Genetic spectrum of Brazilian suspected Bartter Syndrome Patients

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Abstract

Background

This paper's goal is to show the genetic study results of suspected Bartter syndrome (BS) patients followed in a pediatric nephrology reference center in Sao Paulo/Brazil, verify a possible genotype-phenotype correlation and compare the genetic results with those from other regions of the globe.

Results

This descriptive study included 22 patients (21 families) with clinical diagnosis of BS. Pathogenic variants in BS-related genes were detected in 19/22 patients. No BS-related genes were detected in three patients (one case of Congenital Chloride Diarrhea and two siblings with clinical Antenatal BS that, in fact, had Gitelman Syndrome). We observed that 16/19 BS-confirmed patients had *CLCNKB* mutations (BS type 3) with a large phenotypical diversity. Among them, the deletion of the entire gene (del 1–20) was the most frequent variant detected. Interestingly, we observed that patients with homozygous or heterozygous del 1–20 presented earlier manifestations than patients with other *CLCNKB* mutations. They presented no other clinical significant difference.

Conclusion

This study demonstrates the importance of an appropriate investigation of clinically suspected BS patients to rule out pseudo-BS. This data also confirms the difficult to differentiate these patients based just on clinical findings, similar to what has been reported in other studies. There were patients with clinical Antenatal BS in whom the genetic analysis confirmed the final diagnosis of GS or BS type 3. Among BS cases, BS type 3 was the most frequent in this Brazilian cohort and del 1–20 was the most frequently variant detected. In addition, BS type 3 patients with homozygous or heterozygous del 1–20 had earlier manifestations than patients with other *CLCNKB* mutations. Brazilian community has a particular characteristic miscegenation as well as different origins such as Europeans, Africans and Asians and comparing our results with those from other regions we can suppose the genetic background of this Brazilian cohort is related to African and Portuguese inheritance, probably originated in the early period of immigration (colonization and slavery period). Limitations: low number of patients from a single center. However, as a rare disease all data can contribute to the improvement for diagnosis and treatment.

Introduction

Bartter syndrome (BS) encompasses a group of genetic tubular renal diseases characterized by hypokalemia, hypochloremia, hyponatremia, metabolic alkalosis, increased blood levels of renin and aldosterone in patients with normal to low blood pressure [1]. Clinically, one can identify 2 types of presentation: Antenatal BS and Classic BS.

Antenatal BS is considered the most severe form, characterized by polyhydramnios, premature birth, life-threatening episodes of neonatal salt and water loss, failure to thrive, hypercalciuria, and early-onset nephrocalcinosis [2]. Classic BS is supposed to occur in infancy or early childhood and is characterized by remarkable waste of salt and potassium clinically manifested as polyuria, polydipsia, volume contraction, failure to thrive, muscle weakness, growth retardation and, sometimes, nephrocalcinosis [3]. However, before the establishment of BS clinical hypothesis, acquired or genetic pseudo-Bartter conditions, renal or extrarenal, should be ruled out [4–7]. Among them Gitelman Syndrome (GS; OMIM 263800) is an important differential diagnosis. GS is classically described as a milder BS-like renal tubular disease

frequently associated with hypomagnesemia and hypocalciuria that often manifests in teenagers and young adults with cramps, muscle weakness, salt craving, paresthesia, and tetany [8].

It is important to mention the Autosomal Dominant Hypocalcemia (OMIN 601198) that can cause Bartter syndrome-like features. It is a consequence of gain-of-function mutation in *CASR* which encodes the calcium receptor sensor (CaSR) located in the thick ascending Henle's loop leading to secondary disturbances in NKCC2 and ROMK channels and in Na-K-ATPase pump [9]. This disease was named BS type 5 in the past.

The genetic classification of BS is presented in Table 1 and is based on the mutations in the genes that code specific transporters in renal tubular membranes.

Table 1

Genetic classification of Bartter Syndrome, specifying the affected genes, the molecule that is involved and its location in the renal tubules.

Subtypes of BS	Affected gene / Location	Molecule implicated	Location of implicated molecule
Antenatal type 1 (OMIN 601678)	<i>SLC12A1</i> / 15q21.1	Na ⁺ K ⁺ 2Cl ⁻ cotransporter	TAL
Antenatal type 2 (OMIN 241200)	<i>KCNJ1</i> / 11q24	Luminal K ⁺ channel ROMK	TAL
Classic Bartter Type 3 (OMIN 607364)	<i>CLCNKB</i> / 1p36	ClC-Kb channel	DCT
Type 4a with neurosensorial deafness (OMIN 602522)	<i>BSND</i> / 1p31	Barttin, an essential β subunit for chloride channels	DCT, TAL
Type 4b with neurosensorial deafness (OMIN 613090)	<i>CICNKA-CICNKB</i> / 1p36	ClC-Ka and ClC-Kb (chloro channels)	TAL
Antenatal Transient BS type 5 (OMIM 300971)	<i>MAGED2</i> / chromosome X	MAGE-D2 protein	TAL, DCT
TAL = thick ascending loop of Henle, ROMK = potassium recycling channel, DCT = distal cortical tubule.			

These mutations lead to dysfunctional proteins determining the clinical and biochemical abnormalities. Currently, the treatment is just symptomatic, based on potassium, chloride and sodium supplementation, and cyclooxygenase (COX) inhibitors, either nonselective (ex.: indomethacin) or COX2 selective (ex.: celecoxib). [10–11]. A potassium sparing drug such as spironolactone can be associated. These patients can also be treated with renin-angiotensin-aldosterone system (RAAS) blockers replacing the COX inhibitors. However, these drugs can be associated with significant and symptomatic hypotension [11].

Even though these treatments can promote complete or partial electrolyte and metabolic recovery leading to growth improvement, they are associated with significant side-effects [10]. In addition, the patient would always be prone to metabolic and electrolyte severe imbalance during common pediatric diseases.

Difficulties to clinically differentiate the types of BS and GS patients have been recognized and a new name has already been proposed for these diseases, Inherited Salt-Losing Tubulopathies. This group has been classified based on genetic

findings [12]. Therefore, the genetic study in this group of patients is necessary to clarify the true pathway of the disease, and serves as a base to implement specific treatments [13]. In this approach, it is very important to be alert for population differences.

This paper's goal is to show the genetic study results of clinically suspected BS patients followed in a pediatric nephrology reference center in Sao Paulo/Brazil, verify a possible genotype-phenotype correlation and compare the genetic results with those from other regions of the globe.

Patients And Methods

This study included Brazilian patients up to 21 years-old, both genders, with suggestive clinical and laboratory findings of BS followed at Pediatric Nephrology Unit of the Instituto da Criança, University of Sao Paulo. This protocol was approved by the Local Ethical Review Board. The patients were enrolled after they and/or their parents (or guardians) have signed the informed consent form.

Clinical and laboratory data was collected from the patient's records by the same doctors that had been following the patients. Clinical data included gestational age at birth, familial and consanguinity history, age at presentation, presence or not of polyhydramnios, polyuria, polydipsia, episodes of fever, vomiting, seizures, cramps, neurosensorial deafness and failure to thrive. The main laboratory data studied were venous gas analysis, serum potassium, chloride, sodium, ionic calcium, phosphate and magnesium; the urinary exams included excretion fraction of potassium, chloride and sodium as well as morning urinary calcium/creatinine ratio, microalbuminuria and urinary protein/creatinine ratio. Renal ultrasound was also evaluated.

DNA analysis

The blood was collected, genomic DNA was extracted and the sample kept at -20 Celsius degrees until analysis. Exon capture with Nextera Rapid Capture Mendelics Custom Panel V2 followed by next generation sequencing (NGS) with Illumina HiSeq were done. Alignment and identification of variants using bioinformatics protocols, with reference to the GRCh37 version of the human genome. Analyzed genes: *GLA*, *CLCNKA*, *CLCNKB*, *BSND*, *KCNJ1*, *SLC12A1*, *CTNS*, *AQP2*, *AVPR2*, *SLC12A3*, *CLDN19*, *CNNM2*, *CLDN16*, *TRPM6*, *SCNN1G*, *SCNN1B*, *ATP6V1B1*, *ATP6V0A4* and *SLC4A4*.

In addition, copy number variations (CNVs) in *CLCNKA* and *CLCNKB* were verified by Multiplex ligation-dependent probe amplification (MLPA). MLPA analysis was done according to the protocol supplied by the manufacturer of the of the SALSA- MLPA Kit P266 (MRC-Holland, Amsterdam, The Netherlands) which containing 14 probes specific for the *CLCNKB* gene (exon 1, 2, 3, 5, 6, 8, 10, 11, 13, 14, 15, 17, 18, and 19) and 2 probes for the *CLCNKA* gene (exon 5 and 10).

Results

This descriptive study included 22 patients (12 females) from 21 families with clinical diagnosis of BS.

Table 2 shows the initial clinical diagnosis of BS (Antenatal or neonatal BS, Classic BS) and the final genetic diagnosis. In addition, this table shows the characteristics of the variant found for each enrolled patient and the reference in what the variant was previously described or if this is a novel mutation.

Pathogenic variants in BS-related genes were detected in 19 of the 22 study patients. No BS-related genes were detected in three patients and these cases are described below.

Patients with no BS-related genes mutations observed in the current study - Patients with Pseudo-Bartter conditions.

Case 1 - We detected one case of Congenital Chloride Diarrhea (CCD) (OMIM #214700). This patient presented in the neonatal period with polyhydramnios and prematurity, polyuria and BS typical electrolyte and metabolic disturbances. Although she was receiving potassium and sodium supplementation, she evolved with failure to thrive. During one episode of acute diarrhea, CCD was suspected, but the diagnosis was not confirmed by fecal and urinary electrolytes measurements. The records were reviewed and showed that the collections were inappropriate, fecal sample was collected during dehydration status resulting in a false low fecal chloride. Likewise, a urinary sample was collected soon after physiologic solution infusion and potassium reposition, resulting in a high urinary chloride content. These findings led to misdiagnosis BS. During follow up, liquid stools were probably misinterpreted as polyuria. Unfortunately, no other urinary or fecal chloride measurement was performed to confirm the diagnosis of BS during follow up.

As patient presented no mutations in the genes panel applied in this study, whole exome sequencing (WES) was performed and detected a homozygous mutation (c.1487 T>G; p.Leu496Arg) in the solute carrier family 26 (*SLC26A3*) on chromosome 7q31, encoding for a transmembrane Cl⁻/HCO₃⁻ exchanger mainly expressed in the apical brush border of ileum and colonic epithelium [14]. This variant was confirmed by Sanger sequencing. This mutation is considered likely pathogenic according to the American College of Medical Genetics and Genomics (ACMG) standards and guidelines [15] and was reported at least once related to CCD in a family from Hong Kong [14]. This case has already been reported [6].

Cases 21 and 22- Two young male siblings from non-consanguineous parents presented a homozygous *SLC12A3* pathogenic variant, p.Thr648Met, diagnosing GS. They were preterm neonates and after 1 month of age they evolved with vomiting, polyuria, polydipsia, failure to thrive and seizures. Hypocalciuria was observed in both patients and hypomagnesemia was observed in one. Therefore, they presented a completely different clinical syndrome than which is described as classical GS. This finding

shows the importance of the genetic evaluation in such confusing diagnosis, especially when faced with a potential specific therapy in next future.

Pathogenic variants in the BS related genes detected in the current study.

Mutations in BS-related genes were detected in 19/22 potential BS Brazilian patients. All of them are classified as pathogenic variants according to the ACMG [15]. Based on those mutations we were able to genetically classify these patients.

- **Bartter Syndrome type 1** - The boy was born to non-consanguineous healthy parents at 28 weeks of gestation. Severe maternal polyhydramnios had made repeated amniocenteses needed during the last weeks of gestation. He had a long hospitalization with respiratory complications. He evolved with hypokalemic hypochloremic metabolic alkalosis, severe polyuria and failure to thrive. The genetic analysis revealed a compound heterozygous mutation in *SLC12A1* gene (**variant 1**- c.1103A>G, p.Glu368Gly; **variant 2**- c.905G>A, p.Arg302Gln) resulting in loss of function in the Solute Carrier Family 12 Member 1 encoding the apical furosemide-sensitive Na-K-Cl co-transporter, BS type I (OMIM #600839).

- **Bartter Syndrome type 4a** - A homozygous pathogenic *BSND* variant (c.G>A, p.Gly47Arg) was found in one female child establishing the diagnosis of BS type 4a. She was a preterm neonate born to consanguineous (first-degree cousins) healthy parents. After 6 months of age she presented polyuria, polydipsia, failure to thrive and dehydration episodes, associated with neurosensorial deafness. She also presented hypocalciuria and nephrocalcinosis was not detected in renal ultrasound.

- **Bartter Syndrome type 3** - *CLCNKB* pathogenic variants were detected in 16 patients. Among them, the deletion of the entire gene (del 1-20) was the most frequent and was observed in homozygosis (5/16 pts) and compound heterozygosis (6/16 pts). Therefore, the frequency of this variant among all BS-related alleles (19 patients) was 42.1 % (16/38).

One premature girl with homozygous del 1-20 in *CLCNKB* has neurosensorial deafness and was supposed to have an additional mutation in *CLCNKA* gene. Associated mutations in *CLCNKA* and *CLCNKB* can cause BS with neurosensorial deafness, Type 4b BS (Table 1) [16]. However, no pathogenic variant in *CLCNKA* was detected by neither NGS nor MLPA. Therefore, the final genetic diagnosis of this

patient was BS type 3. Sensorial deafness can be considered as a consequence of prematurity in this patient [17].

The other *CLCNKB* variants detected are described in Table 2.

Table 3 shows the main clinical and laboratory data of BS type 3 patients, comparing patients with homozygous or compound heterozygous del 1-20 and patients with other *CLCNKB* mutations. From these tables one can conclude, BS type 3 was the most frequent type of BS diagnosed in this Brazilian cohort. The deletion of the entire *CLCNKB* was the most frequent variant found in this group and its presence was correlated with earlier manifestations.

Table 3- Clinical and laboratory characteristics of Brazilian BS type 3 patients, comparing patients with del 1-20 and those with other *CLCNKB* variants.

Characteristics	1- <i>CLCNKB</i> del 1-20 n= 11	2- Other <i>CLCNKB</i> mutations n= 5	1 Versus 2
Consanguinity	7/11 (63,6%)	3/5 (60%)	
Polyhydramnios	8/11 (72,7%)	2/5 (40%)	p= 0,22
Prematurity	5/11 (45,4 %)	1/5 (20%)	p= 0,3
Age at manifestation (months)	3,4 ± 2,2	15,6 ± 13,6	p= 0,009
X ± DP	3 (0-6)	18 (2-36)	
med(range)			
Creatinine Clearance at presentation (Schwartz formula)	152,1 ± 186 (45-317)	129,3 ± 139,7 (64-178)	p= 0,6
Hypocalciuria	3/11 (27,3%)	3/5 (60%)	p=0,22
Nephrocalcinosis	2/11	0/5	N/A
Neurosensorial deafness	1/11	0/5	N/A

N/A not applicable; del 1-20= deletion of the entire gene *CLCNKB*; GS= Gitelman Syndrome; BS= Bartter Syndrome. Significant p value: p< 0.05

Based on these results, the authors suggested a pathway to investigate BS potential Brazilian patients (Figure 1). The first step is to rule out pseudo-Bartter conditions, acquired or genetic, and this requires a proper anamnesis and exams to locate the waste of sodium, potassium and chloride. In addition, as the deletion of the entire *CLCNKB* was the most frequent variant detected in BS patients, we suggested to begin the investigation performing MLPA to check for CNVs in *CLCNKB*. It is important to emphasize that the MLPA kit used to verify CNVs in *CLCNKB* includes the analysis of *CLCNKA*.

- **Bartter Syndrome type 4b** - Applying the pathway proposed in the Figure 1 and firstly performing MLPA to verify the presence of CNVs in *CLCNKB*, we were able to detect a homozygous deletion of the entire *CLCNKB* and *CLCNKA* in one girl with suspected BS establishing the diagnosis of BS type 4b. Neurosensorial deafness was later diagnosed in this patient. This BS type 4b is a consequence of a digenic defect in the closely adjacent genes encoding ClC-Ka (*CLCNKA*) and ClC-Kb (*CLCNKB*) on chromosome 1p36. [16].

No patient was diagnosed with BS type 2 (*ROMK* mutations), type 5 or BS-like due to gain-of-function mutation in *CaSR*.

Based on this data, it was almost impossible to differentiate clinically suspect BS patients due to the clinical overlap.

Comparison of the variants found in this Brazilian cohort with those identified in some other countries.

In the current study, we also evaluated some of the main genetic reports of suspected Bartter Syndrome patients around the globe. The goal is to compare the Brazilian findings with other countries' findings. Table 4 shows the different genetic findings in different regions of the globe and, when is available, the ancestry or ethnic origin is presented.

Discussion

Bartter syndrome is composed by a group of rare salt-losing tubulopathies with an estimated incidence of 1.2/1 million of the population [34–35]. The BS Brazilian suspected patients included in the current study have a large clinical overlap and this finding has been observed in other populations [36]. In this way, Seyberth proposed a new classification of BS and GS based on the location of the involved channels or transporters, including the subunit Barttin. He named it “Inherited salt-loss tubulopathies” [12].

Therefore, a rational way for investigation is important if we want to achieve the true diagnosis in clinically BS suspected patients. It is noteworthy the importance of the early investigation of pseudo-Bartter conditions in which the treatment would be completely different. Therefore, it is important to measure the urinary sodium, potassium and chloride and to calculate the excretion fraction of these electrolytes and confirm the urinary losses in all suspected BS patients and choose the correct timing to collect the urine sample [6].

After ruling out pseudo-Bartter conditions, one should verify the presence of neurosensorial deafness, a specific feature that can lead to the diagnosis of BS type 4a or BS type 4b. In this study, we detected neurosensorial deafness in 3 patients. One of them with final diagnosis of BS type 4a (homozygous *BSND* mutation), one with final diagnosis of BS type 4b (performing just MLPA to investigate *CLCNKB* and *CLCNKA*), and other patient was genetically diagnosed with BS

type 3 (homozygous deletion in *CLCNKB* and no mutation detected in *CLCNKA* by NGS or MLPA). In the last patient, deafness could just be related to the prematurity which has been reported in literature [6].

We detected one patient with clinical antenatal BS with genetic BS type 1. Polyhydramnios developed during gestation, he was an extreme preterm baby with remarkable metabolic alkalosis, hypokalemia, hypercalciuria and early nephrocalcinosis. However, clinical antenatal BS was also observed in patients with *CLCNKB* mutations, especially in cases of homozygous del 1–20.

We observed that most of the cases had *CLCNKB* mutations (BS type 3) with a large phenotypical diversity. Among these patients, del 1–20 was the most frequent genetic abnormality detected. Interestingly, we observed that patients with del 1–20 presented earlier manifestations than patients with other *CLCNKB* mutations. No other significant difference was detected in the clinical parameters evaluated in this study. However, some authors have already demonstrated that the hypokalemia is more difficult to be corrected in BS type 3 than BS type 1 or type 2 [37–39].

Surprisingly, GS was diagnosed in two siblings with a complete different manifestation that is commonly observed in GS patients as they presented a typical clinical picture of Antenatal BS. The typical clinical history of GS is a milder presentation in adolescents or young adults with cramps. Therefore, this finding denotes the importance of the genetic evaluation in such confusing diagnosis, especially if a specific therapy is to be developed and also emphasizes the phenotypical variation of these patients especially in those with early manifestations. Therefore, we suggest even in young children in which del 1–20 in *CLCNKB* has been ruled out, the genetic investigation of GS should be performed.

No patient was diagnosed with BS type 2 caused by mutations in the potassium voltage-gated channel subfamily J member 1 (*KCNJ1*) gene encoding the apical renal outer medullary potassium channel (ROMK) (OMIM # 600359).

Based on these findings, we suggested a pathway to genetically study Brazilian patients and it is shown in Fig. 1. According to this pathway, performing MLPA for *CLCNKB* we were able to diagnose a new patient with BS type 4b, as she presented del 1–20 in *CLCNKB* and a deletion in *CLCNKA*, using the kit for MLPA to evaluate *CLCNKB* we could also evaluate the *CLCNKA*.

Therefore, one can conclude that genetic analysis is the only way to get the correct diagnosis in suspected BS patients.

In this study, the main conclusion is the group of diseases encompassing clinical and laboratory findings of BS are clinically confusing and the genetic analysis is essential to establish the correct diagnosis. Therefore, the clinical classification of BS seems to be inefficient and should be replaced by the genetic classification. This conclusion has also been achieved by other authors [12]. In addition, based on genetic abnormality a specific therapy for each defect can be developed in next future [13]. As one knows, population differences have been observed among patients with the same clinical diagnosis [21]. Therefore, the knowledge of the particular genetic characteristic of different populations will collaborate with the specific treatment development. In this study, the genetic abnormalities distribution of BS Brazilian patients is detailed. Brazilian community has a particular characteristic miscegenation as well as different origins such as Europeans, Africans and Asians. These characteristics make Brazilian people a very interesting genetic population. The genetic background of Brazilian community, published by Brazilian Institute of Geography and Statistics based on 25 studies of 38 different Brazilian populations described the high miscegenation due to the different colonizers distributed in the five Brazilian regions [40].

The heritage contribution of the population is 62% Europeans ancestry, 21% African and 17% native American. North region still see the highest rate of the native americans (32%) and the South region contains the majority of Europeans (77%), came from Portugal, Italy, Germany, Japan, Middle East and Easter Europe, in the late of 19th and early of 20th centuries. The black African mostly from Bantu and West African went to Northeast region while the Southeast region was colonized by Portugueses and Germans [40].

Conclusion

The del 1–20 in *CLCNKB* was the most frequent variant detected in this group of patients. Evaluating the genetic studies results from different parts of the world we can suppose the genetic background of this Brazilian cohort is more related to African and Portuguese origin and this fact can do one concluded that the genetic BS originated in the early period of immigration (colonization and slavery period).

The study has some limitations as the low number of patients from a single center. However, as a rare disease one must consider that certain points can be concluded even in a small sample population. Evidence-based medicine seems to be not appropriate in the evaluation of rare diseases and the scientists are looking for more appropriate ways to interpret the results in these conditions. Meanwhile, all data of these patients are important to be reported as it can collaborate for a better understanding of these conditions.

Declarations

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Not applicable.

Author's contributions

MHV drafted the study design, the manuscript and made substantial contribution to conception and interpretation of the data, revised and supervised critically the work, ACHLM recruited the patients and contributed general patient data, ACSR and FAMF performed the genetic tests and analysis, JCOAF wrote and revised critically the manuscript. The author(s) read and approved the final manuscript.

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Availability of data and materials

Data generated or analyzed during this study are included in this published article.

Ethics approval and consent to participate

The study was approved by the Local Ethical Review Board, São Paulo, Brazil (reference number 3.261.257).

Consent for publication

The parents/guardians gave a written consent to publication of their anonymized clinical data.

Competing interests

All authors declare that they have no competing interests.

References

1. Bartter FC, Pronove P, Gill JR Jr, MacCardle RC. Hyperplasia of the juxtaglomerular complex with hyperaldosteronism and hypokalemic alkalosis. A new syndrome. *Am J Med.* 1962;33:811–28.
2. Seyberth HW, Schlingmann KP. Bartter- and Gitelman-like syndromes: Salt-losing tubulopathies with loop or DCT defects. *Pediatr Nephrol.* 2011;26:1789–802.
3. Konrad M, Vollmer M, Lemmink HH, van den Heuvel LP, Jeck N, Vargas- Poussou R, et al. Mutations in the chloride channel gene *CLCNKB* as a cause of classic Bartter syndrome. *J Am Soc Nephrol.* 2000;11:1449–59.
4. Najafi M, Kordi-Tamandani DM, Behjati F, Sadeghi-Bojd S, Bakey Z, Karimiani EG, et al. Mimicry and well known genetic friends: molecular diagnosis in an Iranian cohort of suspected Bartter syndrome and proposition of an algorithm for clinical differential diagnosis. *Orphanet J Rare Dis.* 2019;13(1):41. Feb;14(.
5. Matsunoshita N, Nozu K, Yoshikane M, Kawaguchi A, Fujita N, Morisada N, et al. Congenital Chloride Diarrhea needs to be distinguished from Bartter and Gitelman Syndrome. *J Hum Genet.* 2018;Jul;63(8):887–92.
6. Vaisbich MH, Ferreira JCDOA, Messa ACHL, Kok F. Congenital Chloride Diarrhea in a Bartter Syndrome Misdiagnosed Brazilian Patient. *J Rare Disord Diagn Ther.* 2019;5:2–4.
7. Matsunoshita N, Nozu K, Shono A, Nozu Y, Fu XJ, Morisada N, et al. Differential diagnosis of Bartter syndrome, Gitelman syndrome, and pseudo-Bartter/Gitelman syndrome based on clinical characteristics. *Genet Med.* 2016;Feb;18(2):180–8.
8. Simon DB, Nelson-Williams C, Bia MJ, Ellison D, Karet FE, Molina AM, et al. Gitelman's variant of Bartter's syndrome, inherited hypokalaemic alkalosis, is caused by mutations in the thiazide-sensitive Na-Cl cotransporter. *Nat Genet.* 1996;12:24–30.
9. Nesbit MA, Hannan FM, Howles SA, Babinsky VN, Head RA, Cranston T, et al. Mutations affecting G-protein subunit alpha-11 in hypercalcemia and hypocalcemia. *New Eng J Med.* 2013;368:2476–86.
10. Vaisbich MH, Fujimura MD, Koch VH. Bartter syndrome: benefits and side effects of long-term treatment. *Pediatric Nephrology.* 2004;19(8):858–63.
11. Nascimento CLP, Garcia CL, Schwartsman NGS, Vaisbich MH. Treatment of Bartter syndrome. Unsolved issue. *J Pediatr (Rio J)* Sep-Oct. 2014;90(5):512–7.
12. Seyberth HW. An improved terminology and classification of Bartter-like syndromes. *Nat Clin Pract Nephrol.* 2008;4:560–7.
13. Imbrici P, Conte D, Liantonio A. Paving the way for Bartter syndrome type 3 drug discovery: a hope from basic research. *J Physiol.* 2017;595(16):5403–4.
14. Hoglund P, Auranen M, Socha J, Popinska K, Nazer H, et al. Genetic Background of Congenital Chloride Diarrhea in High- Incidence Populations: Finland, Poland, and Saudi Arabia and Kuwait. *Am J Hum Genet.* 1998;63:760–8.
15. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Rehm, Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med.* 2015;17(5):405–24.
16. Schlingmann KP, Konrad M, Jeck N, et al. Salt wasting and deafness resulting from mutations in two chloride channels. *N Engl J Med.* 2004;25(13):1314–9. 350(.

17. Van Naarden K, Decoufle P. Relative and attributable risks for moderate to profound bilateral sensorineural hearing impairment associated with lower birth weight in children 3 to 10 years old. *Pediatrics*. 1999;104:905–10.
18. Brochard K, Boyer O, Blanchard A, Loirat C, Niaudet P, Macher MA, et al. Phenotype–genotype correlation in antenatal and neonatal variants of Bartter syndrome. *Nephrol Dial Transplant*. 2009;24:1455–64.
19. Vargas-Poussou R, Feldmann D, Vollmer M, Konrad M, Kelly L, van den Heuvel LP, et al. Novel molecular variants of the Na-K-2Cl cotransporter gene are responsible for antenatal Bartter syndrome. *Am J Hum Genet*. 1998;62:1332–40.
20. Simon DB, Bindra RS, Mansfield TA, Nelson-Williams C, Mendonça E, Stone R, et al. Mutations in the chloride channel gene *CLCNKB* causes Bartter Syndrome type 3. *Nature Genet*. 1997;17:171–8.
21. Seys E, Andrini O, Keck M, Mansour-Hendili L, Courand PY, Simian C, et al. Clinical and Genetic Spectrum of Bartter Syndrome Type 3. *J Am Soc Nephrol*. 2017;28(8):2540–52.
22. Rodríguez Soriano J, Vallo A, Pérez de Nanclares G, Bilbao JR, Castaño L. A founder mutation in the *CLCNKB* gene causes Bartter syndrome type III in Spain. *Pediatr Nephrol*. 2005;20:891–6.
23. Bettinelli A, Borsa N, Bellantuono R, Syrén ML, Calabrese R, Edefonti A, et al. Patients with Biallelic Mutations in the Chloride Channel Gene *CLCNKB*: Long-Term Management and Outcome. *Am J Kidney Dis*. 2007;49(1):91–8.
24. Lee BH, Cho HY, Lee H, Han KH, Kang HG, Ha IS, et al. Genetic basis of Bartter syndrome in Korea. *Nephrol Dial Transplant*. 2012;27:516–1521.
25. Park CW, et al. Renal dysfunction and barttin expression in Bartter syndrome Type IV associated with a G47R mutation in *BSND* in a family. *Clin Nephrol*. 2011;75:69–74.
26. Steinke KV, et al. Human *CLC-K* Channels Require Palmitoylation of Their Accessory Subunit Barttin to Be Functional. *J Biol Chem*. 2015;290:17390–400.
27. Miyamura N, et al. Atypical Bartter syndrome with sensorineural deafness with G47R mutation of the beta-subunit for *CLC-Ka* and *CLC-Kb* chloride channels, barttin. *J Clin Endocrinol Metab*. 2003;88(2):781–6.
28. Nozu K, Inagaki T, Fu XJ, Nozu Y, Kaito H, Kanda K, et al. Molecular analysis of digenic inheritance in Bartter syndrome with sensorineural deafness. *J Med Genet*. 2007;45(3):182–6.
29. Lemmink HH, Knoers NV, Károlyi L, van Dijk H, Niaudet P, Antignac C, et al. Novel mutations in the thiazide-sensitive NaCl cotransporter gene in patients with Gitelman syndrome with predominant localization to the C-terminal domain. *Kidney Int*. 1998;54:720–30.
30. Tajima T, Nawate M, Takahashi Y, Mizoguchi Y, Sugihara S, Yoshimoto M, et al. Molecular analysis of the *CLCNKB* gene in Japanese patients with classic Bartter Syndrome. *Endocr J*. 2006;53(5):647–52.
31. Nozu K, Fu XJ, Nakanishi K, Yoshikawa N, Kaito H, Kanda K, et al. Molecular Analysis of Patients with Type III Bartter Syndrome: Picking Up Large Heterozygous Deletions With Semiquantitative PCR. *Pediatr Res*. 2007;62:364–9.
32. Nozu K, Iijima K, Kanda K, Nakanishi K, Yoshikawa N, Satomura K, et al. The Pharmacological Characteristics of Molecular- Based Inherited Salt-Losing Tubulopathies. *J Clin Endocrinol Metab*. 2010;95:E511–8.
33. García Castaño A, Pérez de Nanclares G, Madariaga L, Aguirre M, Madrid A, Chocroón S, et al. Poor phenotype-genotype association in a large series of patients with Type III Bartter syndrome. *PLoS One*. 2017;12(3):e0173581.
34. Bokhari SRA, Mansur A. Bartter Syndrome. In *StatPearls*. Treasure Island (FL): StatPearls Publishing LLC; 2018.
35. Ji W, Foo JN, O’Roak BJ, et al. Rare independent mutations in renal salt handling genes contribute to blood pressure variation. *Nat Genet*. 2008;40:592–9.
36. Al Shibli A, Narchi H. Bartter and Gitelman syndromes: Spectrum of clinical manifestations caused by different mutations. *World J Methodol*. 2015;5(2):55–61.
37. Han Y, Lin Y, Sun Q, Wang S, Gao Y, Shao L. Mutation spectrum of Chinese patients with Bartter syndrome. *Oncotarget*. 2017;8(60):101614–22.

38. Han Y, Zhao X, Wang S, Wang C, Tian D, Lang Y, et al. Eleven novel SLC12A1 variants and an exonic mutation cause exon skipping in Bartter syndrome type I. *Endocrine*. 2019;64(3):708–18.
39. Liu T, Wang C, Lu J, Zhao X, Lang Y, Shao L. Genotype/phenotype analysis in 67 Chinese patients with Gitelman's syndrome. *Am J Nephrol*. 2016;44(2):159–68.
40. Instituto Brasileiro de Geografia e Estatística. Notas técnicas: histórico da investigação sobre cor ou raça. Resultados da Amostra. 2008. [Internet] [cited 2020 May 29]. Available from: <http://www.ibge.gov.br>.

Tables 2 And 4

Table 2- Initial clinical diagnosis, final genetic diagnosis (type of BS, GS or pseudo-Bartter)), variants detected in this Brazilian cohort of suspected BS, characteristics of the variants, level of pathogenicity and references in what the variant was described or if it is a novel mutation.

Case number	Ethnics/ last name origin	Clinical diagnosis	Final Diagnosis	Gene	Position	Protein	Level of pathogenicity	Reference
1	caucasian	Antenatal BS	Pseudo-Bartter CCD	<i>SLC26A3</i>	c.1487 T>G (homozygosis)	p.Leu496Arg	-likely pathogenic -the position is highly conserved among species - "in silico" predictors consider this a deleterious variant. -This mutation was reported at least once in literature related to CCD in a patient from Hong Kong and is absent in 138 mil subjects of the world population.	14
2	caucasian Portuguese/ Spanish	Antenatal BS	BS type 1	<i>SLC12A1</i>	c.1103A>G; c.905G>A (compound heterozygosis)	p.Glu368Gly; p.Arg302Gln	Variant 1 - pathogenic; c.1103A>G was described in a caucasian female child and is present in heterozygosis in 1: 141 mil subjects of the world population; Variant 2 - pathogenic; c.905G>A has been reported in German population and is present in heterozygosis in 6:141 mil subjects of the world population.	18 - Variant 1 19- Variant 2
3	black Portuguese/ Gallic	Antenatal BS/ND	BS type 3	<i>CLCNKB</i>	c.(? _1)_(*1_?)del (homozygosis)	del exons 1 a 20	-pathogenic -deletion of the entire gene	20 21
4	brown Portuguese/ Portuguese	Classic BS	BS type 3	<i>CLCNKB</i>	c.(? _1)_(*1_?)del (homozygosis)	del exons 1 a 20	- pathogenic -deletion of the entire gene	20 21
5	brown	Classic BS	BS type 3	<i>CLCNKB</i>	c.(? _1)_(*1_?)del	del exons 1 a 20	-pathogenic	20 21

	Afroamerican- (Nigerian)/ Portuguese/ Spanish				(homozygosis)		-deletion of the entire gene	
6	Iberian Peninsula/ Portuguese	Classic BS	BS type 3	<i>CLCNKB</i>	c.(? _1)_(*1_?)del (homozygosis)	del exons 1 a 20	- pathogenic -deletion of the entire gene	20 21
7	caucasian Portuguese/ ?	Antenatal BS	BS type 3	<i>CLCNKB</i>	c.(? _1)_(*1_?)del (homozygosis)	del exons 1 a 20	- pathogenic -deletion of the entire gene	20 21
8	Arabian/ Lebanese	Classic BS	BS type 3	<i>CLCNKB</i>	c.610G>A (homozygosis)	p.Ala204Thr	- pathogenic - Spanish founder mutation	20 22
9	caucasian Italian/ Portuguese	Antenatal BS	BS type 3	<i>CLCNKB</i>	c.610G>A (homozygosis)	p.Ala204Thr	- pathogenic - Spanish founder mutation	20 22
10	caucasian Portuguese/ Portuguese	Classic BS	BS type 3	<i>CLCNKB</i>	c.673G>T (homozygosis)	p.Glu225*	- definitively pathogenic - c.1560T>G p.Tyr520* is a novel mutation, but the molecular mechanism, characteristics of the region and clinical correlation make it pathogenic.	novel mutation
11	caucasian Italian/ Portuguese- Spanish	Antenatal BS	BS type 3	<i>CLCNKB</i>	c.610G>A; c.(? _1)_(*1_?)del (compound heterozygosis)	p.Ala204Thr; del exons 1- 20	Variant 1- pathogenic; -Spanish founder mutation; Variant 2 - pathogenic; -deletion of the entire gene	20 22
12	Spanish	Classic BS	BS type 3	<i>CLCNKB</i>	c.610G>A; c.(? _1)_(*1_?)del (compound heterozygosis)	p.Ala204Thr; del exons 1- 20	Variant 1 - Spanish founder mutation; Variant 2 - deletion of the entire gene	20 22
13	Portuguese	Classic BS	BS type 3	<i>CLCNKB</i>	c.18dupG; c.(? _1)_(*1_?)del (compound heterozygosis)	p.Leu7Ala fs*3; del exons 1-20	Variant 1 - pathogenic - p.Leu7Ala fs*is present in heterozygosis in 3:123 mil	23

							<p>subjects of the world population and has been reported.</p> <p>Variant 2-pathogenic</p> <p>deletion of the entire gene.</p>	
14	Portuguese	Classic BS	BS type 3	<i>CLCNKB</i>	<p>c.18dupG; c.(?_1)_(*1_?)del</p> <p>(compound heterozygosis)</p>	p.Leu7Alafs*3; del exons 1-20	<p>Variant 1-pathogenic</p> <p>- p.Leu7Alafs*3 is presente in heterozygosis in 3:123 mil subjects of the world population and has been reported.;</p> <p>Variant 2-pathogenic;</p> <p>- deletion of the entire gene</p>	23
15	caucasian ?/ Portuguese	Antenatal BS	BS type 3	<i>CLCNKB</i>	<p>c.910C>T; c.(?_1)_(*1_?)del</p> <p>(compound heterozygosis)</p>	p.Arg304*; del exons 1 a 20	<p>Variant 1-pathogenic;</p> <p>- It is a novel mutation present in heterozygosis in 10:123 mil subjects of the world population.</p> <p>Variants leading to abnormal protein transcription are associated with BS. (Ref.1, PMID: 9326936) -</p> <p>Variant 2-pathogenic;</p> <p>-deletion of the entire gene</p>	novel mutation
16	Portuguese (nórdico)/ Portuguese	Antenatal BS	BS type 3	<i>CLCNKB</i>	<p>c.1400C>T; c.(?_1)_(*1_?)del</p> <p>(compound heterozygosis)</p>	p.Ala467Val; del exons 1 a 20	<p>Variant 1-likely pathogenic;</p> <p>- The position is highly conserved among species;</p>	novel mutation

							<p>- "in silico" predictors consider this a deleterious variant.</p> <p>- This is a novel mutation and is absent in 123 mil subjects of the world population;</p> <p>Variant 2- pathogenic;</p> <p>- deletion of the entire gene</p>	
17	Portuguese	Classic BS	BS type 3	<i>CLCNKB</i>	<p>c.1783C>T; c.1560T>G</p> <p>(compound heterozygosis)</p>	<p>p.Arg595*; p.Tyr520*</p>	<p>Variant 1- pathogenic.</p> <p>Variant 2- This variant was not described previously, but the molecular mechanism, the characteristics of the region and the clinical correlation are enough to classify this variant as pathogenic.</p>	<p>5</p> <p>novel mutation</p>
18	<p>brown</p> <p>Portuguese/ Spanish- Portuguese</p>	Antenatal BS	BS type 3	<i>CLCNKB</i>	<p>c.1309G>A; Chr1:6.374.838-16.383.003.</p> <p>(compound heterozygosis)</p>	<p>p.Gly437Arg; del exons 6 a 19</p>	<p>Variant 1- pathogenic</p> <p>detected in heterozygosis in 7: 123 thousand subjects of the world population and has been described previously.</p> <p>Variant 2- considerada patogênica.; não consegui achar descrição desta deleção.</p>	<p>24</p> <p>novel mutation?</p> <p>20, 21</p>

19	Iberian Peninsula/ Portuguese-Spanish	Antenatal BS/ND	BS type 4A	<i>BSND</i>	c.139G>A (homozygosis)	p.Gly47Arg	pathogenic - This mutation is present in heterozygosis in 25:138.000 subjects of the world population. Functional studies have demonstrated this mutation leads to a decrease in membrane expression of the protein. The severity of the renal phenotype of this mutation is variable and has been previously reported.	25-27
20	Portuguese/ Portuguese-Spanish	Antenatal BS/ND	BS type 4b	<i>CLCNKB/CLCNKA</i>	del 1-20; del exons 5 and 10 (digenic inheritance)	del exons 1-20/ del exons 5 and 10	Digenic inheritance Gene 1 (CLCNKB)-pathogenic (deletion of the entire gene) Gene 2 (CLCNKA)-pathogenic (del exons 5 and 10)	28
21	brown Italian/ Spanish	Antenatal BS	GS	<i>SLC12A3</i>	c.1943C>T (compound heterozygosis)	p.Thr648Met	-likely pathogenic. This is a novel mutation and is present in heterozygosis in 10: 123 thousand subjects in the world population. A variant in the same codon (p.Thr648Arg) has been previously reported in homozygosis related to GS.	novel mutation 29
22	brown Italian/ Spanish	Antenatal BS	GS	<i>SLC12A3</i>	c.1943C>T (compound heterozygosis)	p.Thr648Met	- likely pathogenic This is a novel mutation and is present in heterozygosis	novel mutation 29

							in 10: 123 thousand subjects in world population. A variant in the same codon (p.Thr648Arg) has been reported in homozygosis related to GS.	
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Table 4- Results of some genetic studies in suspected Bartter syndrome patients in different regions of the globe. Ethnic origin is reported when it is available.

1 st author (year)	Country of the study; – study group according to the type of bs (n)	Genetic type of BS [results for non-typed suspected bs patient's studies] (n)	<i>CLCNKB</i> mutations N (n - all genetic diagnosed bs patients)	Del 1-20 -n with homozygous del 1-20 (n bs type 3); - number of alleles (total number of study alleles)	Other <i>CLCNKB</i> variants Type (n with homozygous variants + heterozygous variants / (n bs type 3) - number of alleles (total number of study alleles)	Conclusion	Reference
Simon et al (1997)	USA, Portugal, Spain, Saudi Arabia, UK, Turkey (66 families)	-BS type 1 or 2 (22 kindreds) -BS type 3 (17 kindreds) -no mutation found (27 families)	17 (39)	-10 (17); ancestry: African American (5) Portugal (3) Saudi Arabia (1) Yemen (1) -20 (132) 15.5%	-p.A204T homozygosis (2); ancestry Spain (2) -p.P124L homozygosis (2) ancestry Turkey (2)	Among 17 families with BS type 3, the deletion of the entire gene (del 1-20) was detected in 59%, mainly in patients with African American and Portugal ancestries.	20
Konrad et al (2000)	Germany, France, USA, The Netherlands - Non-BS type 1 or 2 (45 patients; 39 families)	-BS type 3 (36; 30 families)	36; 30 families (N/A)	- 9 families (30 families); ancestry: African American (1) West Indian (1) Dutch (1) Turkish (4) German (2) -18 (60) 30%	-according to the ancestry (N families): Caucasian American -p.S337F (1); p.R538P (1); p.A77T (1); p.S573Y (1); p. [R438H] - [splice donor] (1) German -c.816-2(A>G); splice acceptor (1) fs463 X478 (1) -901-1(G T); splice acceptor (1) -Unequal cross (LOF) (1) -p.[fs630>X644] - [L139P] (1) Italy -p.P124L (1) North African - S297R (1) -c.611del78bp (1)	homozygous del 1-20 was the most frequent mutation found (30% of the studied families)	3

					<p>Dutch</p> <p>-c.816-2 (A>G); splice acceptor (1)</p> <p>-c.901-1 (G>A); splice acceptor (1)</p> <p>-fs463>X478 (1)</p> <p>French</p> <p>-c.1588ins4bp; p.fs518>X522 (1)</p> <p>Saudi Arabian</p> <p>K560M (1)</p> <p>Hispanic</p> <p>K560M (1)</p> <p>French Canadian</p> <p>-H357Q (1)</p> <p>Turkish</p> <p>-p.P124L (1)</p>		
Bettinelli et al (2006)	Italy -BS type 3 (13 patients)	N/A	13 (N/A)	<p>-2 (13); 5 (26) 19.2%</p> <p>del 1-20 @ ancestry:</p> <p>2 from Italy and 1 from Pakistan</p>	<p>homozygous variants</p> <p>-p.R438H (1); ancestry: Italy</p> <p>-p.A242E (2); ancestry: Italy</p> <p>-del 1-6 (2); ancestry: Italy</p> <p>-p.Leu7AlafsX3 (1); ancestry: Brazil</p> <p>-p.P124L (1); ancestry: Italy</p> <p>-p.G433E (1); ancestry: Italy</p> <p>heterozygous variants</p> <p>-(p.[R438H]-[A242E]) (1); ancestry: Italy</p> <p>-(p.[R595X]-[del 1-20]) (1); ancestry: Italy</p> <p>-(p.[M412I]-[C667X]) (1); ancestry: Italy</p>	<p>Del 1-20 was detected in 19.2 % of the studied alleles.</p> <p>No predominant variant was found in these population.</p>	23

Tajima et al. (2006)	Japan; BS type 3 (7 patients)	N/A	7 (N/A)	2 (7); 4 (14) 28.5%	- homozygous W610X (1) - heterozygous W610X (2) - L130 heterozygosis (1) - heterozygous del exons 1 and 2 (1)	The authors concluded that the findings corroborate other Japanese studies showing the meaning of W610X in this population.	30
Nozu et al (2007)	Japan; -BS type 3 (5 patients; 4 families)	N/A	5 (N/A)	-0 (5); -1 (10) 10%	- (p.W391X) (homozygosis in 2 + heterozygosis in 3). - 7 (10 alleles)	- (p.W391X) was the most frequent. It is considered a founder Japanese mutation. - del 1-20 was detected in just 1 allele.	31
Brochard K et al. (2009)	France; - French Antenatal and Neonatal BS patients (42 patients; 37 families)	-BS type 1 (13; 11 families)); BS type 2 (19; 15 families); BS type 3 (6; 6 families); BS type 4a (4; 4 families))	6 (37 families)	-3 (6) These 3 families are included in Seys's report (2017) -6 (74 alleles) 8.1%	- C.1107+1G>T in homozygosis (1 Tunisian) - C.1588ins4bp; p.518fsX4 in homozygosis (1 caucasian) - c.343A>C; p.Thr115Pro in homozygosis (1 caucasian)	Patients with <i>CLCNKB</i> mutations can present with antenatal and neonatal BS. In this study group BS type 3 was diagnosed in 16.2% of patients with Antenatal or Neonatal BS and in this group homozygous del 1-20 was detected in 50% (3/6 families).	18
Nozu K et al (2010)	Japan; - non-specified suspected BS (16 patients; 14 families)	-BS type I (2), -BS type II (2), -BS type III (9, 7 families) - GS (3).	9, 7 (16)	-0 (16); -0 (32)	- (p.W391X) (homozygosis in 4 patients + heterozygosis in 1)/ (9). - 9 (32 alleles) and 9/18 <i>CLCNKB</i> alleles)	- (p.W391X) was the most frequent. It is considered a founder Japanese mutation. - del 1-20 was not present in this cohort.	32
Lee et al. (2012)	Korea; - non-specified suspected BS (26 patients)	-BS type I (1) - BS type II (1) -BS type III (23) - BS type IVa (1)	23 (26)	-1 (23); -6 (52) 11.5%	-(p.W610X) (homozygosis in 7 + heterozygosis in 11) -25 (52)	-(p.W610X) was the most frequent variant and del 1-20 was the second one, corresponding to 54.3 and 17.4% of the 46 <i>CLCNKB</i> alleles.	24
García Castaño et al (2017)	Spain; - BS type 3 patients (30 patients)	N/A	30 (N/A)	-1 (30); -6 (60) 10%	p.Ala204Thr (homozygous mutation in 16 patients [16 families] + heterozygous mutation in 8): (p.[Ala204Thr] - [1-20 del] (3); (p.[Ala204Thr] - [Glu442Gly]) (4 patients [3 siblings]).	p.Ala204Thr was the most frequent variant found and it is considered a Spanish founder mutation. - del 1-20 was detected in 10% of the study alleles.	33

					<p>(p.[Ala204Thr] - [Val170Met]) (1)</p> <p>- 40 alleles (60)</p> <p><i>Other variants:</i></p> <p>-(p. [Ile398_Thr401del] - [1-20 del]) (1);</p> <p>-(p. [Ser343Alafs*6] - p.[Glu442Gly]) (1);</p> <p>-(p.[Leu252fs] - p. [Leu252fs]) (1);</p> <p>-(p.[Arg595*] - [Arg595*]) (1)</p> <p>-(p.[Ala210Val] - [?]) (1);</p> <p>-(p.[Ala204Thr] - [Val170Met]) (1)</p>		
Seys et al (2017)	<p>French</p> <p>BS type 3 (115 patients; 111 families)</p>	N/A	115;111 families (N/A)	<p>-29 families (111 families) 26.1 %;</p> <p>geographic origin:</p> <p>Caucasian (10)</p> <p>Central Africa (5)</p> <p>North Africa (3)</p> <p>Turkey (3)</p> <p>nd (3)</p> <p>Asia (2)</p> <p>Western Africa (1)</p> <p>Guadeloupe (1)</p> <p>Cape Verde (1)</p> <p>-72 (222) 32.4%</p>	<p>27 novel mutations/ 60 mutations detected</p>	<p>The presence of large deletions is related an earlier presentation.</p> <p>60 mutations were identified.</p> <p>Del 1-20 was detected in 32.4% of the studied alleles</p>	21
Najafi et al (2019)	<p>Iran;</p> <p>- non-specified suspected BS</p> <p>(17 patients)</p>	<p>-BS type 3 (12)</p> <p>- pseudo-BS (4)</p> <p>- no mutation (1)</p>	12 (12)	<p>-12 (12);</p> <p>-24 (24) 100%</p>	<p>-0 / (12)</p> <p>-0 (24 alleles)</p>	<p>- BS type 3 was the most frequent type of BS and</p> <p>del 1-20 was the most frequent variant detected</p>	4

This study (2020)	Brazil;	-BS type 1 (1)	17 (22) 77.2%	- 5 (17); -16 (44) 36.4%	- p.Ala204Thr (homozygous 2 + heterozygous 2)	- BS type 3 was the most frequent type of BS detected in this Brazilian cohort. Del 1-20 was the most common variant detected. Authors suggested a fluxogram for genetic analysis of Brazilian suspected-BS patients.
	-non-specified BS (22; 21 families)	-BS type 2 (0)			- p.Glu225* (homozygous 1)	
		-BS type 3 (17)			- p.Leu7Ala fs*3 (heterozygous 2)	
		-BS type 4a (1)			- p.Arg304* (heterozygous 1)	
		-BS type 4b (1)			- p.Ala467Val (heterozygous 1)	
		-GS (2)			- p.Arg595* (heterozygous 1)	
		pseudo-BS (1)			- p.Tyr520* (heterozygous 1)	
					- p.Gly437Arg (heterozygous 1)	
					- del exons 6 a 19 (heterozygous 1)	

Figures

Figure 1 – Algorithm for the investigation of clinically suspected Bartter Syndrome Brazilian Patients.

Figure 1

[See figure.]