule (DCT). GS is relatively frequent, and more than 100 mutations scattered through *SLC12A3* have been identified thus far. Although the disease is recessively inherited, up to 40% of patients are found to carry only a single mutation, instead of being compound heterozygous or homozygous. The phenotype of GS is highly heterogeneous in terms of age at presentation, and nature/severity of the biochemical abnormalities and clinical manifestations. This phenotypical heterogeneity is observed not only between all patients harbouring *SLC12A3* mutations but also among family members or patients with identical mutations. In this review, we discuss the potential explanations for the failure to identify mutant alleles in *SLC12A3*, as well as the different mechanisms that can account for the inter- and intra-familial phenotype variability in GS, including genetic heterogeneity, position and nature of the mutations, functional consequences, compensatory mechanisms, and modifying genes.

E. Riveira-Munoz · O. Devuyst (✉)
Division of Nephrology,
Université catholique de Louvain Medical School,
10 Avenue Hippocrate,
1200 Brussels, Belgium
e-mail: Devuyst@nefr.ucl.ac.be

Q. Chang · R. J. Bindels
Department of Physiology, Nijmegen Centre for Molecular Life Sciences, Radboud University Nijmegen Medical Centre,
Nijmegen, The Netherlands

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Introduction: Gitelman's syndrome and salt-losing tubulopathies

In 1966, Gitelman and co-workers described a new familial disorder in which patients presented with hypokalemic alkalosis and a peculiar susceptibility to carpopedal spasm and tetany due to hypomagnesemia [1]. For decades, the disease was included in a group of closely related disorders, refered to as hypokalemic salt-losing tubulopathies or Bartter's-like syndromes (BS). All these disorders are associated with secondary aldosteronism, responsible for hypokalemia and metabolic alkalosis, but they markedly differ in terms of age of onset, severity of clinical manifestations, presence of urinary concentrating defect, other electrolyte abnormalities, and magnitude of urinary calcium excretion. Based on clinical manifestations, the salt-losing tubulopathies were grouped into two major groups: the hyperprostaglandin-E syndrome [HPS, also named antenatal Bartter's syndrome (aBS)], associated or not with sensorineural deafness (SND); and the classic Bartter's and Gitelman's syndromes (cBS and GS, respectively). Despite some overlapping features, it also appeared that the HPS group included disorders affecting the thick ascending limb (TAL) of Henle's loop, with furosemide-like manifestations, whereas the second group—and particularly the GS—was related to a defect in the distal convoluted tubule (DCT), with thiazide-like manifestations [2]. In 1992, Bettinelli and co-workers showed that a distinctive feature of GS was the dissociation of renal Ca^{2+} and Mg^{2+} handling, leading to hypocalciuria and hypo-

magnesemia [3]. Over the past 10 years, crucial developments in molecular genetics, initiated by the group of R. Lifton, led to the identification of mutations in transporters and channels responsible for these inherited tubulopathies. The aBS was associated to inactivating mutations in the genes encoding the apical Na^+–K^+–$2Cl^-$ cotransporter NKCC2 or the apical K^+ channel ROMK, whereas inactivating mutations in barttin, a regulatory beta-subunit of the basolateral ClC-Ka and ClC-Kb channels, were detected in aBS with SND. On the other hand, inactivating mutations of ClC-Kb, which is located both in the TAL and DCT, were associated with the cBS, whereas GS was found to be associated with mutations of the thiazide-sensitive sodium-chloride cotransporter NCCT (Fig. 1). A comprehensive classification of these salt-losing tubulopathies, based on the clinical, physiological, and molecular insights discussed above [2, 4, 5], provides a basis to understand the distinct phenotypes of these disorders (Table 1).

The purpose of this editorial is to review the molecular basis of GS, defined as a salt-losing tubulopathy affecting the DCT, in order to discuss the striking phenotype variability that is associated with that peculiar disease.

Molecular defect in Gitelman's syndrome: *SLC12A3* and NCCT

In 1996, Simon and co-workers used linkage analysis in 12 unrelated families to demonstrate that loss of function mutations in the *SLC12A3* gene were responsible for GS [6]. The *SLC12A3* gene is located on the long arm of chromosome 16 (gene locus: 16q13) and comprises 49 kb with 26 exons. It encodes the thiazide-sensitive Na^+–Cl^- cotransporter (NCCT, or TSC), an integral membrane protein of 1,021 amino acids expressed in the apical membrane of cells lining the DCT. NCCT belongs to the nine-member family of electroneural cation-chloride coupled cotransporters (*SLC12*) that also includes the bumetanide-sensitive Na^+–K^+–$2Cl^-$ cotransporter and the K^+–Cl^- cotransporters. Members of this family share a high degree of homology in their amino acid sequence and proposed topology [7]. Analysis of NCCT cDNAs revealed that the cotransporter contains a central hydrophobic region, comprising 12 putative transmembrane domains flanked by a short N-terminus and a long hydrophilic intracellular C-terminus [6–8] (Fig. 2a). GS is transmitted as an autosomal recessive trait, and the majority of patients are compound heterozygous for different mutations within the paternal and maternal *SLC12A3* allele. The disease is not rare, since the prevalence of heterozygotes is approximately 1% in various European populations [9].

Mutation detection in Gitelman's syndrome

To date, more than 100 mutations scattered through the *SLC12A3* gene have been identified in patients with GS (Fig. 2b). The majority of these mutations have been collected in the Human Gene Mutation Database (HGMD, http://www.hgmd.cf.ac.uk/). Most (81/113) are missense mutations substituting conserved amino acid residues within putative functional domains of the NCCT, whereas nonsense, frameshift and splice-site defects, and gene rearrangements are less frequent (32/113).

In spite of the growing number of causative mutations identified in patients with GS, up to 40% of patients are still found to carry only a single mutation in *SLC12A3*, instead of being compound heterozygous or homozygous [10]. Because GS is inherited as a recessive trait, with heterozygous relatives of GS patients being clinically and metabolically asymptomatic, it is likely that there is a failure to identify the second mutant allele. Several explanations for this deficit of detection can be proposed. First, mutations may be located in regulatory fragments of the *SLC12A3*

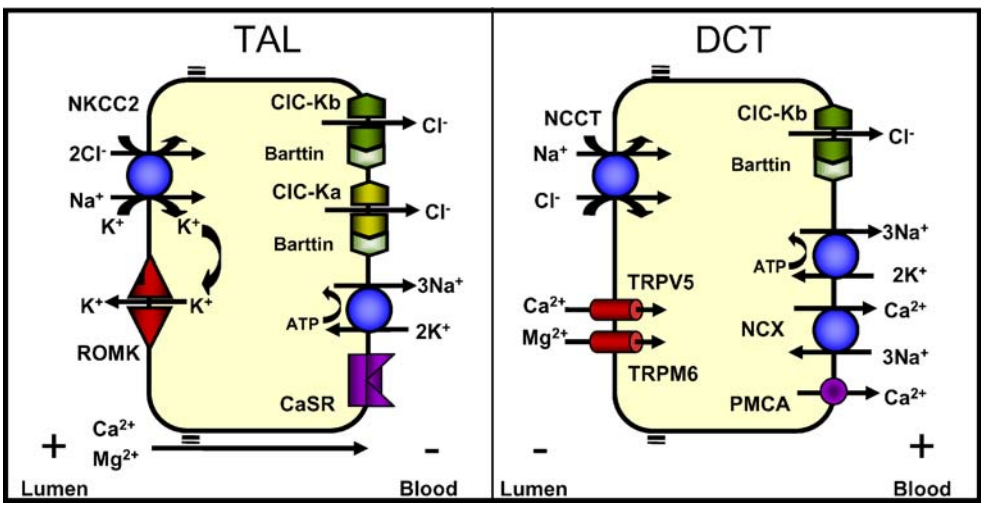

Fig. 1 Schematic representation of ion transporters operating in the cells lining the thick ascending limb (TAL) of Henle's loop and the distal convoluted tubule (DCT), supporting the phenotypical and molecular classification of salt-losing tubulopathies

Table 1 Classification of inherited salt-losing tubulopathies originating in the thick ascending limb of Henle's loop and the distal convoluted tubule. The classification is adapted from Jeck et al. [2], Reinalter et al. [4] and Phillips et al. [5] (*HPS* hyperprostaglandin-E syndrome, *aBS* antenatal Bartter's syndrome, *SND* sensorineural deafness, *cBS* classical Bartter's syndrome, *GS* Gitelman's syndrome, *TAL* thick ascending limb, *DCT* distal convoluted tubule)

Clinical disorder	Affected tubular segment	Analogy with diuretics	Bartter-like classification[a]	Mutated gene	Gene product
HPS (aBS)	TAL	Furosemide	I	*SLC12A1*	NKCC2
HPS (aBS)	TAL	Furosemide/amiloride (initial hyperkalemia, less marked hypokalemia)	II	*KCNJ1*	ROMK
HPS (aBS)+SND	TAL+DCT	Furosemide/thiazide (without hypercalciuria)	IV	*BSND*	Barttin[b]
cBS	DCT+TAL	Thiazide/furosemide (variable calciuria)	III	*CLCNKB*	ClC–Kb
GS	DCT	Thiazide	–	*SLC12A3*	NCCT

[a] This classification is based on the chronological order of gene discovery
[b] A digenic disorder with inactivating mutations of *CLCNKA* and *CLCNKB* has been associated with a phenotype indistinguishable from patients with barttin mutations

gene, 5′ or 3′UTRs, or in deeper intronic sequences which are not routinely screened for mutations. Second, there may be large genomic rearrangements, involving one or more exons that will not be identified by mutation detection techniques based on individual exons analysis. Third, the expression of the NCCT cotransporter could be influenced by epigenetic modifications and/or silent polymorphisms that could interfere with its function. Fourth, other genes may be involved in GS. Mutations in the *CLCNKB* gene encoding ClC-Kb have been detected in three unrelated patients presenting simultaneous features of cBS (presentation within the first year of life with episodes of dehydration) and GS [11] and in patients from a large inbred Bedouin family presenting a similar phenotype heterogeneity [12]. The distribution of ClC-Kb in both the TAL and DCT, and a potential compensation by ClC-Ka in the TAL, may probably explain these overlapping syndromes [2]. In any case, these studies show that GS is indeed genetically heterogeneous, raising the possibility of a concurrent heterozygous mutation in a gene other than *SLC12A3*. In addition to *CLCNKB*, other genes participating in the complex handling of NaCl, Ca^{2+} and Mg^{2+} in DCT, or its regulation, are potential candidates. Of note, mutations in *CLCNKB* have also been associated with the closely adjacent gene *CLCNKA*, encoding the chloride channel ClC-Ka, causing a digenic disease that mimics the aBS with SND phenotype observed in patients with inactive barttin [13].

Phenotype variability in Gitelman's syndrome

Classically, GS has been considered as a benign variant of salt-losing nephropathies, usually detected during adolescence or adulthood, often asymptomatic or presenting with mild symptoms, such as weakness, fatigue, salt carving, thirst, nocturia, or cramps. This view has been challenged by recent reports emphasizing the phenotype variability and the potential severity of the disease. In a detailed evaluation of 50 adult GS patients with proven mutations in *SLC12A3*, Cruz et al. [14] showed a high prevalence of patient-reported symptoms, with about half of the patients rating their symptoms as moderate to severe. Furthermore, GS was associated with a significant reduction in the quality of life—comparable in intensity to that associated with congestive heart failure or diabetes. Severe manifestations, such as early onset (before age 6 years), growth retardation, invalidating chondrocalcinosis, tetany, rhabdomyolysis, seizures, and ventricular arrhythmia have been described, although in a limited number of cases [14–16]. Based on the large number of patients with proven mutations in *SLC12A3*, the phenotype of GS is highly heterogeneous in terms of age at presentation, nature/severity of biochemical abnormalities, and nature/severity of the clinical manifestations (Table 2). The phenotype variability has been documented not only among all patients carrying a wide variety of *SLC12A3* mutations but also when a common underlying mutation is present [17] and within families [18]. Cardinal features of GS, such as hypocalciuria and hypomagnesemia, might also change during the life cycle of a given patient, reflecting dietary changes or compensatory mechanisms [2, 18].

A possible explanation for the intra-familial variability observed in GS patients is that gender may play a role, since it has been suggested recently that males are more severely affected than females [18]. That observation is supported by earlier studies showing that the density of NCCT in DCT can be influenced by estrogen in rats [19]. Such a positive effect of estrogen on the regulation of NCCT may explain the less severe phenotype observed in female patients carrying the same mutations as their affected brothers. Other potential factors include regulatory or modifier genes that could affect

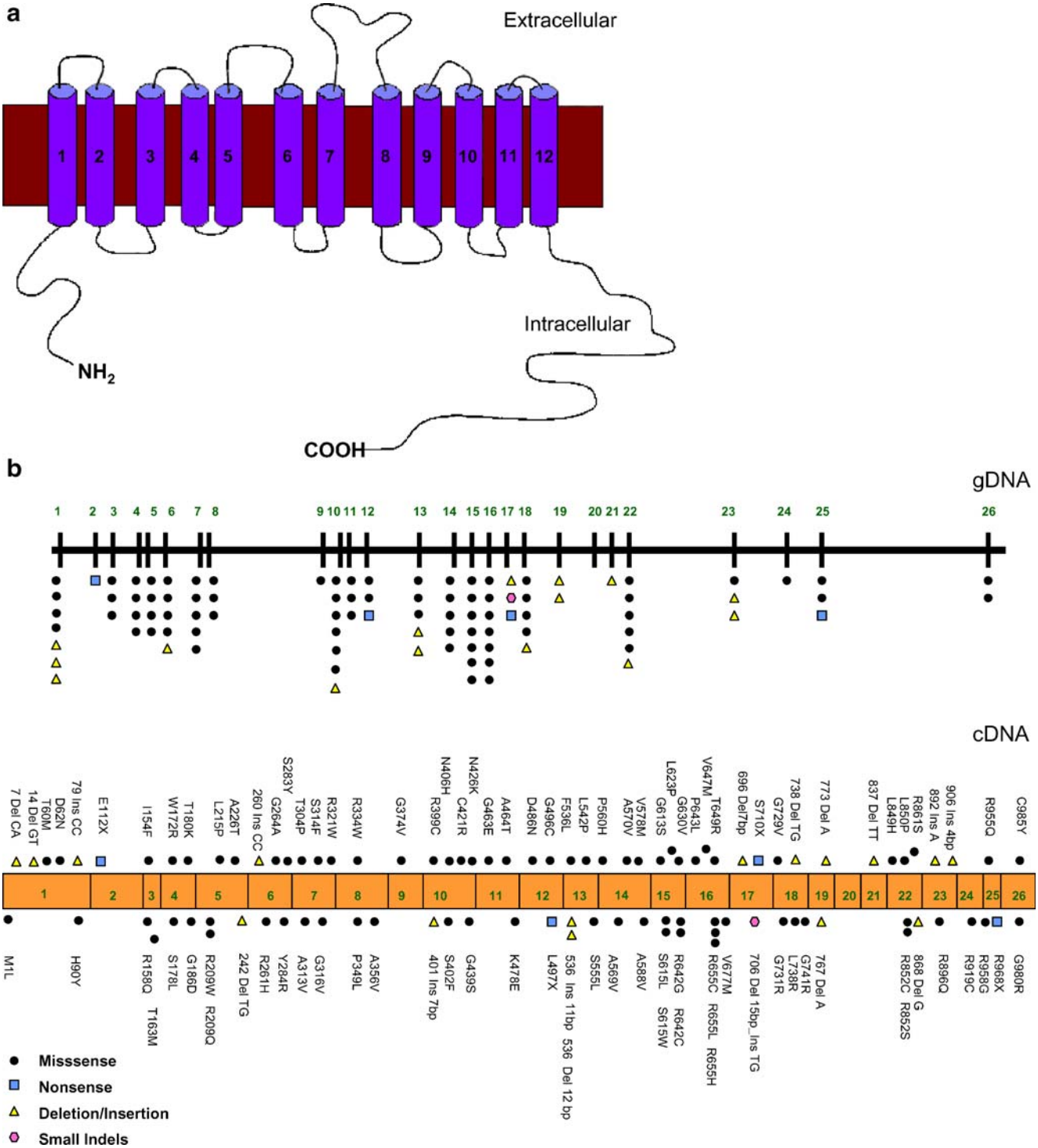

Fig. 2 **a** Predicted topology of the human NCCT cotransporter encoded by the *SLC12A3* gene. **b** Type of mutations in *SLC12A3* that have been associated with Gitelman's syndrome to date

the regulation or activity of NCCT, as well as environmental factors (intake of NaCl, Ca^{2+}, Mg^{2+}, ...) [2]. Compensatory mechanisms operating in other nephron segments should also be considered. For instance, recent studies have shown that the hypocalciuric response to thiazides is due to a compensatory increase in Na^{+} reabsorption and passive Ca^{2+} transport in the proximal tubule, secondary to the initial natriuresis due to NCCT inhibition in the DCT [20]. Provided it can be extrapolated to GS, the above compensatory mechanism could participate in phenotype variability.

More fundamentally, considering that most of patients with GS are compound heterozygous harbouring a wide

Table 2 Prevalence and nature of the clinical manifestations that have been associated with genetically proved Gitelman's syndrome. The prevalence data are compiled from Cruz et al. [14], Peters et al. [15], Pachulski et al. [16] and Lin et al. [18]

Most common (>50% of patients)	Prominent (20–50% of patients)	Occasional (<20% of patients)	Rare (case reports)
Salt carving	Fainting	Early onset (before age 6)[a]	Seizure
Cramps, muscle weakness, pain	Polyuria	Failure to thrive[a]	Ventricular tachycardia
Fatigue	Arthralgia	Growth retardation[a]	Rhabdomyolysis
Dizziness	Chondrocalcinosis	Vertigo, ataxia	Blurred vision
Nocturia	QTc prolongation	Carpopedal spasm, tetany	Pseudotumor cerebri
Thirst, polydipsia	Febrile episodes[a]	Vomiting	Sclerochoroidal calcification
Paresthesia, numbness		Constipation	
Palpitations		Enuresis	
Low blood pressure		Paralysis	

[a] In children

variety of mutant *SLC12A3* alleles (Fig. 2), the phenotype variability in GS raises the question whether peculiar symptoms could be related to the nature/position of the underlying mutation(s). In particular, nonsense, frameshift, splice-site defects and gene rearrangements will introduce premature translation stop codons that are likely to have a loss-of-function effect on the NCCT cotransporter. By analogy with other autosomal recessive disorders, one can predict that the combination of mutations present in each allele may also play a role in the phenotype variability of GS. In cystic fibrosis, for instance, several studies suggest that there are relationships between the CFTR genotype and the extent of lung and pancreatic disease [21].

Functional studies: heterologous expression systems

As discussed above, a potential mechanism for interfamilial phenotype heterogeneity in GS could be differences in the functional consequences of mutations in *SLC12A3*. Heterologous expression of NCCT using *Xenopus laevis* oocytes has permitted functional effects of GS mutants to be tested. Like many integral plasma membrane proteins, NCCT undergoes N-linked glycosylation, with a first step in the endoplasmic reticulum, followed by cleavage of some sugar residues and acquisition of more complex glycosylation in the Golgi apparatus, leading to the mature protein. The full glycosylation of NCCT is essential for its normal function (and thiazide binding) in the apical plasma membrane of cells lining the DCT [22]. Expression of mutant NCCT in *Xenopus* oocytes revealed the existence of two different types of mutations [23–25]. Class I mutants are considered as "non-functional", since the metolazone-sensitive $^{22}Na^{+}$ uptake rate is completely abolished. These mutant cotransporters show only partial glycosylation, resulting in the retention of the protein inside the cell, probably due to defective folding. By contrast, class II mutations are considered as "functional", since they show partial activity as metolazone-sensitive $^{22}Na^{+}$ uptake. These mutant proteins are fully glycosylated, but partially impaired in their routing to the cell surface. Using this system, we have recently identified a third class of mutations involved in GS. This class III includes mutants that are fully glycosylated and partly retained inside the cell, like "functional" mutants. Nevertheless, when the mutant cotransporter reaches the cell surface, it does not show any activity. The latter results imply that a processing defect of the mutant NCCT is not the only pathogenic mechanism underlying GS [26]. Furthermore, preliminary studies suggest that class I or class III mutants, associated with splicing mutants, are preferentially detected in a subset of GS patients with a severe phenotype [26]. It must be noted that expression studies using stably transfected Madin-Darby canine kidney (MDCK) cells, which express predominantly the complex glycosylated NCCT, may be an alternative to investigate the regulation of the apical trafficking of the transporter. However, the latter system appears to be limited by the relatively low expression levels obtained (the increase over background is below 25%) [27].

NCCT knockout mouse model

In order to better understand the role of NCCT and the pathophysiology of GS, Schultheis and co-workers generated a mouse model with a null mutation in the *Slc12a3* gene on a mixed background [28]. The NCCT null mice showed hypocalciuria and hypomagnesemia at baseline but, in marked contrast to the GS, no disturbances of K^{+} and acid-base homeostasis. The NCCT-deficient mice had no signs of hypovolemia on a standard Na^{+} diet, but they showed a lower blood pressure than wild-type when fed a Na^{+}-depleted diet for 2 weeks, suggesting a subtle hypovolemia compensated at baseline [28]. Subsequent

studies performed on colonies backcrossed into a homogeneous C57BL/6 background showed that NCCT null mice had a mild compensated alkalosis with increased levels of plasma aldosterone [29] and an increased sensitivity to develop hypokalemia when exposed to reductions in dietary K^+ [30]. Collectively, these studies demonstrated that mice lacking NCCT faithfully recapitulate many of the physiological findings observed in patients with GS. They helped to better understand the molecular mechanisms operating in the DCT, and confirmed the importance of secondary structural damage or tubular compensation that may blunt the primary defect [28–30]. Finally, they underlined the importance of the genetic background for the NCCT null phenotype, which is also potentially relevant for understanding phenotypic variability in GS.

Conclusions

Despite the remarkable clinical and molecular insights that have been provided since its initial description, the GS retains part of its mystery. The clinical features of the disease (including its potential severity) are increasingly recognized, and *SLC12A3* genotyping used to ascertain the diagnosis. However, we still do not understand why the phenotype of GS may vary to such extent, not only between patients harbouring different *SLC12A3* mutations but also between individuals harbouring the same mutation. Part of the individual phenotypic variability is probably explained by gender, regulatory or modifier genes, compensatory mechanisms, as well as environmental factors or dietary habbits. On the other hand, understanding how mutations in *SLC12A3* disturb the activity of NCCT remains of crucial importance in GS. Based on the functional classification obtained in heterologous expression systems, one could expect that different types of mutations will be reflected by distinct phenotypes. In parallel, the importance of the nature of the mutation cannot be minimized. Mutations that introduce stop codons in the initial part of the protein, or those that result in missplicing, frameshift and gene rearrangements, which are likely to have a loss-of-function effect, have not yet been characterized. Furthermore, such mutations could possibly lead to unstable transcripts, and changes in the level of mutant mRNA expression may also contribute to the phenotypic variability of the disease. Thus, further efforts are needed to substantiate the phenotype variability of GS and establish correlations with the *SLC12A3* genotype. These studies should include large cohorts of patients with careful phenotype evaluation, in relation to functional classification and molecular analysis of the mutant alleles. The tools for such detailed studies are readily available, ideally complemented by the increased clinical awareness for GS and salt-losing tubulopathies in general.

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References

1. Gitelman HJ, Graham JB, Welt LG (1966) A new familial disorder characterized by hypokalemia and hypomagnesemia. Trans Assoc Am Physicians 79:221–235
2. Jeck N, Schlingmann KP, Reinalter SC, Komhoff M, Peters M, Waldegger S, Seyberth HW (2005) Salt handling in the distal nephron: lessons learned from inherited human disorders. Am J Physiol Regul Integr Comp Physiol 288:R782–R795
3. Bettinelli A, Bianchetti MG, Girardin E, Caringella A, Cecconi M, Appiani AC, Pavanello L, Gastaldi R, Isimbaldi C, Lama G, Marchesoni C, Matteucci C, Patriarca P, DiNatale B, Setzu C, Vitucci P (1992) Use of calcium excretion values to distinguish two forms of primary renal tubular hypokalemic alkalosis: Bartter and Gitelman syndromes. J Pediatr 120:38–43
4. Reinalter SC, Jeck N, Peters M, Seyberth (2004) Pharmacotyping of hypokalaemic salt-losing tubular disorders. Acta Physiol Scand 181:513–521
5. Phillips DR, Ahmad KI, Waller SJ, Meisner P, Karet FE (2006) A serum potassium level above 10 mmol/l in a patient predisposed to hypokalemia. Nat Clin Pract Nephrol 2:340–346
6. Simon DB, Nelson-Williams C, Bia MJ, Ellison D, Karet FE, Molina AM, Vaara I, Iwata F, Cushner HM, Koolen M, Gainza FJ, Gitelman HJ, Lifton RP (1996) Gitelman's variant of Bartter's syndrome, inherited hypokalemic alkalosis, is caused by mutations in the thiazide sensitive Na-Cl cotransporter. Nat Genet 12:24–30
7. Hebert SC, Mount DB, Gamba G (2004) Molecular physiology of cation-coupled Cl^- cotransport: the SLC12 family. Pflugers Arch 447:580–593
8. Gamba G, Saltzberg SN, Lombardi M, Miyanoshita A, Lytton J, Hediger Ma, Brenner BM, Hebert SC (1993) Primary structure and functional expression of a cDNA encoding the thiazide-sensitive, electroneutral sodium-chloride cotransporter. Proc Natl Acad Sci USA 90:2749–2753
9. Reissinger A, Ludwig M, Utsch B, Prömse A, Baulmann J, Weisser B, Vetter H, Kramer HJ, Bokemeyer D (2002) Novel NCCT gene mutations as a cause of Gitelman's syndrome and a systematic review of mutant and polymorphic NCCT alleles. Kidney Blood Press Res 25:354–362
10. Lemmink HH, Knoers NV, Karolyi L, van Dijk H, Niaudet P, Antignac C, Guay-Woodford LM, Goodyer PR, Carel JC, Hermes A, Seyberth HW, Monnens LA, van den Heuvel LP (1998) Novel mutations in the thiazide-sensitive NaCl cotransporter gene in patients with Gitelman syndrome with predominant localization to the C-terminal domain. Kidney Int 54:720–730
11. Jeck N, Konrad M, Peters M, Weber S, Bonzel KE, Seyberth HW (2000) Mutations in the chloride channel gene, *CLCNKB*, leading to a mixed Bartter-Gitelman phenotype. Pediatr Res 48:754–758
12. Zelikovic I, Szargel R, Hawash A, Labay V, Hatib I, Cohen N, Nakhoul F (2003) A novel mutation in the chloride channel gene *CLCNKB* as a cause of Gitelman and Bartter syndromes. Kidney Int 63:24–32
13. Schlingmann KP, Konrad M, Jeck N, Waldegger P, Reinalter SC, Holder M, Seyberth HW, Waldegger S (2004) Salt wasting and deafness resulting from mutations in two chloride channels. N Engl J Med 350:1314–1319

14. Cruz DN, Shaer AJ, Bia MJ, Lifton RP, Simon DB; Yale Gitelman's and Bartter's Syndrome Collaborative Study Group (2001) Gitelman's syndrome revisited: an evaluation of symptoms and health-related quality of life. Kidney Int 59:710–717
15. Peters M, Jeck N, Reinalter S (2002) Clinical presentations of genotypically defined patients with hypokalemic salt-losing tubulopathies. Am J Med 112:183–191
16. Pachulski RT, Lopez F, Sharaf R (2005) Gitelman's not so benign syndrome. N Engl J Med 353:850–851
17. Coto E, Rodriguez J, Jeck N, Alvarez V, Stone R, Loris C, Rodriguez LM, Fischbach M, Seyberth HW, Santos F (2004) A new mutation (intron 9+1G>T) in the *SLC12A3* gene is linked to Gitelman syndrome in gypsies. Kidney Int 65:25–29
18. Lin SH, Cheng NL, Hsu YJ, Halperin ML (2004) Intrafamilial phenotype variability in patients with Gitelman syndrome having the same mutations in their thiazide-sensitive sodium/chloride cotransporter. Am J Kidney Dis 43:304–312
19. Verlander JW, Tran TM, Zhang L, Kaplan MR, Hebert SC (1998) Estradiol enhances thiazide-sensitive NaCl cotransporter density in the apical plasma membrane of the distal convoluted tubule in ovariectomized rats. J Clin Invest 101:1661–1669
20. Nijenhuis T, Vallon V, van der Kemp AW, Loffing J, Hoenderop JG, Bindels RJ (2005) Enhanced passive Ca^{2+} reabsorption and reduced Mg^{2+} channel abundance explains thiazide-induced hypocalciuria and hypomagnesemia. J Clin Invest 115:1651–1658
21. Welsh MJ, Ramsey BW, Accurso F, Cutting GR (2001) Cystic fibrosis. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The metabolic and molecular bases of inherited disease. McGraw-Hill, New York, pp 5121–5188
22. Hoover RS, Poch E, Monroy A, Vazquez N, Nishio T, Gamba G, Hebert SC (2003) N-Glycosylation at two sites critically alters thiazide binding and activity of the rat thiazide-sensitive Na(+):Cl(−) cotransporter. J Am Soc Nephrol 14:271–282
23. Kunchaparty S, Palcso M, Berkman J, Velazquez H, Desir GV, Bernstein P, Reilly RF, Ellison DH (1999) Defective processing and expression of thiazide-sensitive Na-Cl cotransporter as a cause of Gitelman's syndrome. Am J Physiol 277:F643–F649
24. de Jong JC, van der Vliet WA, van den Heuvel LP, Willems PH, Knoers NV, Bindels RJ (2002) Functional expression of mutations in the human NaCl cotransporter: evidence for impaired routing mechanisms in Gitelman's syndrome. J Am Soc Nephrol 13:1442–1448
25. Sabath E, Meade P, Berkman J, de los Heros P, Moreno E, Bobadilla NA, Vazquez N, Ellison DH, Gamba G (2004) Pathophysiology of functional mutations of the thiazide-sensitive Na-Cl cotransporter in Gitelman disease. Am J Physiol Renal Physiol 287:F195–F203
26. Riveira-Munoz E, Dahan K, Godefroid N, Chang Q, HoenderopJG, Bindels RJ, Devuyst O (2006) A new class of mutations involved in Gitelman's syndrome affects the intrinsic activity of the Na-Cl cotransporter NCCT. Nephrol Dial Transplant 21:iv6
27. de Jong JC, Willems PH, van den Heuvel LP, Knoers NV, Bindels RJ (2003) Functional expression of the human thiazide-sensitive NaCl cotransporter in Madin-Darby canine kidney cells. J Am Soc Nephrol 14:2428–2435
28. Schultheis PJ, Lorenz JN, Meneton P, Nieman ML, Riddle TM, Flagella M, Duffy JJ, Doetschman T, Miller ML, Shull GE (1998) Phenotype resembling Gitelman's syndrome in mice lacking the apical Na+–Cl− cotransporter of the distal convoluted tubule. J Biol Chem 273:29150–29155
29. Loffing J, Vallon V, Loffing-Cueni D, Aregger F, Richter K, Pietri L, Bloch-Faure M, Hoenderop JG, Shull GE, Meneton P, Kaissling B (2004) Altered renal distal tubule structure and renal Na(+) and Ca(2+) handling in a mouse model for Gitelman's syndrome. J Am Soc Nephrol 15:2276–2288
30. Morris RG, Hoorn EJ, Knepper MA (2006) Hypokalemia in a mouse model of Gitelman's syndrome. Am J Physiol Renal Physiol 290:F1416–F1420