Bartter syndrome (BS) is a heterogeneous disorder, caused by mutations in several genes which mostly encode proteins involved in ions transportation across renal cells in the thick ascending limb of the nephron. It is characterized by deficient renal reabsorption of sodium and chloride, which results in a group of certain symptoms. Different types of BS can be distinguished from different clinical manifestations, and most importantly, via analyzing possible affected gene(s) for its confirmation. A close associated syndrome which was primarily considered as a mild variant of BS, Gitelman syndrome (GS), is characterized by hypokalemic metabolic alkalosis with hypocalciuria, and hypomagnesemia. In this review, we discuss different features of BS and also GS, including clinical and genetic alterations which correspond to each type.

**Keywords:** Bartter Syndrome; Molecular Genetics; Child.
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confirmation. A close associated syndrome which was primarily considered as a mild variant of BS, Gitelman syndrome (GS), is characterized by hypokalemic metabolic alkalosis with hypocalciuria, and hypomagnesemia [8]. In this review, we discuss different features of BS and also GS, including clinical and genetic alterations which correspond to each type. Briefly and interestingly, the clinical and genetics classification of BS have been developed in parallel and consistently.

Pathology of Bartter syndrome

BS consists of a number of different inherited tubular disorders that each result from impairment of different parts of the transportation machinery of the thick ascending Henle’s loop and/or distal convoluted tubule, leading to salt-losing and volume depletion disorders [9]. BS was first categorized to two major types of neonatal and classical. Neonatal BS (also called Hyperprostaglandine E syndrome [10]) is the most severe form, which is mostly characterized by polyhydramnios, premature birth, growth retardation, intrauterine and postnatal polyuria concomitant with intense dehydration, and recurrent vomiting [11]. Of other symptoms, hypercalciuria, nephrocalcinosis, and sometimes end-stage renal failure and deafness can be noted [12-15]. In some cases, special appearances like a triangular-shaped face with a distinguished forehead, large eyes, strabismus, a drooping mouth, protruding ears, emaciation, and small muscles can be present [16,14]. Osteopenia, diarrhea, fever, and increases susceptibility to infection are also systematic symptoms of neonatal BS [17]. Neonatal BS is generally an autosomal recessive (AR) disorder, from which a great genetic heterogeneity has been reported. This includes types I and II of BS [7] which can be clinically distinguished from one another. In both cases, symptoms like nephrocalcinosis and hypercalciuria are markedly detectable, while massive renal NaCl excretion, metabolic alkalosis, and hypokalemia mostly exist in type I patients [18]. This speculation may not be always right, as patients bearing SLC12A1 mutations may be diagnosed after the neonatal period [19]. Classical BS, however, manifests milder symptoms, starting from infancy to adolescence. This type has been categorized as type III BS, which has been reported to be transmitted in an AR manner [20]. Classical BS is generally associated with growth retardation (in the absence of early therapy), polyuria, polydysplasia, constipation, salt craving, vomiting, dehydration, and less frequently facial appearances [7]. Increased fractional excretion of Na, K, and Cl are the most typical findings. In addition, the presence of normal or mildly high Ca excretion is important as it may lead to distinction from GS, in which hypocalciuria is always present [21].

GS is mostly asymptomatic; however, transitory occurrences of weakness and tetany, concomitant with abdominal pain, fever, and vomiting have been reported in some cases. In addition, growth retardation and polyuria are mild or absent, and chondrocalcinosis is occasionally reported. The prolonged intervals without symptoms may result in the diagnosis during adult age for many cases [22-24]. Prematurity or the lack of maternal polyhydramnios are important factors to distinct GS from classical BS. Hypomagnesemia, hypokalemia, and modest metabolic alkalosis, as well as a moderate elevation in uric acid, are among detectable blood features. Notably, renal function is normal. GS can be misdiagnosed by classical BS, when hyperreninism and hyperaldosteronism are present. In those cases, normal excretion of prostaglandin E2 (PGE2) can be considered as one of the differentiations [25,7]. GS is also transmitted as an AR disorder [8]. Beside types I, II, and III, two other types (IV and V) are classified beyond neonatal or classical types, as BS with sensorineural deafness and BS associated with autosomal dominant (AD) hypocalcemia, respectively. It was centrally based on the finding of two additional genes, BSND and CASR, which are reported to cause BS with the unifying pathophysiology, loss of salt transportation by the thick ascending limb, showing the great impact of genetics on the classification of BS [26-28]. Differences in the phenotypes of BS patients are associated to the specific roles of each gene in the kidney and other organs, which may lead to specific diagnosis and treatment.

Genetics of Bartter Syndrome

BS represents a group of heterogeneous diseases consisting of several AR syndromes plus an AD type. Different genes are reported to be associated with different types of BS whose alterations, individually or sometimes in combination, lead to this heterogeneity. Genetic based classification of BS has led to emergence of five different types, plus GS as an individual territory. Notably, each type of BS can be diagnosed by considering certain clinical features, which may lead to analysis of corresponding genes for confirmation and thus
result in defined procedures for specialized treatments. The phenotype-genotype correlation in different types of BS has been indicated, leading to genetic categorization of BS [28-32].

**Type I**

BS type I (BS-I) (neonatal BS) is a life-threatening disease, with both renal hypokalemic alkalosis and intense systemic symptoms. The abnormalities like polyhydramnios between 24-30 weeks of gestation (resulting from fetal polyuria) and premature delivery can be observed in the neonatal period. In this period, high concentrations of chloride, versus normal sodium, potassium, calcium, and PGE2, are observed in the amniotic fluid. Neonates with BS-I have intense salt wasting and hypochloremia, plus hyperprostaglandinuria, failure to thrive, and moderate hypokalemic metabolic alkalosis. In the affected infants, vomiting, fever, and diarrhea have been ascribed to the stimulation of the renal and systemic PGE2 activity. These conditions can be suitably treated by inhibition of PGE2 synthesis [33,34,10].

Linkage of BS to a particular gene, which led to its distinction from GS, was first reported by Simon and colleagues who showed some functions of SLC12A1 gene [35]. SLC12A1 is located on chromosome 15q15, has a length of about 113 kb and 26 exons, and produces 8 protein coding products as a result of alternative splicing. Sequencing the whole coding regions of this gene is the first molecular procedure in order to find possible mutation and confirm BS-I.

**SLC12A1** belongs to the Na⁺-dependent subgroup of solute carrier 12 (SLC12) family of transporters, and encodes renal Na-K-2Cl co-transporter (also known as NKCC2). SLC12A2 is highly expressed in the kidney, and is responsible for the vast majority of NaCl reabsorption in the apical membrane of epithelial cells in the thick ascending limb of Henle [35]. SLC12A1 couples with its close member SLC12A2 to actively mediate the electroneutral transportation of K⁺ and Na⁺, together with transportation of Cl⁻ across the cell membrane. Therefore, the result of SLC12A1 disruption is hypercalciuria and severe volume depletion with early presentation in BS patients. Some observed (frameshift and nonsense) mutations in highly conserved regions of SLC12A1 co-segregate with the disease.

SLC12A1 is regulated by with-no-lysine kinase (WNK)-3 and angiotensin II, which may play important roles in fine-tuning of renal transepithelial NaCl transportation and kidney associated modulation of hypertension via phosphorylation by OSR1 (odd-skipped related transcription factor 1), respectively [36,37]. SLC12A1 may be blocked by bumetanide and furosemide, which are commonly used loop diuretic drugs [38,39].

**Type II**

BS Type II (BS-II) (neonatal BS) is an AR hypokalemic renal salt-wasting disease caused by alterations in the KCNJ1 (also known as ROMK), which encodes KCNJ1 channel. KCNJ1 protein plays crucial roles in potassium recycling located in the thick ascending limb of Henle’s loop, and potassium secretion in the distal tubule and cortical collecting duct. Infants with BS-II are transiently hyperkalemic, which results from KCNJ1 channel dysfunction in potassium secretion in the distal convoluted tubule and cortical collecting duct. BS-II is described by symptoms like elevated Ca²⁺ excretion leading to nephrocalcinosis, hypokalemic metabolic alkalosis, natriuresis, diuresis, and elevated prostaglandin E2 and renin [40-42,30].

KCNJ1 channel is responsible for a remarkable portion of reabsorption of sodium and potassium that are unfiltered at glomerulus, and conflacts the pathway for potassium recycling that is essential for SLC12A1 co-transporter [43,44]. It was first speculated that in kindreds of BS who did not show mutations in SLC12A1, dysfunctional regulators of the co-transporter might also lead to BS, as well. Interestingly and notably, as the first evidence of the heterogeneity of BS [41], KCNJ1 mutation showed the latter. In order to regulate renal potassium handling, KCNJ1 interacts with WNK kinases [45,46], Src family protein tyrosine kinase [47], POSH (plenty of SH3) [48], and protein kinase C (PKC) [49].

KCNJ1 is located on chromosome 11q24, has a length of about 34 kb, and consists of just two exons. It produces seven different isoforms, among which there are six protein codings. Sequencing the entire coding region of KCNJ1 is the first molecular procedure for mutation detection, and therefore for proving BS-II. Mutations in KCNJ1 drastically alter the conformation of the channel, resulting in loss of potassium channel activity through the phosphorylation sites alteration, or frameshift the gene open reading frame (ORF) [41].

Prenatal diagnosis is available through biochemical examinations of amniotic fluid and maternal urine, in which electrolytes in the amniotic fluid is shown to be high, except for...
potassium. On the other hand, urinary chloride, calcium, and sodium are low, suggesting predicting parameters in prenatal diagnosis of BS-II [50].

**Type III**

BS Type III (BS-III) or classical BS is an AR disorder, which is centrally characterized by hypokalaemic alkalosis with salt-wasting, low blood pressure, normal magnesium, hyper/normocalciuria, and absence of nephrocalcinosis. Patients harbor mutations in the renal chloride channel gene, \(CLCNKB\), which cause loss of function and consequently impairment in the reabsorption of chloride in the thick ascending limb of the Henle’s loop [20]. \(CLCNKB\), which encodes a member of the family of voltage-gated chloride channels, is located on chromosome 1p36, has a length of about 14 kb, and consists of 20 exons. Resulting from alternative splicing, three different isoforms are produced from this gene. A close member of this family, \(CLCNKA\), is also located in close proximity and has shown to play roles in the pathogenesis of the disease. Different kinds of alterations including missense or nonsense mutations, and large deletions have been reported as loss of function mutations. In some cases, large deletions have been driven from unequal crossing over between \(CLCNKB\) and \(CLCNKA\) [20]. Notably, it has been recently shown that in mild phenotypes, certain mutations can lead to specific behaviors in the channel. Mutations around the selectivity filter can result in hyperactivity of heterologous expression systems, and decreased surface expression [51,52]. Certain polymorphisms/mutations in \(CLCNKB\) gene has been shown to be associated to essential hypertension [53].

**Type IV**

BS type IV (BS-IV), which is the most severe life-threatening among different types of BS, is an AR disorder generally characterized by neonatal volume depletion, premature birth imputable to polyhydramnios, sensorineural deafness, severe renal salt-wasting, and polyuria [14,27]. Mutation in \(BSND\), which encodes barttin (BSND), is shown to be responsible for this condition. \(BSND\) is a crucial accessory subunit of chloride channels, and is expressed in the kidney and inner ear. A variety of \(BSND\) mutations cause phenotypes of different severity that lead to its dysfunctional interactions with CIC-K channel, causing impairment of chloride reabsorption in the nephron as well as potassium secretion in the stria vascularis and the vestibular labyrinth [31,54,55]. \(BSND\) is located on chromosome 1p32, with only one transcript, 4 exons and a length of about 13kb. Sequence analysis of its entire coding region and deletion/duplication analysis is available for the genetic confirmation of BS-IV. Laboratory manifestations also include hypokalemic metabolic alkalosis, normotensive hyperreninemic hyperaldosteronism, and increased urinary sodium, potassium, and chloride [56]. BS-IV is also shown to have a digenic inheritance resulting from double homozygous mutations in \(CLCNKA\) and \(CLCNKB\), resulting in indistinguishable phenotypes from \(BSND\) mutations. This is probably due to the proximity of these two genes, which in some cases can be the target of large deletions involving both. Dysfunctional \(BSND\) results in ‘double knockout’ of \(CLCNKA\) and \(CLCNKB\), and rationalizes the outcome of both \(CLCNKA\) and \(CLCNKB\) dysfunctions to cause the same disease [57,58]. Notably, it has been reported that a certain kind of mutation can interfere with the wild functional \(BSND\) in the inner ear but not in the kidney, resulting in non-syndromic deafness [59].

**Type V**

In BS type V (BS-V), gain-of-function mutations in the extracellular \(\text{Ca}^{2+}\)-sensing receptor gene \((\text{CASR})\) lead to AD hypocalcemia, characterized by decreased chloride reabsorption, negative balance of sodium chloride, secondary hyperaldosteronism, and hypokalemia [28,60]. Different clinical presentations in BS-V patients can be determined from different activating mutations in \(\text{CASR}\) [61]. In these patients, calcium metabolism is abnormal, the level of parathyroid hormone is low, and renal calcium excretion is high. They are secondary to \(\text{CaSR}\) hyperactivation at low serum calcium concentrations that leads to abnormal inhibition of parathyroid hormone [62,63]. The \(\text{CaSR}\) is a member of the G protein–coupled receptor (GPCR) family and stimulates MAPK (mitogen-activated protein kinase)-ERK (extracellular signal-regulated kinases) signaling cascades. Hyper-activated \(\text{CaSR}\) leads to excessive signal transduction pathways, and therefore an increased cytosol calcium gradient excreted from the endoplasmic reticulum, which explains the higher sensitivity of mutant \(\text{CaSR}\) to external calcium [64]. \(\text{CASR}\) is located on chromosome 3q13 with a length of 104kb, contains seven
exons, and produces four different transcripts. Sequence analysis of the entire coding region of CASR is the test of choice for genetic confirmation of BS-V.

**Gitelman syndrome**

Gitelman syndrome (GS) represents an AR salt-losing tubulopathy with hypokalemic hypomagnesemic hypocalciuria, metabolic alkalosis, and hyperreninemic hyperaldosteronism, associated with normal blood pressure. GS is shown to be caused by mutations in SLC12A3, which is a renal thiazide-sensitive co-transporter, mediating the reabsorption of sodium and chloride in the distal convoluted tubule. A variety of different non-conservative loss-of-function mutations have been shown to cause GS [8].

SLC12A3 is located on chromosome 16q13, with a length of 51kb and 26 exons. It produces six different transcripts including four protein coding isoforms.

Modifier genes and/or environmental factors have also been identified to play important roles in the outcome function of affected genes. Mutations in CLCNKB, for example, have been shown to result in two different phenotypes among two sisters, one with severe symptoms of classic BS in the neonatal period accompanied by deafness, and the other with no symptoms [65]. A distinct mutation in BSND among three brothers has also been shown to cause different phenotypes, again indicating the possible role for other genes and/or environmental factors [56].

**Conflict of Interest**

None declared

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None declared

**References**

14. Landau D, Shalev H, Ohaly M, Carmi R.
46. Murthy M, Cope G, O'Shaughnessy KM. The acidic motif of WNK4 is crucial for its interaction with the K channel ROMK. Biochemical and biophysical research communications 2008;375(4):651-654.
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<table>
<thead>
<tr>
<th>Types (mode of inheritance)</th>
<th>Symptoms</th>
<th>Linked gene(s)</th>
<th>Gene description</th>
<th>Also known as</th>
<th>Locus</th>
<th>Neighboring genes</th>
<th>Gene(s) function(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BS Type I (AR)</td>
<td>Severe volume depletion, hypokalemia, metabolic alkalosis with high prenatal mortality, nephrocalcinosis</td>
<td>SLC12A1</td>
<td>Solute carrier family 12 (sodium/potassium/chloride transporter), member 1</td>
<td>NKCC2, RSC1</td>
<td>15q15-q21.1</td>
<td></td>
<td>Reabsorption of NaCl in kidney [35], colonic Cl- absorption coupled with HCO3- secretion [67], maintaining cell volume homeostasis under hypertonic conditions [46].</td>
</tr>
<tr>
<td>BS Type II (AR)</td>
<td>Elevated Ca2+ excretion leading to nephro-calcinosis, hypokalemic metabolic alkalosis, natriuresis, diuresis, and elevated prostaglandin E2 and renin</td>
<td>KCNJ1</td>
<td>Potassium inwardly rectifying channel, subfamily J, member 1</td>
<td>ROMK, ROMK, KIR1.1</td>
<td>11q24</td>
<td>KNCN5, FLI1, C11orf45, TP53AP1, SENC</td>
<td>Resting membrane potential and cell excitability [49], potassium recycling in thick ascending limb of Henle’s loop, and potassium secretion in the distal tubule and cortical collecting duct [48].</td>
</tr>
<tr>
<td>BS Type III (AR)</td>
<td>Hypokalemic alkalosis with salt-wasting, low blood pressure, normal magnesium, hyper/normo-calciiuria, and absence of nephrocalcinosis</td>
<td>CLCNKB, CLCKB</td>
<td>Chloride channel, voltage-sensitive Kb</td>
<td>CLCNK1, CIC-K2, CLC-Kb</td>
<td>1p36</td>
<td>CLCNKA, FAM131C, EPHA2, LOC44056</td>
<td>Regulation of cell volume, membrane potential stabilization, signal transduction and transepithelial transport, renal salt reabsorption</td>
</tr>
<tr>
<td>BS Type IV (AR)</td>
<td>Neonatal volume depletion, premature birth imputable to polyhydramnios, sensorineural deafness, severe renal salt-wasting, and polyuria</td>
<td>BSND</td>
<td>Bartter syndrome, infantile, with sensorineural deafness (Barttin)</td>
<td>BART, DFN7B73</td>
<td>1p32.1</td>
<td>TMEM61, PCSK9, TRNA-R, C16O, USP24</td>
<td>Cl- channel beta-subunit responsible for renal Cl reabsorption and secretion of K+ inner ear [54]</td>
</tr>
<tr>
<td>BS Type V (AD)</td>
<td>Decreased chloride reabsorption, negative balance of sodium chloride, secondary hyperaldosteronism, and hypokalemia</td>
<td>CASR</td>
<td>Calcium-sensing receptor</td>
<td>CAR, HH, FH, HH, EGR, HH1, NHTP, PCAR1, GPRC2, HYPOC1</td>
<td>3q13</td>
<td>HRNPAP, Z3, CSTA, CD86, ILD1</td>
<td>G protein-coupled receptor signaling, maintenance of mineral ion homeostasis</td>
</tr>
<tr>
<td>Gitelman syndrome (AR)</td>
<td>Hypokalemic hypomagnesemic hypocalciuria, etiologic alkalosis, and hyperreninemic hyperaldosteronism, associated with normal blood pressure</td>
<td>SLC12A3</td>
<td>Solute carrier family 12 (sodium/chloride transporter), member 3</td>
<td>NCC, TSC, NCCT</td>
<td>16q13</td>
<td>BPS24P17, MIR13B-2, HERPUD1, NUP53, CETP</td>
<td>Regulation of cell volume, membrane potential stabilization, signal transduction and transepithelial transport, renal salt reabsorption</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CLCNKB</td>
<td>Chloride channel, voltage-sensitive Kb</td>
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</tr>
</tbody>
</table>

### Table 1. Different types of Bartter and Gitelman syndrome. Key symptoms, linked gene(s), and corresponding information for each gene, regarding description, other names, locus, neighboring genes, and functions, for different types of Bartter syndrome and Gitelman syndrome have been included.