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REVIEW

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The molecular genetic approach to "Bartter's syndrome"

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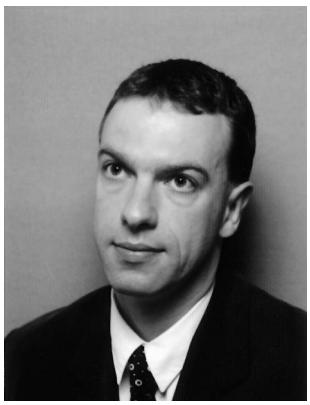

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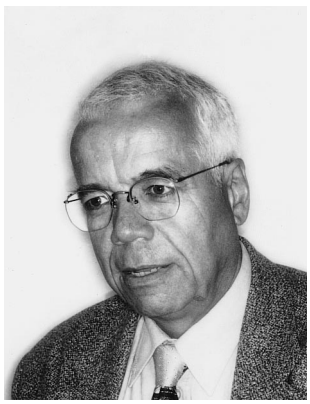

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Abstract The term "Bartter's syndrome" comprises a set of autosomal recessively inherited renal tubular disorders characterized by hypokalemia, metabolic alkalosis, hyperreninism, and hyperaldosteronism but normal blood pressure. Additional clinical and biochemical features led to a classification into phenotypically different tubulopathies: Gitelman's syndrome, hyperprostaglandin E syndrome (antenatal Bartter's syndrome), and classic Bartter's syndrome. Gitelman's syndrome results from mutations in the *SLC12A3* gene encoding the human thiazide-sensitive sodium chloride cotransporter, leading to impaired reabsorption of sodium chloride in the distal convoluted tubule. Genetic heterogeneity of hyperprostaglandin E syndrome has been demonstrated by identification of mutations in the *SLC12A1* gene as well as in the *KCNJ1* gene. Mutations in *SLC12A1* coding for the bumetanide-sensitive sodium potassium 2 chloride cotransporter (NKCC2) cause defective reabsorption of sodium chloride in the thick ascending limb of Henle's loop. Mutations in *KCNJ1* leading to loss of function of the potassium channel ROMK disrupt potassium recycling back to the tubule lumen and inhibit thereby the NKCC2 activity. A third gene for hyperprostaglandin E syndrome has been mapped to the short arm of chromosome 1, and it remains to be evaluated whether other genes are involved in the pathogenesis of this disease. Classic Bartter's syndrome has been demonstrated to result from defective chloride transport across the basolateral membrane in the distal nephron due to mutations in the chloride channel gene *CLCNKB*. This article reviews the molecular genetic approach that has led to identification of genetic defects underlying the different hypokalemic tubulopathies.

Key words Bartter's syndrome · Antenatal Bartter's syndrome · Hyperprostaglandin E syndrome · Gitelman's syndrome · *SLC12A1* · *SLC12A3* · *KCNJ1* · *CLCNKB*

Abbreviations *NCCT* Sodium chloride cotransporter · *NKCC2* Sodium potassium 2 chloride cotransporter

Introduction

The term Bartter's syndrome comprises a spectrum of hereditary renal tubular disorders characterized by chronic hypokalemia and metabolic alkalosis. In 1962 Bartter and colleagues reported on two patients who presented with hypokalemic metabolic alkalosis, hyponatremia, hypochloremia, and normal blood pressure despite of hyperaldosteronism [1]. Histology of the kidneys revealed hyperplasia of the juxtaglomerular apparatus in both patients. In the following years numerous studies described a variety of additional clinical and biochemical anomalies which occur in association with hypokalemic metabolic alkalosis [2–4]. The variety of additional findings led to a classification into at least three phenotypically different hypokalemic tubulopathies which have been referred to as different variants of Bartter's syndrome: (a) Gitelman's syndrome, or familial hypokalemia-hypomagnesemia syndrome, (b) hyperprostaglandin E syndrome (antenatal Bartter's syndrome), and (c) so-called classic Bartter's syndrome [5–7]. The various hypokalemic tubulopathies have been reported to be familial, and the inheritance pattern is most consistent with autosomal recessive transmission [8, 9]. On the pathophysiological level many alterations have been identified, but despite extensive investigations the pathogenesis of the various disorders remained uncertain [5, 10]. Advances of molecular biology offered a new pathway to elucidate the molecular basis of these diseases. Several genes had been cloned whose products are involved in renal electrolyte homeostasis. Based on preexisting information from pathophysiological studies these genes were thought to be candidates for the different hypokalemic tubulopathies. This review describes the molecular genetic approach that was successfully used to localize and identify genes involved in various phenotypes of Bartter's syndrome.

Phenotypic variability of "Bartter's syndrome"

Gitelman's syndrome

In 1966 Gitelman and colleagues reported on three patients with hypokalemic metabolic alkalosis associated with hypomagnesemia and impaired renal conservation of magnesium and potassium [2]. This disorder subsequently designated as Gitelman's syndrome, or familial hypokalemia-hypomagnesemia syndrome, is inherited as an autosomal recessive trait, although genetic heterogeneity with autosomal dominant transmission in a subset of families has been discussed [8, 11]. The prevalence of the disease is estimated to be 1:50,000 [12].

Gitelman's syndrome has frequently been referred to as a variant of Bartter's syndrome since laboratory features including hypokalemia, metabolic alkalosis, hyperreninism and hyperaldosteronism are the same as those described in Bartter's original report [11, 13]. Gitelman's syndrome patients, however, can be discriminated from those with classic Bartter's syndrome and hyperprostaglandin E syndrome by permanently decreased serum magnesium levels and characteristic hypocalciuria [2, 14]. Typically the renal concentrating capacity is normal or slightly diminished in patients with Gitelman's syndrome [13, 14]. Urinary excretion of prostaglandin E_2 has been reported to be within the normal range, and prostaglandin synthetase inhibitors seem to be of limited benefit in these patients [13, 15]. The clinical course of Gitelman's syndrome is relatively mild, with a disease onset from childhood to juvenile age or even later. Patients may be completely asymptomatic, and diagnosis is sometimes made when serum electrolytes are measured [11, 13]. Clinical findings include occasional episodes of muscle weakness, tetany, carpopedal spasms, paresthesia, chronic dermatitis, and short stature [2, 11, 13, 14]. Gitelman's syndrome appears to have a benign prognosis with respect to renal function [13].

Hyperprostaglandin E syndrome (antenatal Bartter's syndrome)

Hyperprostaglandin E syndrome is the most severe form of the autosomal recessive hypokalemic tubulopathies. The prevalence of this disease is estimated to be about 1:50,000–1:100,000 [9, 16]. Affected individuals exhibit hypokalemic metabolic alkalosis and hyperrenemic hyperaldosteronism but normotensive blood pressure, thus resembling the patients in Bartter's original report. Characteristic additional features include hypercalciuria and isosthenuria [3, 17–19]. Based on the findings of increased renal and systemic prostaglandin E_2 synthesis and the effective treatment with cyclo-oxygenase inhibitors the term hyperprostaglandin E syndrome was introduced to describe this syndrome [7, 18, 20]. The disorder typically manifests itself in utero with marked fetal polyuria, leading to polyhydramnios between the 22th and 30th week of gestation, premature labor, and preterm delivery [3, 21]. The neonatal period is characterized by severe renal loss of salt and water [3, 7, 21, 22]. During the first weeks or months of life most infants develop nephrocalcinosis [3, 16, 23]. Further clinical features include polyuria, polydipsia, and recurrent episodes of fever and vomiting, and patients often fail to thrive [5, 7, 24]. Recently, sensoneurinal deafness has been reported in patients with hyperprostaglandin E syndrome [9, 25]. Our own observations indicate that sensoneurinal hearing impairment may be as frequent as 10% in patients with this disorder (Károlyi and Seyberth, unpublished data).

Classic Bartter's syndrome

Among the hypokalemic tubulopathies classic Bartter's syndrome is the most heterogeneous and least defined disorder. Typically the diagnosis of classic Bartter's syndrome is considered when criteria for Gitelman's syndrome or hyperprostaglandin E syndrome are not ful-

filled [6, 7]. Some patients exhibit only the core features of Bartter's original description, with chronic hypokalemic alkalosis, hyperrenemic hyperaldosteronism but normal blood pressure [1]. Serum magnesium concentrations are normal but may be intermittently slightly diminished. Nephrocalcinosis is an uncommon finding, and investigation of renal concentrating ability shows no gross abnormality [6]. In other patients precise clinical diagnosis may be difficult because of additional features overlapping the different tubulopathies.

The clinical course of patients with classic Bartter's syndrome is relatively benign with respect to renal electrolyte loss and volume depletion. Patients can be completely asymptomatic or present with muscular weakness, fatigue, or growth retardation [6]. In most cases the diagnosis is not made before school age or adolescence. The prevalence of classic Bartter's syndrome is unknown, and inheritance pattern best fits with autosomal recessive transmission.

Pathophysiology and candidate genes

Numerous studies have identified a variety of abnormalities at the pathophysiological level in the different hypokalemic tubulopathies, but clearly different pathomechanisms could not be identified [5, 10]. Several patterns of aberrant renal ion transport have been described which suggested a defect of sodium chloride reabsorption in either the ascending limb of the loop of Henle [26, 27], the proximal [28] or early distal [29] tubule, or the cortical collecting duct [30]. Other studies report normal urinary chloride excretion and abnormal renal potassium handling to be the more prominent feature [10, 17, 23]. Thus information on the pathophysiology of these disorders suggested several genes which had been cloned in humans or other mammalian species as candidates for molecular studies. These candidate genes included genes coding for sodium, chloride or potassium channels expressed in kidney, as well as members of the electroneutral renal sodium-(potassium)-chloride cotransporter family [31, 32].

The pathogenetic role of prostaglandins in hypokalemic tubulopathies has been much debated [27, 33, 34]. Excessive renal and systemic synthesis of prostaglandin E_2 has been well documented in patients with hyperprostaglandin E syndrome [4, 20]. Prostaglandins are known to inhibit sodium chloride reabsorption in the thick ascending limb and inhibit renal concentrating ability [35, 36]. Therefore the candidate genes also included genes whose products are involved in prostaglandin metabolism. Vascular hyporesponsiveness to the pressure activity of angiotensin has been described in various patients [3, 19]. Therefore the gene encoding the angiotensin II type I receptor was screened for mutations in five patients diagnosed as having Bartter's syndrome [37]. In one of these patients a sequence abnormality was found that predicted a single amino acid substitution in the angiotensin receptor, but the study could not demonstrate that this sequence variant was disease related [37].

Mutations in the *SLC12A3* gene causing Gitelman's syndrome

Typical features of autosomal recessive Gitelman's syndrome resemble the state induced by administration of thiazide diuretics [38]. In consequence it was hypothesized that the defect in Gitelman's syndrome affects a similar region of the cortical distal tubule as that affected by thiazides [38, 39]. From rat kidney and flounder urinary bladder cDNAs encoding an absorptive thiazide-sensitive sodium-chloride cotransporter (NCCT) had been cloned [32, 40]. In rats NCCT is expressed in the renal cortex, consistent with localization in apical cell membranes in the distal convoluted tubule [32]. To test this cotransporter as a candidate for Gitelman's syndrome the human NCCT cDNA was cloned, and genomic clones containing the corresponding gene (*SLC12A3*) were identified [41, 42]. Fluorescence in situ hybridization and linkage analysis in reference families (CEPH) with a genetic marker close to the *SLC12A3* gene assigned the human locus to chromosome 16q13 [41, 42]. Families affected with Gitelman's syndrome showed significant linkage with microsatellite markers close to this locus [41]. Locus homogeneity of Gitelman's syndrome has been confirmed in subsequent linkage studies, and there is no evidence for a further gene locus so far [43–45].

The exon-intron organization of the *SLC12A3* gene has been determined and revealed that the human NCCT is encoded in 26 exons. Sequence analysis predicted a cotransporter protein comprising 1,021 amino acids and containing 12 putative transmembrane domains with long intracellular amino- and carboxytermini [41, 42]. A screening for mutations was performed in affected individuals, and various mutations were detected that segregated with the disease phenotype, and that were proven to be absent in normal controls [41, 46]. These mutations include base substitutions, altering single amino acids at residues that are highly conserved among species or affecting the exon-intron splice sites. Furthermore, nonsense mutations introducing a premature stop codon as well as deletions or insertions causing a frameshift were identified [41, 44, 46, 47] (Fig. 1). About 100 different mutations have been reported so far which are spread evenly throughout the *SLC12A3* gene. In some kindreds one of the parents of the index case was found to be affected with Gitelman's syndrome, suggesting an autosomal dominant mode of inheritance [11]. However, molecular analysis of one of these families demonstrated the respective parent to be compound heterozygous for mutations in the *SLC12A3* gene, while the affected children were either homozygous or compound heterozygous, having inherited one mutation from each parent [44, 46]. Therefore the inheritance pattern in this family confirms autosomal recessive inheritance of the disease.

The identification of mutations in *SLC12A3* has demonstrated the molecular basis of Gitelman's syndrome. Frameshift mutations and splice-site mutations lead to a truncated polypeptide, resulting in an altered or absent

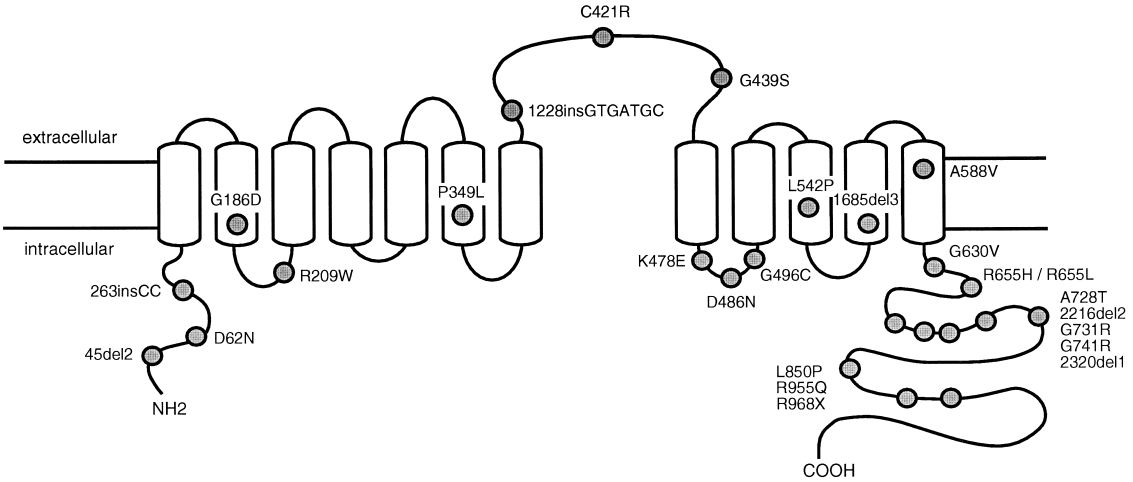

Fig. 1 Protein structure of the thiazide-sensitive sodium chloride cotransporter (NCCT or TSC) and location of amino acid substitutes identified in patients with Gitelman's syndrome [41, 44, 46]. *Circles*, positions of mutations

protein product. These mutations are predicted to result in loss of cotransporter function, leading to impaired reabsorption of sodium chloride in the distal convoluted tubule in patients with Gitelman's syndrome. The effects of single point mutations on protein function remain to be investigated in functional tests.

Mutations in the *SLC12A1* gene causing hyperprostaglandin E syndrome (antenatal Bartter's syndrome)

By analogy to Gitelman's syndrome, a candidate gene based approach was used to clone a gene responsible for other hypokalemic tubular disorders [48]. The administration of loop diuretics such as furosemide produces electrolyte disturbances similar to those seen in patients with hyperprostaglandin E syndrome, providing evidence of impairment of a specific tubular function at least in a subset of these patients [49, 50]. From rat, rabbit, and mouse kidney an absorptive bumetanide- and furosemide-sensitive sodium potassium 2 chloride cotransporter (NKCC2) had been cloned [32, 51, 52]. NKCC2 is expressed in apical cell membranes in the thick ascending limb of the loop of Henle [51, 52]. To investigate this cotransporter for its potential role in hypokalemic tubulopathies the homologous human gene (*SLC12A1*) was cloned [48].

Characterization of the exon-intron organization of the *SLC12A1* gene demonstrated that the human NKCC2 is encoded by 26 exons and predicts a cotransporter protein of 1,099 amino acid residues with 12 potential membrane-spanning helices flanked by large cytosolic domains at the amino- and carboxytermini [48]. The genomic locus of *SLC12A1* has been assigned to chromosome 15q15-q21 [48]. Significant genetic linkage with microsatellite markers from this chromosomal region was shown in a set of families with hyperprostaglandin E syndrome, providing strong evidence for a defect in the *SLC12A1* gene in these families [48]. However, linkage analyses also revealed genetic heterogeneity of this disorder since not all families showed linkage to this gene locus [53, 54]. Affected individuals were screened for mutations in the *SLC12A1* gene. Different mutations were identified which segregated with the disease phenotype and were not observed in unaffected controls (Fig. 2) [48]. The mutations that were detected include point mutations that alter highly conserved amino acid residues as well as insertions or deletions causing a frameshift [48]. Whether specific point mutations result in lost or significantly reduced cotransporter function has not been investigated in functional studies yet. The frameshift mutations that have been identified lead to a truncated protein product and will cause a loss of function of NKCC2.

The results of these molecular studies demonstrate that defects in the *SLC12A1* gene coding for the bumetanide-sensitive NKCC2 cause hyperprostaglandin E syndrome. Loss of cotransporter function leads to impaired potassium-coupled reabsorption of sodium chloride in the thick ascending limb of the loop of Henle. These findings are consistent with impaired response to the administration of furosemide, as has been demonstrated in patients affected with this syndrome [55].

Genetic heterogeneity of hyperprostaglandin E syndrome revealed by mutations in the *KCNJ1* gene

Genetic heterogeneity of hyperprostaglandin E syndrome (antenatal Bartter's syndrome) had been demonstrated by linkage analyses in affected families, providing evidence for a further gene in which defects may cause this disease [53, 54]. Sodium chloride reabsorption in the thick ascending limb via the NKCC2 depends on adequate supply of luminal potassium. The ATP-regulated inwardly rectifying potassium channel ROMK (Kir 1.1) recycles the potassium entering the cells via the NKCC2 back to the tubule lumen and is therefore essential for the function of this cotransporter [56]. Therefore the gene encoding this

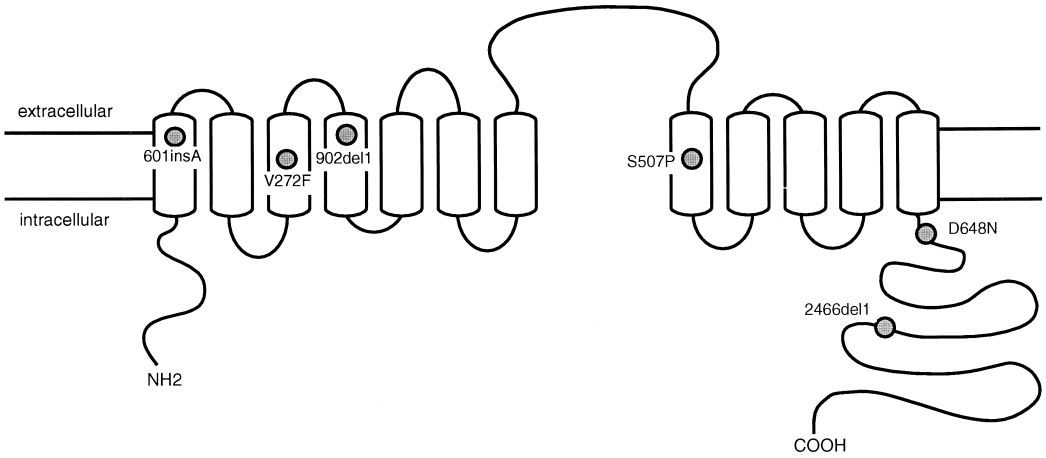

Fig. 2 Structural model of the human bumetanide-sensitive sodium potassium 2 chloride cotransporter protein (NKCC2 or BSC) and location of amino acid residues affected by mutations in patients with hyperprostaglandin E syndrome (antenatal Bartter's syndrome) [48]. *Circles*, mutations

potassium channel was a further candidate for hyperprostaglandin E syndrome. From rat and human kidney ROMK cDNAs had previously been cloned [57–59]. In humans the *KCNJ1* gene coding for the ROMK channel contains five exons which produce five distinct transcripts [58, 59]. These transcripts predict three ROMK proteins which share a core sequence of 372 amino acids encoded in exon 5 and vary in length from 372 to 391 amino acids at their NH_2-termini [58, 59]. The core structure of the ROMK proteins features two transmembrane spanning domains, a pore-forming domain, and intracelluar amino- and carboxytermini. In rats ROMK isoforms are differentially expressed in the macula densa and in apical membranes from medullary thick ascending limb to cortical collecting duct [60, 61]. In distal nephron segments ROMK isoforms have been proposed to participate in potassium secretion [60]. In humans no data exist about differential expression of the ROMK isoforms.

The genomic locus of the *KCNJ1* gene has been localized to human chromosome 11q24-11q25 by fluorescence in situ hybridization [59, 62]. Families with hyperprostaglandin E syndrome in which linkage to the *SLC12A1* gene had been excluded were typed with microsatellite markers spanning the region containing *KCNJ1* [54]. Haplotype analysis in these families provided evidence for a defect in this region in the families studied [54]. This was confirmed by identification of mutations in the *KCNJ1* gene which were shown to segregate with the disease phenotype and were not found in unaffected controls [53, 54]. The mutations comprise substitutions of single bases that either lead to single amino acid substitutions or introduce a premature stop codon, and deletions or insertions causing a frameshift [53, 54]. The mutations affect amino acid residues of the transmembrane segments, the pore-forming domain, the putative ATP-binding domain as well as the amino- and carboxytermini of the ROMK channel protein (Fig. 3) [53, 54].

All mutations that have been reported so far are located in exon 5 of the *KCNJ1* gene, therefore altering amino acid residues contained in all predicted isoforms of the channel. [53, 54]. One of the mutations identified to be homozygous in a patient with hyperprostaglandin E syndrome causes a substitution of glutamic acid for glycine at position 167 in the second transmembrane segment [54]. Prior in situ mutagenesis studies had suggested this amino acid residue to face the channel pore and to be essential for its geometrical structure [63]. The observed mutation implies that it alters the channel pore and eliminates the channel function [54]. Other mutations introduced in rat ROMK cDNA and expressed in COS-7 cells have been demonstrated to abolish or significantly impair electrophysiological properties of the channel protein [64]. Loss of function of the ROMK channel prevents recycling of potassium across the apical membrane from cells back into the tubule lumen. In the thick ascending

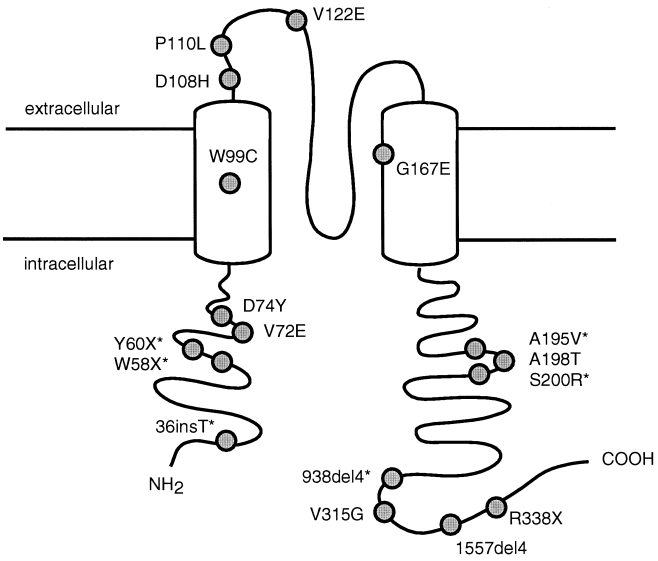

Fig. 3 Schematic drawing of the renal potassium channel ROMK1 and location of mutations in patients with hyperprostaglandin E syndrome (antenatal Bartter's syndrome) [53, 54]. *Star*, mutations taken from [53] in which nomenclature is based on ROMK isoform 2

Table 1 Phenotypes of hypokalemic tubulopathies that have been referred to as variants of Bartter's syndrome and corresponding genes and proteins in which defects have been identified

Phenotype	Gene	Defective Protein
Gitelman's syndrome	SLC12A3	Sodium-chloride cotransporter (NCCT, TSC)
Hyperprostaglandin E-syndrome (antenatal Bartter's syndrome)	SLC12A1	Sodium potassium 2 chloride cotransporter (NKCC2, BSC)
	KCNJ1	Potassium channel (ROMK)
	Unidentified (gene locus chromosome 1p)	Unknown
	Unknown	Unknown
Classic Bartter's syndrome	CLCNKB	Chloride channel (hClC-Kb)

limb inadequate supply with intraluminal potassium inhibits the activity of the NKCC2, resulting in defective potassium-coupled reabsorption of sodium-chloride in this nephron segment and renal salt wasting.

Initial clinical data suggest that patients with hyperprostaglandin E syndrome and a defect in ROMK have milder hypokalemia than patients with a defect in NKCC2 [53, 64]. This could be explained by assuming that inhibition of potassium secretion in distal nephron segments via ROMK causes a less severe potassium loss, or that potassium recycling in the thick ascending limb is partially maintained by other potassium channels [64, 65]. However, not in all patients with hyperprostaglandin E syndrome have defects in either the NKCC2 or the ROMK potassium channel been identified, indicating that there may exist further mechanisms involved in the pathogenesis of this disorder [53, 54, 66].

A third gene for hyperprostaglandin E syndrome maps to chromosome 1p

A third gene for hyperprostaglandin E syndrome has recently been mapped to chromosome 1p32.1-p35 by positional cloning in a large inbred Bedouin family [67]. In this family hyperprostaglandin E syndrome was reported to be associated with sensoneurinal hearing loss [25]. The association between the two disorders has been proposed to be due to a pleiotropic effect of a single genetic defect or coincidental inheritance of two disease genes at this locus [25]. This hypothesis requires further investigation since no candidate gene in this chromosomal region has been identified so far. Furthermore, future studies must evaluate whether this gene locus accounts for hyperprostaglandin E syndrome in patients who are thought to have a defect in neither the *SLC12A1* nor the *KCNJ1* gene [53, 54].

Mutations in the *CLCNKB* gene causing classic Bartter's syndrome

Laboratory features of patients with classic Bartter's syndrome including hypokalemic metabolic alkalosis, occasionally low serum magnesium levels, and a relatively mild clinical course may resemble the phenotype of Gitelman's syndrome. However, on the molecular level classic Bartter's syndrome has been shown to be genetically distinct from Gitelman's syndrome by linkage analyses in affected families [43]. Mutations in the chloride channel gene *CLCNKB* have recently been demonstrated in patients with hypokalemic metabolic alkalosis and hyper- or normocalciuria without nephrocalcinosis [66]; serum magnesium levels were reported to be normal. Unfortunately, this article provided no information on the antenatal period.

The mutations in these patients comprised large deletions as well as missense and nonsense mutations in *CLCNKB* on chromosome 1p36 [66, 68, 69]. These mutations are predicted to significantly impair transport of reabsorbed chloride from epithelial cells across the basolateral membrane via the chloride channel hClC-Kb in the distal nephron [31, 70].

Conclusions

Appplication of molecular genetics to inherited hypokalemic tubulopathies has led within 2 years to the identification of genetic defects underlying the pathogenesis in different forms of Bartter's syndrome (Table 1). Gitelman's syndrome results from mutations in the *SLC12A3* gene encoding the human thiazide-sensitive NCCT, leading to impaired reabsorption of sodium chloride in the distal convoluted renal tubule. Gitelman's syndrome appears to be a genetically homogeneous disease without evidence for an additional gene locus. Hyperprostaglandin E syndrome has been confirmed to be genetically a heterogeneous disorder arising from mutations either in the *SLC12A1* or the *KCNJ1* gene. Mutations in *SLC12A1* coding for the bumetanide-sensitive NKCC2 cause defective potassium-coupled reabsorption of sodium chloride in the thick ascending limb of Henle's loop. Loss of function mutations in the *KCNJ1* gene disrupt potassium recycling via the potassium channel ROMK from the cells back to the tubule lumen and inhibit thereby the NKCC2 activity. A third gene for hyperprostaglandin E syndrome has been mapped to chromosome 1p, and it re-

mains to be evaluated whether this disease can also arise from defects in other genes involved in the regulation of renal salt transport.

Mutations in the chloride channel gene *CLCNKB* underlie the pathogenesis of classic Bartter's syndrome, causing defective chloride transport across the basolateral membrane from epithelial cells in the distal tubule.

In the future molecular genetic methods will permit a precise diagnosis for these disorders and will allow patients to be classified unambiguously. A diagnosis on the molecular level can be established either by identification of mutations in affected individuals or by genotyping larger families with polymorphic genetic markers tightly linked to the different genes. For the *SLC12A3* gene an intragenic tetranucleotide microsatellite marker is available, making it possible to type families suspected to have Gitelman's syndrome without restrictions due to recombination events [46]. The mutations that have been identified so far suggest that each family has its private mutation making mutation screening time consuming and labor intensive. New mutation detection techniques may help to overcome this problem in the future [71]. The desirability of performing prenatal diagnostics by molecular methods in hyperprostaglandin E syndrome, for example, with regard to intrauterine treatment, remains debatable. In this disorder polyhydramnios in affected individuals is a characteristic feature and can be detected by ultrasonography.

Further studies will have to evaluate the contribution of additional genetic factors to associated findings such as sensoneurinal hearing loss. Hearing loss in patients with hyperprostaglandin E syndrome has been thought to be due to a to a pleiotropic effect of a genetic defect or coincidence of two genetic defects [25]. However, it remains unclear whether this complication is a consequence of preterm delivery, or whether it results from episodes of severe electrolyte imbalance and massive dehydratation.

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