The Bartter-Gitelman Spectrum: 50-Year Follow-up With Revision of Diagnosis After Whole-Genome Sequencing

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Abstract

Bartter syndrome (BS) and Gitelman syndrome (GS) are renal tubular disorders affecting sodium, potassium, and chloride reabsorption. Clinical features include muscle cramps and weakness, in association with hypokalemia, hypochloremic metabolic alkalosis, and hyperreninemic hyperaldosteronism. Hypomagnesemia and hypocitruria are typical of GS, while juxtaglomerular hyperplasia is characteristic of BS. GS is due to SLC12A3 variants, whereas BS is due to variants in SLC12A1, KCNJ1, CLCNKA, CLCNKB, BSND, MAGED2, or CASR. We had the opportunity to follow up one of the first reported cases of a salt-wasting tubulopathy, who based on clinical features was diagnosed with GS. The patient had presented at age 10 years with tetany precipitated by vomiting or diarrhea. She had hypokalemia, a hypochloremic metabolic alkalosis, and normal or low blood pressure despite hyperreninemia and hyperaldosteronism. Hypomagnesemia and hypocalciuria are typical of GS, while hypocalciuria is characteristic of BS. GS is due to mutations of BSND (Barttin CLCNK-type accessory channel) encoding Kir1.1 (ATP-dependent potassium channel; also referred to as renal outer medullary channel); BS type 4A (MIM: 602522) affects less than 10% of patients and is due to mutations of BSND (Barttin CLCNK-type accessory channel). BS, which represents a group of genetically heterogeneous disorders with 7 subtypes reported, and GS are caused by variants in genes encoding ion transporters or channels most commonly causing loss of function (Table 1). BS type 1 (MIM: 601678) affects approximately 25% of patients and is associated with mutations of SLC12A1 (solute carrier family 12 member-1) encoding NKCC2 (sodium-potassium-2 chloride cotransporter); BS type 2 (MIM: 241200) affects 10% to 25% of patients and is due to mutations of KCNJ1 (potassium channel, inwardly rectifying, subfamily-J, member-1) encoding K_1.1 (ATP-dependent potassium channel; also referred to as renal outer medullary channel); BS type 3 (MIM: 607364) affects approximately 20% to 30% of patients and is due to mutations of CLCNKB (chloride channel, kidney-B) encoding CLC-Kb (voltage-gated chloride channel-Kb); BS type 4A (MIM: 602522) affects less than 10% of patients and is due to mutations of BSND (Barttin CLCNK-type accessory channel).
subunit-beta) encoding an essential subunit for voltage-gated chloride channel-Ka (CLC-Ka) and CLC-Kb; BS type 4B (MIM: 613090) affects less than 5% of patients who also have sensory nerve deafness due to mutations of both CLCNKA and CLCNKB encoding CLC-Ka and CLC-Kb respectively; BS type 5 (MIM: 300971), which occurs as a transient fetal form in approximately 10% of BS patients, is due to mutations of MAGED2 (melanoma-associated antigen family member-D2); and BS occurring in association with autosomal dominant hypocalcemia (ADH) (MIM: 601198), formerly referred to as BS type 5, results from gain-of-function mutations of CASR (calcium-sensing receptor) [4, 5, 9-12]. GS in approximately 80% to 95% of patients is associated with mutations in SLC12A3 encoding NCCT (thiazide-sensitive Na-Cl cotransporter); KCNJ10, sodium-potassium-2 chloride cotransporter; SLC12A1, solute carrier family 12 member-1; SLC12A3, solute carrier family 12 member-3; XR, X-linked recessive.

Abbreviations: ADH, autosomal dominant hypocalcemia; BS, Bartter syndrome; BSND, Barttin CASR, calcium-sensing receptor; CLC-Ka, voltage-gated chloride channel-Ka; CLC-Kb, voltage-gated chloride channel-Kb; CLCNK-type accessory subunit-beta; CLCNKA, chloride channel, kidney-A; CLCNKB, chloride channel, kidney-B; DAR, digenic autosomal recessive; FXYD2, FXYD-domain containing ion transport regulator-2; GS, Gitelman syndrome; HNF1B, hepatocyte nuclear factor-1 homeobox-B; KCNJ1, potassium channel, inwardly rectifying, subfamily-J, member-1; KCNJ10, potassium channel, inwardly rectifying, subfamily-J, member-10; Kir1.1, ATP-dependent potassium channel; Kir4.1, ATP-sensitive inward rectifier potassium channel 10; MAD, monogenic autosomal dominant; MAGED2, melanoma-associated antigen family member-D2; MAR, monogenic autosomal recessive; MIM, Mendelian Inheritance in Man; NaK, sodium/potassium)-transporting ATPase subunit-gamma; NCCT, thiazide-sensitive Na-Cl cotransporter; NKCC2, sodium-potassium-2 chloride cotransporter; SLC12A1, solute carrier family 12 member-1; SLC12A3, solute carrier family 12 member-3; XR, X-linked recessive.

**Table 1. Genetic basis of types of Bartter syndrome and Gitelman syndrome**

| Disorder | MIM* | Gene | Protein | Inheritance |
|---------|------|------|---------|-------------|
| BS type 1 | 601678 | SLC12A1 | NKCC2 | MAR |
| BS type 2 | 241200 | KCNJ1 | K_1.1 | MAR |
| BS type 3 | 607364 | CLCNKB | CLC-Kb | DAR |
| BS type 4A | 602522 | BSND | Barttin | MAR |
| BS type 4B | 613090 | CLCNKA and CLCNKB | CLC-Ka | MAR |
| BS type 5 | 300971 | MAGED2 | MAGE-D2 antigen | XR |
| BS in association with ADH | 601198 | CASR | CASR | MAD |
| GS | 263800 | SLC12A3 | NCCT | MAR |
| GS-like | 601678 | KCNJ10, FXYD2, and HNF1B | K_4.1, NaK ATPase subunit gamma, HNF1B | MAR |

*All entries within Online Mendelian Inheritance in Man (OMIM: OMIM.org) are given a unique and stable MIM number.

Materials and Methods

Ethical Considerations

Informed consent and leukocyte DNA samples were obtained from the proband and unrelated healthy individuals using protocols approved by Multicentre Research Ethics Committee (UK) (No. MREC/02/2/93) and local and national ethics committees (Australia).

Genome Sequencing and Variant Confirmation

Leukocyte DNA was extracted from venous blood (Gentra Puregene blood kit, Qiagen) and assessed for integrity by agarose gel electrophoresis. WGS was performed on an Illumina HiSeqX machine, reads mapped to hg19 and variants called with GATK HaplotypeCaller v3.4 and filtered based on variant quality, population allele frequency, and effect on encoded protein [24]. Variants were confirmed by DNA Sanger sequence analysis using polymerase chain reaction products generated using CLCNKB.
forward (5’-AAATCCTGCCCCGACGCTTA-3’) and re-
verse (5’-AGTGTTATAGGGAAGTGCCCCC-3’) primers
(Life Technologies); the BigDye Terminator v3.1 Cycle
Sequencing Kit (Life Technologies); and automated detection
system (ABI3730 Automated capillary sequencer; Applied
Biosystems). Further validation was performed by AvaII
(New England Biolabs) restriction endonuclease digest ana-
lysis of polymerase chain reaction products. The frequency
of the CLCNKB variant was assessed in public databases
dbSNP and gnomAD v2.1.1.

Bioinformatic Analysis of Homozyogosity
Possible continuous regions of homozygosity (ROH) were de-
tected in each autosome based on a hidden Markov model
and the genotype likelihoods as implemented in bcftools roh
v1.1 [25] using allele frequencies and genetic maps from the
1000G phase 3 data set [24].

Results
Patient and Clinical Findings
A 10-year-old girl, whose parents were reportedly
nonconsanguineous and came from Nisyros in the Greek
Dodecanese island group, presented with 2 years of repeated
episodes of cramps in her hands and feet with Trouseau and
Chvostek signs; and abdominal pain associated with inter-
current viral infections and sometimes eating licorice, which
can result in apparent mineralocorticoid excess [23, 26,
27]. Physical examination was normal with blood pressure
110/70. Serum biochemical results indicated hypokalemia
with hypochloremic metabolic alkalosis, in association with
hyponatremia, mild hypercalcemia, and normomagnesemia
(Table 2) [23]. However, she subsequently developed
hypocalciuria and hypomagnesemia (0.5 mmol/L). A renal
biopsy was reported as normal with no evidence of juxtaglo-
merular hyperplasia, which is characteristic of BS [1, 28]. GS
was diagnosed based on clinical features and she was treated
with nonsteroidal anti-inflammatory drugs and continued
oral potassium and magnesium supplementation [26].

She remained relatively asymptomatic with no manifest-
tions of pseudogout or ectopic calcification, but had 1 mis-
carriage following appendicitis, followed by 2 uneventful
pregnancies [26]. She underwent partial gastrectomy at age
34 years for a large gastric ulcer secondary to nonsteroidal
anti-inflammatory use. At age 55 years she had developed
chronic renal impairment, and a renal biopsy demonstrated
moderate parenchymal injury consisting of tubular atrophy
and interstitial scarring. Furthermore, she developed: type
2 diabetes mellitus; secondary hyperparathyroidism, osteo-
porosis, and vitamin D deficiency; glucose-6-phosphate de-
hydrogenase (G6PD) deficiency with hemolytic anemia from
Pyridium (phenazopyridine hydrochloride); poor bladder
control managed by a pacemaker; melanosis coli with colonic
polyps; and depression.

The patient complained of sore eyes without visual symp-
toms, and was referred for ophthalmic examination at age
66 years and again at age 68 years, after which scleral cal-
cification was noted incidentally on computed tomography
brain scanning. On examination, visual acuity was 6/7.5 in
each eye. Anterior segments were unremarkable, with
no cataracts. Fundoscopy demonstrated focal yellow chor-
oidal deposits superior to the arcades in both eyes (Fig. 1).
Furthermore, optical coherence tomography, B-scan ultra-
sound, and computed tomography scanning demonstrated
striking sclerochoroidal calcification (SCC) calcification in
both eyes (see Fig. 1), which has been associated with both
BS and GS [29, 30].

At age 68 years the patient weighed 54 kg, blood pressure
was 135/80 with no postural drop, and pulse was 60/minute

| Table 2. Serum clinical biochemistry of proband |
|-----------------------------------------------|
|                                            | Aged 10 y pretreatment | Normal range in children | Aged 68 y on treatment | Normal range in adults |
| Sodium, mmol/L                              | 129-144              | 133-144                   | 143                   | 135-145                |
| Potassium, mmol/L                           | 1.9-3.0              | 3.5-5.3                   | 3.8                   | 3.5-5.5                |
| Chloride, mmol/L                            | 84-97                | 98-111                    | 96                    | 95-110                 |
| Bicarbonate, mmol/L                         | 29-38                | 19-28                     | 33                    | 20-32                  |
| Calcium corrected for albumin, mmol/L       | 2.75                 | 2.10-2.56                 | 2.59                  | 2.15-2.55              |
| Phosphate, mmol/L                           | 2.9                  | 0.6-1.9                   | 0.85                  | 0.8-1.5                |
| Magnesium, mmol/L                           | 0.98                 | 0.64-1.09                 | 0.77                  | 0.7-1.05               |
| Creatinine, μmol/L                          | 119                  |                            | 45-85                 |                        |
| Urea, mmol/L                                | 10.4                 | 1.6-6.0                   | 10.9                  | 3.0-8.5                |
| Albumin, g/L                                | 44                   |                            | 33-44                 |                        |
| Parathyroid hormone, pmol/L                 | 12.5                 |                            | 1.6-6.9               |                        |
| Vitamin D, nmol/L                           | 83                   |                            | 50-250                |                        |
| Cholesterol, mmol/L                         | 6.2-7.2              | 2.8-6.0                   | 6.7                   | 3.5-5.5                |
| Triglycerides, mmol/L                       | > 3.75               | 0.4-2.1                   | 4.7                   | < 1.5                  |
| Serum pH                                     | 7.45                 | 7.35-7.45                 | 41                    | 90-120                 |
| Glomerular filtration rate, mL/min/1.7 m²   | 135                  | 85-150                    | 2770                  | 100-950                |
| Aldosterone, upright, pmol/L                | > 480                |                            | 3.3-41                |                        |
| Aldosterone:renin ratio                     | > 6                  |                            | < 70                  |                        |
and regular. Examination was unremarkable with no peripher al edema. Biochemical results indicated elevated serum aldosterone and renin concentrations (see Table 2). Imaging of the parathyroids and thyroid revealed no abnormalities. Her medications included potassium chloride extended-release 2.4 g twice a day orally (123 mmol/24 hours), magnesium 480 mg three times a day orally, sodium bicarbonate 100 mg twice a day orally, amiloride 10 mg twice a day orally, metformin 1 g twice a day orally, rosuvastatin 40 mg orally daily, iron polymaltose 100 mg IM, ostein 1 daily (vitamin D and/or calcium), and denosumab 60 mg subcutaneous every 6 months. SCC was being managed conservatively, and dry eye syndrome with lid hygiene, hypromellose 3mg/g, and carbomer-980 2.2mg/g eye gel. She was also recommended to cease smoking.

At age 72 years, her weight has increased to 63 kg with a healthy body mass index of 23.0. Her serum cholesterol, high-density lipoprotein cholesterol (HDLC), low-density lipoprotein cholesterol, cholesterol/HDLC, and non-HDLC levels were within the normal range, whereas her serum triglycerides remained high at 2.0 mmol/L (normal range < 1.5 mmol/L). It is unlikely that the type 2 diabetes mellitus and dyslipidemia are related to the salt-wasting condition, although diabetic patients can develop a range of electrolyte disorders that had occurred in this patient and included hyponatremia, hypokalemia, hypercalcemia, hypocalcemia, hypomagnesemia, and hypophosphatemia [31].

**Genetic Analysis**

WGS analysis of leukocyte DNA from the patient revealed an absence of abnormalities in SLC12A3, KCNJ10, FXYD2, and HNF1B, which are reported to be associated with GS, and in KCNJ1 or BSND, which are associated with BS type 2 and BS type 4A, respectively (see Table 1). Moreover, variants with an allele frequency (AF) of 16% to greater than 50% were identified in genes associated with BS types 1, 4B, 5, and ADH, thereby indicating that these commonly occurring variants were unlikely to be the cause of the disease in the patient. Thus, the variants with an AF greater than 50% in Europeans included SLC12A1 (n = 2), which is associated with BS type 1; CLCNKA (n = 3), which is associated with BS type 4B; CLCNKB (n = 5), which is associated with BS type 4B; and CASR (n = 2), which is associated with ADH. A MAGED2 variant with an AF of 16% in Europeans, which is associated with BS type 5, was also found. In contrast, WGS analysis identified a homozygous C-to-T transition rare (AF < 1%) variant at nucleotide c.226 in exon 3 of CLCNKB (NM_000085.5), located on chromosome 1p36.13. The C-to-T transition, which was confirmed by DNA Sanger sequence analysis (Fig. 2A), predicted the occurrence of a nonsense mutation (p.Arg76Ter) of the CLC-Kb protein and a loss of an AvaII restriction endonuclease site (Fig. 2B). Subsequent restriction endonuclease digest analysis confirmed the presence of the homozygous CLCNKB c.226C > T variant in the proband while 3 unrelated wild-type controls had only wild-type alleles (see Fig. 2B). Furthermore, this CLCNKB variant (c.226C > T), which is present only as a rare, heterozygous variant with allele frequencies of 0.00003 and 0.00001 in dbSNP (rs370985865) (n = 17 950 individuals) and gnomAD v2.1.1 (n = 125 563 individuals), respectively, was the sole candidate variant fitting with an autosomal recessive mode of inheritance. Thus, the homozygous occurrence of this rare CLCNKB c.226C > T variant in the patient, and its absence as a homozygote in more than 140 000 individuals in dbSNP and gnomAD v2.1.1, supported its pathogenic role in causing the disease of BS type 3 in the patient.

The patient was sequenced as a singleton because the parents were not available, and so phasing of variants to confirm compound heterozygosity was not possible. In addition, an examination for genes harboring at least 2 high-confidence heterozygous variants with moderate in silico support, using a Combined Annotation Dependent Deletion (CADD) score of greater than 20, which is typical of the top 1% deleterious variants in the human genome, identified only a single gene, HMCN1 (Hemicentin-1; NM_031935.3), with 2 rare variants that were both missense changes p.(Q1174E;p2332R) with an AF less than 1%. However, HMCN1 is reported to be associated with age-related macular degeneration and therefore does not represent a strong candidate in comparison to the known pathogenic p.Arg76Ter variant in CLCNKB. Thus, based on the American College of Medical Genetic and Genomics and the Association for Molecular Pathology guidelines [32], the CLCNKB c.226C > T (p.Arg76Ter) nonsense variant would be placed in the pathogenic, very strong 1 (PVSI) category. The PVSI category includes null variants (nonsense, frameshift, canonical ± 1 or 2 splice sites, initiation...
codon, single or multiexon deletion) in a gene whose loss of function is a known mechanism of the disease, and this is consistent with a pathogenic role for the CLCNKB c.226C>T (p.Arg76Ter) nonsense variant in BS type 3, as it is predicted to result in loss of approximately 90% of the protein (Fig. 2C), with likely loss of its function in facilitating the flow of chloride currents through the channel, which is a known mechanism for the disease. Moreover, the CLCNKB c.226C>T variant has also been reported i) as a homozygous p.Arg76Ter mutation in 2 unrelated patients with BS type 3; and ii) in 3 unrelated patients with BS type 3 as compound heterozygous mutations with p.Val170Met, p.Trp610Ter, and a...
c.1228-1G > A mutation involving the splice acceptor site at the boundary of CLCNKB intron 12 and exon 13, which is predicted to result in an in-frame deletion of 30 amino acids [33-36]. In addition, the homozygous CLCNKB p.Arg76Ter variant has been reported in ClinVar (ID975076.2) to be associated with familial epilepsy, hypocaliuria, and proteinuria, although plasma magnesium and calcium concentrations were not reported, and hypomagnesemia and hypocalcemia, associated with GS and BS, are known metabolic causes of seizures [37].

The CLCNKB c.226C>T transition, and it is possible this transition may have involved deamination of a methylated cytosine (5-methylcytosine), resulting in formation of a thymine [38]. Thus, occurrence of the homozygous CLCNKB mutation in the patient may be due to 2 mutations arising de novo via deamination of 5-methylcytosines on independent haplotypes; or 1 mutation arising de novo with the other being inherited from a parent; or both mutations being inherited from heterozygote parents. We considered this latter possibility of parental heterozygosity for the CLCNKB p.Arg76Ter variant most likely because the parents, although reportedly nonconsanguineous, came from the Greek island of Nisyros with fewer than 1500 inhabitants, thereby increasing the likelihood of sharing a common ancestor. Since DNA from both deceased parents was unavailable, we analyzed WGS data from the proband for occurrence of genome-wide ROH, and also near the CLCNKB locus. This revealed 13 ROH greater than 1 Mb [39] and 3 of these, including the largest observed ROH comprising 8.7 Mb, were located on chromosome 1p34.3 to q36.21 and in the vicinity of CLCNKB (Fig. 2D). Another 5.1-Mb ROH was found on chromosome 6 [39, 40]. ROH greater than 4 Mb are uncommon in demonstrably outbred individuals [41], and our findings of 2 ROH greater than 4 Mb suggest a degree of unreported parental consanguinity due to a shared ancestor, which is not surprising given the small population of their island origin. Thus, distant relatedness of the parents with heterozygosity of the CLCNKB p.Arg76Ter mutation appears a plausible explanation for the homozygous mutation.

**Discussion**

The diagnosis of GS, as the cause of the salt-wasting tubulopathy in this patient approximately 60 years ago, led to her becoming one of the first individuals described with this condition [23, 26]. However, as a result of our analysis that revealed a homozygous nonsense mutation (c.226C>T; p.Arg76Ter) in CLCNKB, a revised diagnosis of BS type 3 is warranted based on current classification.

The parents of the proband are reportedly nonconsanguineous. However, they originate from a Greek island with fewer than 1500 inhabitants, and may share a common ancestor who had the CLCNKB mutation. ROH tracts greater than 4 Mb are rare in demonstrably outbred individuals [41], and studies of consanguineous families have suggested that pathogenic homozygous variants are overrepresented within the 10 largest ROH tracts in a genome of a child with related parents [42]. Thus, identification of an 8.7-Mb ROH on chromosome 1 proximal to a 1.1-Mb ROH containing CLCNKB, and another 5.1-Mb ROH on chromosome 6 is consistent with the suggestion the patient’s parents are distantly related. Thus, the CLCNKB p.Arg76Ter may be more common in the Grecian Dodecanese group and lead to a higher incidence of BS type 3.

The original report of BS described 2 patients with hypokalemic alkalosis associated with hyperreninemic hyperaldosteronism and juxtaglomerular hyperplasia, but normal blood pressure [1]. Symptoms included failure to thrive, severe polyuria, and polydipsia due to a renal concentrating defect [1]. Subsequently, in another patient cohort, a second nephropathy was identified comprising loss of renal magnesium, intermittent episodes of tetany, normal urinary concentration, minimal involvement of the renin-angiotensin system, and an unusually low urinary calcium, that was termed GS to distinguish it from BS [2, 26]. However, as more cases of GS and BS type 3 (classic BS) have been reported, it is clear there is considerable overlap between these conditions [4, 5, 43, 44]. For example, hypomagnesemia and hypocaliuria, once considered pathognomonic for GS, have since been reported as features of BS [45]. Furthermore, while magnesium and calcium concentrations in blood and urine were not reported in Bartter’s original description, tetany, carpopedal spasms, and positive Chvostek signs, which are symptoms of hypomagnesemia, were present in one of Bartter’s index cases, although these features could also be attributed to hypokalemia [46].

Thus, our patient has some phenotypic features more commonly associated with GS, namely low urinary calcium excretion, and other features such as renal failure and tubulointerstitial atrophy and interstitial fibrosis, which are uncommon in GS but more prevalent in BS type 3. Furthermore, similar to the clinical progression in our patient, a report of a mixed BS-GS phenotype in 3 unrelated patients described the coexistence of hypomagnesemia and hypocaliuria that was absent at presentation but subsequently detected, reflecting a transition from BS type 3 to GS [43].

These phenotypic overlaps may indicate physiological cooperation of the apical NCCT and basolateral CLC-Kb for salt reabsorption in the distal convoluted tubule (DCT). Since ion transport mechanisms are coupled to each other, loss-of-function mutations affecting one element of transepithelial transport may lead to the breakdown of absorption in affected epithelial cells. Coexistence of hypomagnesemia and hypocaliuria in GS, and less frequently in BS type 3, suggests that the dissociation of renal calcium and magnesium handling may not always be caused by NCCT dysfunction as originally thought, but possibly because of a more general impaired transepithelial salt reabsorption in the DCT involving CLC-Kb [3].

The current terminology of BS subgroups is based on the chronological order of discovery rather than their pathophysiological basis and clinical presentation. Our data support the revised classification of salt-losing tubulopathies proposed by Seyberth [3], in which milder forms of BS type 3 and GS are grouped into a single disease category distinct from more severe forms of BS. This classification thus reflects differences in DCT dysfunction (GS and BS type 3), loop disorders (BS type 1 and 2), and compound disorders (BS type 4A and 4B).

SCC, associated both with BS and GS [29, 30], is not associated with progressive visual loss, but is associated with disturbed calcium metabolism. Systemic calcification, hypomagnesemia, and chronic kidney disease, which are
symptoms found in this patient, have been reported to be associated with long-term use of proton pump inhibitors [47-49]; however, this patient had not been treated with proton pump inhibitors in the preceding 10 years before SCC was detected. Therefore, genetic analysis and identification of a causative mutation were useful in excluding salt-losing conditions that occur secondary to nongenetic causes.

In summary, GS and BS type 3 have overlapping phenotypes making diagnosis based on phenotypic data alone challenging. Indeed, a recent study simultaneously sequencing 37 genes in 174 children with BS or GS revealed a discrepancy in clinical and genetic diagnosis in 10 children, with 3 cases of clinically diagnosed GS revised to BS type 3 following genetic identification of a CLCNKB mutation [11]. Molecular diagnosis is therefore valuable in diagnosing disorders with overlapping clinical and laboratory manifestations.

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Disclosures

The authors have nothing to disclose.

Data Availability

The data that support the findings of the study are available from the corresponding author on reasonable request.

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