

Description of the novel variant c.784delG;p. (Ala262Profs*68) at *BSND* gene and its association with Bartter Syndrome Type Iva

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Abstract. Bartter syndrome (BS) is a group of diseases caused by variants in genes related to salt reabsorption in the thick ascending limb of the loop of Henle. It causes dysregulation in salt homeostasis and is characterized by hyperplasia and hypertrophy of the juxtaglomerular apparatus, hyperaldosteronism, hypokalemic alkalosis and impaired growth and development. BS type IVa is caused by variants in *BSND*, which encodes Barttin, a subunit for chloride channels (CLC). This specific subtype also causes sensorineural deafness due to its impact on CLC-kidney a (Ka), a channel important for the production of endolymph in the inner ear. In the present study, the case of a Brazilian girl diagnosed with BS Iva was presented, whose molecular diagnosis revealed a novel variant, c.784delG;p.(Ala262Profs*68), compound heterozygous with c.139G>A;p.Gly47Arg. This novel variant appears to be the first BS IVa causing variant described in exon 4, which encodes for the later part of the cytoplasmatic C-terminal unstructured tail. *In silico* analysis of this variant predicted the resulting frameshift as disease causing, due to alteration in significant portion of the protein. While the authors suggest this variant may cause erroneous membrane sorting of the CLC type channels, further studies are necessary to elucidate the mechanism.

Introduction

Bartter syndrome (BS) is a rare autosomal recessive disease caused by pathogenic variants in the genes coding for proteins related to salt transport and regulation in the thick ascending limb of Henle's loop in the nephrons. It was first described in 1962, characterized by hyperplasia and hypertrophy of the juxtaglomerular apparatus, hyperaldosteronism and hypokalemic alkalosis, associated with impaired growth and development (1,2). Later studies divided BS into classic, as described by Bartter *et al* (1), and antenatal (3). Both types were further divided into subtypes, according to the affected gene: *SLC12A1* (antenatal, type I) (4-6), *KNJI* (antenatal, type II) (7,8), *CLCNKB* (classic, type III) (9,10), *BSND* or *CLCNKA* and *CLCNKB* at the same time (antenatal, type IV) (11-14).

BS type IVa (OMIM 602522) is used to refer to BS type IV caused by variants in the gene *BSND*. BS type IV usually presents with severe clinical manifestations and is the only subtype which incurs sensorineural deafness. It is typically presented by premature birth, polyhydramnios, hyper-PGE2, severe hypokalemic hypochloremic metabolic alkalosis, iso- or hyposthenuria, and previously mentioned sensorineural deafness (12,15-18). The *BSND* gene, located in 1p32.3, is composed of 4 exons, and encodes for Barttin, the β -subunit of chloride channel (CLC)-kidney a (Ka) and CLC-Kb channels. These largely similar channels are part of the CLC family of voltage-gated chloride channels and were named after appearing to be two different channels of this family that were kidney-specific (chloride channel-kidney; letters 'a' and 'b' being used solely to differentiate the two). Further studies demonstrated that they are expressed in basolateral membranes of renal tubules, in the thick ascending limb of Henle's loop, and also the stria vascularis of the inner ear (11,19-21).

Barttin is composed of 320 amino acids: A short N-terminus (aa1-8) which affects CLC-K trafficking and activation, two transmembrane helices (aa9-26 and aa35-54), an extracellular linker between them (aa27-34),

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and a cytoplasmatic C-terminal tail (aa 55-320) of which 17 amino acids (aa55-72) are needed for CLC-K conductivity (11,19,22-24). Barttin is essential for CLC-Ka and CLC-Kb function and altered or loss of Barttin function will alter or impede function of these channels. This will in turn cause an ion imbalance in the thick ascending limb (TAL) of Henle's loop, where all proteins associated with BS act in conjunction with one another to promote ion reabsorption in the nephron (18,25-27). The differentiation of BS type IV from the other types is associated with the expression of Barttin along with CLC-Ka and CLC-Kb in the inner ear, where these channels help maintain K⁺ levels in the endolymph. Therefore loss of Barttin function will also cause hearing impairment (11,20).

Several pathogenic variants are described in the *BSND* gene and most of them are located in exon 1, which is the portion that codifies the N-terminus and transmembrane helices (Table I). These variants have significant effects on the structure and function of the Barttin protein, potentially interfering with its ability to interact with CLC-K channels or impairing its stability within the cell membrane (11,12,28-43).

In the present study, an uncommon association between a novel variant in exon 4 of the *BSND* gene (c.784delG;p. (Ala262Profs*68)) together with the known pathogenic variant in exon 1 (c139G>A, rs74315289) was described. The variant was found in a 10-year-old Brazilian girl diagnosed with typical BS type IV. DNA sequencing revealed that this is the unique alteration found in this gene and that the compound heterozygous variant was potentially responsible for the development of BS type IVa in this patient.

Materials and methods

Subject and case report. The patient is female, 10 years old at the time of diagnosis. The patient and mother were informed, and the mother signed a consent form. Patient's father is deceased and there are no known affected relatives. The patient was clinically diagnosed and cared for in University Hospital of Brasilia (HUB), in July 2017, when peripheral blood was collected from patient and mother for molecular diagnosis and participation in the present study.

The patient was born from non-consanguineous parents, premature (31 weeks), weighing 1,715 g, with the mother presenting with polyhydramnios. Symptoms onset occurred at ~7 years of age, but renal tubulopathy was not suspected at the time, despite a history of inadequate growth, sensorineural deafness and repeated convulsions, leading to a diagnosis of epilepsy. Referral to nephrology services was due to nephrocalcinosis detected by abdominal ultrasonography. Clinical diagnosis of Bartter type IV with neurosensorial deafness occurring at the age of 10 years and 10 months, presenting not only the aforementioned moderate inadequate growth (Z-score of -2.06; percentile 2%; corrected for birth at 31 weeks), sensorineural deafness and epilepsy, but also intestinal constipation, hyperreninemia, hypokalemic hypochloremic metabolic alkalosis, hypercholesterolemia, nephrocalcinosis, hypocitraturia, but no renal insufficiency (more details in Table II).

The patient has been monitored and treated in the Pediatric Nephrology Services of the University Hospital of Brasilia in the following years. Treatment was prescribed with potassium

citrate, hydrochlorothiazide, amiloride, indomethacin and potassium supplementation with potassium chloride. The patient was also undergoing treatment with sodium valproate for epilepsy control. During clinical follow-up, hospitalizations were required for the management of hypokalemia episodes. Socio-economic factors caused difficulties for the family in adhering to medical instruction and in obtaining the prescribed medications, which led to discontinuation of treatment in 2021. At the most recent follow-up, at 15 years of age, the patient was using: potassium citrate 20 mEq three times daily, hydrochlorothiazide 25 mg daily, amiloride 2.5 mg daily, potassium chloride 600 mg three times daily, indomethacin 50 mg daily, and had discontinued sodium valproate on her own initiative. On physical examination, the patient had a weight of 50.9 kg, height of 158 cm, blood pressure of 90/60 mmHg, was in favorable general condition, presented with sensorineural hearing loss and speech impairment. Bilateral nephrocalcinosis and nephrolithiasis persisted, with normal renal function and hypokalemia (creatinine 0.5 mg/dl; potassium 3.02 mEq/l). The present study was approved (approval no. 81191617.3.0000.5504, opinion no. 2.550.807) by the Ethics Committee of the Federal University of São Carlos (São Paulo, Brazil).

DNA extraction and purification. Peripheral blood samples from patient and mother were collected. Genomic DNA was extracted using Gen Elute Blood Genomic DNA kit (MilliporeSigma), following the manufacturer's instructions. The coding sequences of *BSND* gene were amplified by polymerase chain reaction, using primers previously described (11): 5'-GAGCAGAGAGAAGACCGAGTC-3' and 5'-TGTCTTCTCTCCCTGTGTAAGC-3' for exon 1; 5'-TGCCTAACTCACAGAATTGAGAG-3' and 5'-ACAGAGGCTGTCTCTCCTTTG-3' for exon 2; 5'-CTCTCCTTTTAAACCCTTGAAC-3' and 5'-GACCACATACCCAAAGCAAC-3' for exon 3; 5'-CCATTTTGCAGATAGGGAAAC-3' and 5'-CGGGAAGGTGGATTATCCTAC-3' for exon 4. The reactions were carried out using Taq High Fidelity Polymerase master mix (Cellco) following the manufacturer's instructions. Amplification was performed under the following conditions: an initial denaturation step at 95°C for 1 min; followed by 35 cycles of denaturation at 95°C for 30 sec, annealing at 58°C for 30 sec, and extension at 72°C for 1 min; with a final extension step at 72°C for 3 min. Agarose gel (1%) electrophoresis was performed following amplification to check for the presence and quality of the fragments of interest, using ethidium bromide for visualization. For sequencing, the PCR product was purified using Agarose Gel Extraction Kit (Cellco) or following a previously described method (44). Sequencing was performed using Sanger's methodology by MacroGen Inc.

Genetic analysis. Variants were identified by comparison with the reference sequence for *Homo sapiens* chromosome 1, GRCh38.p14 Primary Assembly NC_000001.11 (54998883.55017222) and for *BSND* transcript NM_057176.3. Previously described variants were sourced from the literature and ensemble variation table, which sources data from several public databases including dbSNP (<https://www.ncbi.nlm.nih.gov/snp/>), ClinVar (<https://www.ncbi.nlm.nih.gov/clinvar/>) and HGMD-public (<https://www.hgmd.cf.ac.uk/ac>). Variant

Table I. Bartter's Syndrome type IVa described in databanks and literature.

First author/s, year	Variant ID	Position (GRCh38)	Class	DNA consequence	Exon	Intron	Protein consequence	(Refs.)
Birkenhäger <i>et al</i> , 2001	rs74315284 (dbSNP) CM013300 (HGMD-PUBLIC)	1:54999187	SNP (START lost)	c.1A>T	1	-	p.0	(11)
Ozlu <i>et al</i> , 2006	CM065989 (HGMD- PUBLIC)	1:54999188	SNP (START lost)	c.2T>A	1	-	p.0	(36)
Birkenhäger <i>et al</i> , 2001; Ashton <i>et al</i> , 2018; Zaffanello <i>et al</i> , 2006, Riazuddin <i>et al</i> 2009	rs74315286 (dbSNP) CM013299 (HGMD-PUBLIC) rs121908145 (dbSNP) CM094872 (HGMD-PUBLIC)	1:54999189 1:54999196	SNP (START lost) SNP (STOP gained)	c.3G>A c.10G>T	1 1	- -	p.0 p.Glu4*	(11,37,38) (39)
Birkenhäger <i>et al</i> , 2001; Wang <i>et al</i> , 2017	rs74315285 (dbSNP) CM013302 (HGMD-PUBLIC)	1:54999208	SNP (missense)	c.22C>T	1	-	p.Arg8Trp	(11,40)
Elrharchi <i>et al</i> , 2018	rs74315285 (dbSNP) CM1812478 (HGMD-PUBLIC)	1:54999208	SNP (missense)	c.22C>G	1	-	p.Arg8Gly	(41)
Birkenhäger <i>et al</i> , 2001	rs74315288 (dbSNP) CM013301 (HGMD-PUBLIC)	1:54999209	SNP (missense)	c.23G>T	1	-	p.Arg8Leu	(11)
Birkenhäger <i>et al</i> , 2001	rs74315287 (dbSNP) CM013303 (HGMD-PUBLIC)	1:54999214	SNP (missense)	c.28G>A	1	-	p.Gly10Ser	(11)
Kitanaka <i>et al</i> , 2006	CM063882 (HGMD-PUBLIC)	1:54999280	SNP (STOP gained)	c.94C>T	1	-	p.Gln32*	(42)
Elrharchi <i>et al</i> , 2018	rs777656311 (dbSNP)	1:54999293	SNP (missense)	c.107C>A	1	-	p.Thr36Asn	(41)
Ashton <i>et al</i> , 2018	-	1:54999311	SNP (missense)	c.125G>A	1	-	p.Ser42Asn	(37)
Wang <i>et al</i> , 2017	rs34561376 (dbSNP)	1:54999313	SNP (missense)	c.127G>A	1	-	p.Val43Ile	(40)
Walsh <i>et al</i> , 2018; Lee <i>et al</i> , 2012; Miyamura <i>et al</i> , 2003; Brum <i>et al</i> , 2007; García- Nieto <i>et al</i> , 2006; Kitanaka <i>et al</i> , 2015; Heilberg <i>et al</i> , 2015	rs74315289 (dbSNP) CM035675 (HGMD-PUBLIC)	1:54999325	SNP (missense)	c.139G>A	1	-	p.Gly47Arg	(28-32,42,43), present study
Birkenhäger <i>et al</i> , 2001	rs1389952796 (dbSNP) CG015703 (HGMD-PUBLIC)	1:54999343- 5499383	Indel (splicing region)	c.157-177+2- del	1	1	-	(11)

Table I. Continued.

First author/s, year	Variant ID	Position (GRCh38)	Class	DNA consequence	Exon	Intron	Protein consequence	(Refs.)
Walsh <i>et al</i> , 2018	-	1:54999365	SNP (splicing region)	c.177+2T>A	-	1	-	(28)
Zaffanello <i>et al</i> , 2006	CS066269 (HGMD-PUBLIC)	1:54999368	SNP (splicing region)	c.177+5G>C	-	1	-	(38)
Ozlu <i>et al</i> , 2006	rs771232166 (dbSNP) CM065990 (HGMD-PUBLIC)	1:55005106	SNP (STOP gained)	c.262G>T	2	-	p.Glu88*	(36)
de Pablos <i>et al</i> , 2014	CI145856 (HGMD-PUBLIC)	1:55007026	Indel (frameshift)	c.302dup	3	-	p.Trp102Valfs*7	(33)
Shajan <i>et al</i> , 2023; Walsh <i>et al</i> , 2018; Plumb <i>et al</i> , 2016	rs765135576 (dbSNP)	1:55007175- 55007176	indel (frameshift)	c.452delC	3	-	p.Pro151Leufs*27	(12,28,34)
	CG094068 (HGMD-PUBLIC)	-	Loss of exon 2-4	-	2-4	-	-	(35)
Birkenhäger <i>et al</i> , 2001	CG015704 (HGMD-PUBLIC)	-	Loss of exons 3 and 4	-	3-4	-	-	(11)
	-	1:55008449	Indel (frameshift)	c.784delG	4	-	p.(Ala262Profs*68)	Present study

pathogenicity was predicted via online tool MutationTaster2 (<http://www.mutationtaster.org>) (45), and CADD Score (<https://cadd.gs.washington.edu/score>) (46).

Homology analysis. The sequence for the wild-type Barttin was used as a query on pBLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastp&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome). The complete sequences for matches with identity >90%, excluding synthesized sequences, were selected. Coding sequences of the selected sequences were used for DNA homology analysis. ClustalW (<https://www.genome.jp/tools-bin/clustalw>) multiple alignment tool was used to align the selected sequences with the wild-type *BSND*; Barttin sequence, coding and primary sequences for c.139G>A;p.Gly47Arg and the sequence resulting from c.784delG;p.(Ala262Profs*68).

Results

Clinical diagnosis of BS type IV guided the genetic analysis to the *BSND* gene, as this is the most common gene related to BS with sensorineural deafness, with a few reported cases of recessive pathogenic variants affecting both *CLCNKA* and *CLCNKB* genes (47,48). Sequencing analysis revealed that in our patient, the *BSND* gene was indeed the reason for the

present syndrome, caused by the compound heterozygous variants. In exon 1, analysis revealed the pathogenic variant c.139G>A;p.Gly47Arg (rs 74315289), which has been previously described in patients with BS type IV (28-32,42,43,49). In exon 4, the novel variant c.784delG;p.(Ala262Profs*68) was found (Fig. 1A). Variant analysis on the patient's mother, revealed that she is heterozygous for c.139G>A;p.Gly47Arg, but the c.784delG;p.(Ala262Profs*68) variant was not found (Fig. 1B). It was not possible to determine if the novel variant c.784delG;p.(Ala262Profs*68) is a *de novo* mutation or was inherited from the father, since he was deceased when the present study was carried out and variant analysis was not possible.

In silico analysis of deletion of c.784delG;p. (Ala262Profs*68) in MutationTaster2 (39) classified it as 'disease causing' and showed that the resulting frameshift modifies 58 residues in the C-terminal region. It then causes the loss of the original stop codon, adding another 9 residues in comparison to the wildtype protein. CADD analysis was also performed (46), obtaining a scaled (PHRED-like) score of 18.80. Barttin's structure has not yet been established empirically. However, prediction models indicate the cytoplasmic C-terminal region is unstructured. The variants found in the *BSND* gene including the new variant found in the present study are described in Fig. 2.

Table II. Patient's clinical presentation at diagnosis (10 years old).

Parameter	Patient	Reference range
Sex	F	-
Age	10	-
Height	136 cm	-
Weight	25.5 kg	-
Blood Pressure	90x60 mmHg	-
Creatinine	0.5 mg/dl	0.12-1.06 mg/dl
Sodium	138 mmol/l	136-145 mmol/l
Potassium	2.97 mmol/l	3.5-5.5 mmol/l
Chloride	91 mmol/l	95-105 mmol/l
Calcium	10.1 mg/dl	8.8-10.1 mg/d
Magnesium	2.2 mg/dl	1.6-2.2 mg/dl
Blood pH	7.412	7.32-7.42
Blood Bicarbonate	33.8 mmol/l	21-28 mmol/l
Serum Renin	>500 μ UI/ml	6-70.8 μ UI/ml
Aldosterone	21 ng/dl	$\leq$ 40 ng/dl
Urine calcium	78 mg/24 h	42-353 mg/24 h
Urine Sodium	58 mmol/24 h	40-180 mmol/24 h
Urine Potassium	20 mmol/24 h	40-80 mmol/24 h
Urine Chloride	54 mmol/24 h	15-40 mmol/24 h

Discussion

The present case represents the first description of the pathogenic variant c.784delG;p.(Ala262Profs*68). To the best of our knowledge, it is the first reported variant affecting exon 4 exclusively. Homology analysis showed that there is conservation in this region with other primates (Fig. 3), suggesting it may have a significant function. The CADD scaled score of 18.8 also indicates the likelihood of pathogenicity. It is not advisable to use hard cut-off values for CADD scores as evidence of pathogenicity. However, the developers of the tool suggest that a value between 10 and 20 would be adequate, as a score of 10 would indicate the variant is among the 10% most deleterious, and a score of 20 represents the top 1% in this parameter (46). The CADD score for the novel deletion variant is comparable to the score of 26.7 for c.139G>A;p.Gly47Arg.

There are currently 21 *BSND* variants described in patients with BS type IV, including the aforementioned c.139G>A;p.Gly47Arg, SNPs, small indels and gross deletions (Table I and Fig. 2) (11,12,28-43). Of the 21 variants, three cause loss of START codon, eight are missense SNPs (three located in the N-terminal stretch, one in the first helix, four in the second helix), three affect splicing regions, three result in premature STOP codons, two are gross deletions involving at least two entire exons, and two cause frameshift which alter or truncates the major part of the cytoplasmatic C-terminal.

A total of 14 out of 18 described pathogenic variants of *BSND* alter the protein prior to aa72. This is not surprising since the C terminal region has little function according to the literature. Studies have shown that truncations of the protein

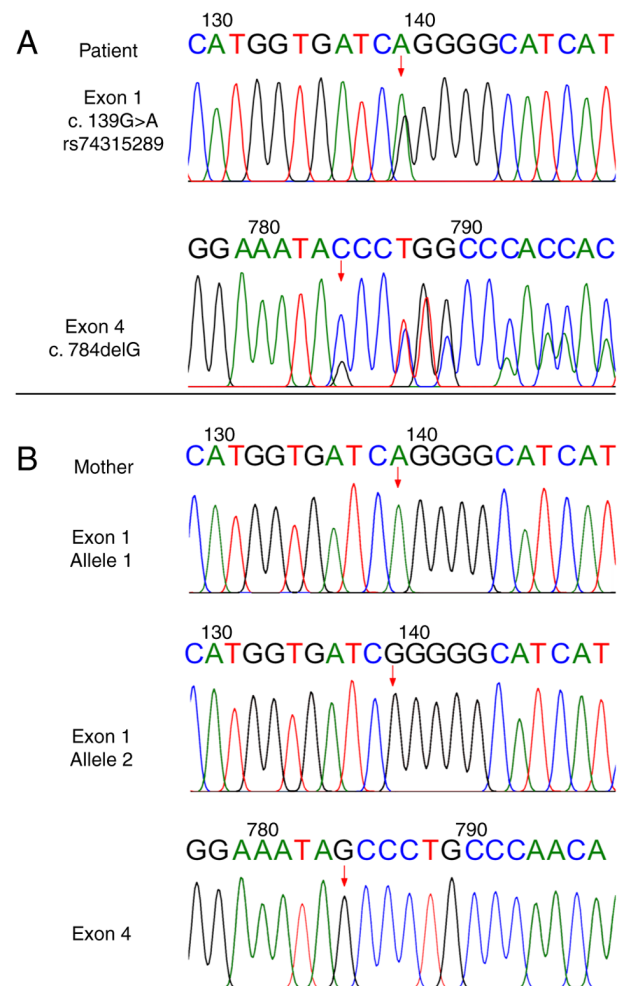


Figure 1. *BSND* variants found in patient using Sanger sequencing. The variant (A) c.139G>A;p.Gly47Arg (rs74315289) in exon 1 and (B) c.784delG;p.(Ala262Profs*68) at exon 4 of the *BSND* gene from an affected female individual. Arrows indicate the nucleotide change in exon 1 or the location of the deletion in the sequence of exon 4.

anterior to residue 72 result in the loss of the stimulatory function over CIC channels; however, truncations beyond this residue have little to no influence in membrane insertion and activation of CLC-K channels (19,22,49,50). Some studies question this assumption, as they show the C-terminus region is important for membrane localization and normal CLC-K channel function (22,51). Frameshift variants are reported in several genetic disorders, and usually result in significant truncations (52,53) and/or loss of important motifs (54). In a study presented by Al Salmani *et al* (55), however, it was shown how a frameshift in the largely disordered cytoplasmatic C-tail of a potassium channel affects its function through several different mechanisms.

The present study revealed pathogenic variant c.784delG;p.(Ala262Profs*68). This frameshift substitutes the final 58 residues of the wild-type protein for 68 different residues. The variant c.452delC (rs765135576) is similar to the deletion found in the present study, as it is also a single base deletion resulting in frameshift, affecting solely the C-terminal tail (beyond aa262). The patient in that study was homozygous for the variant but no hypothesis for the mechanism responsible for its pathogenicity was

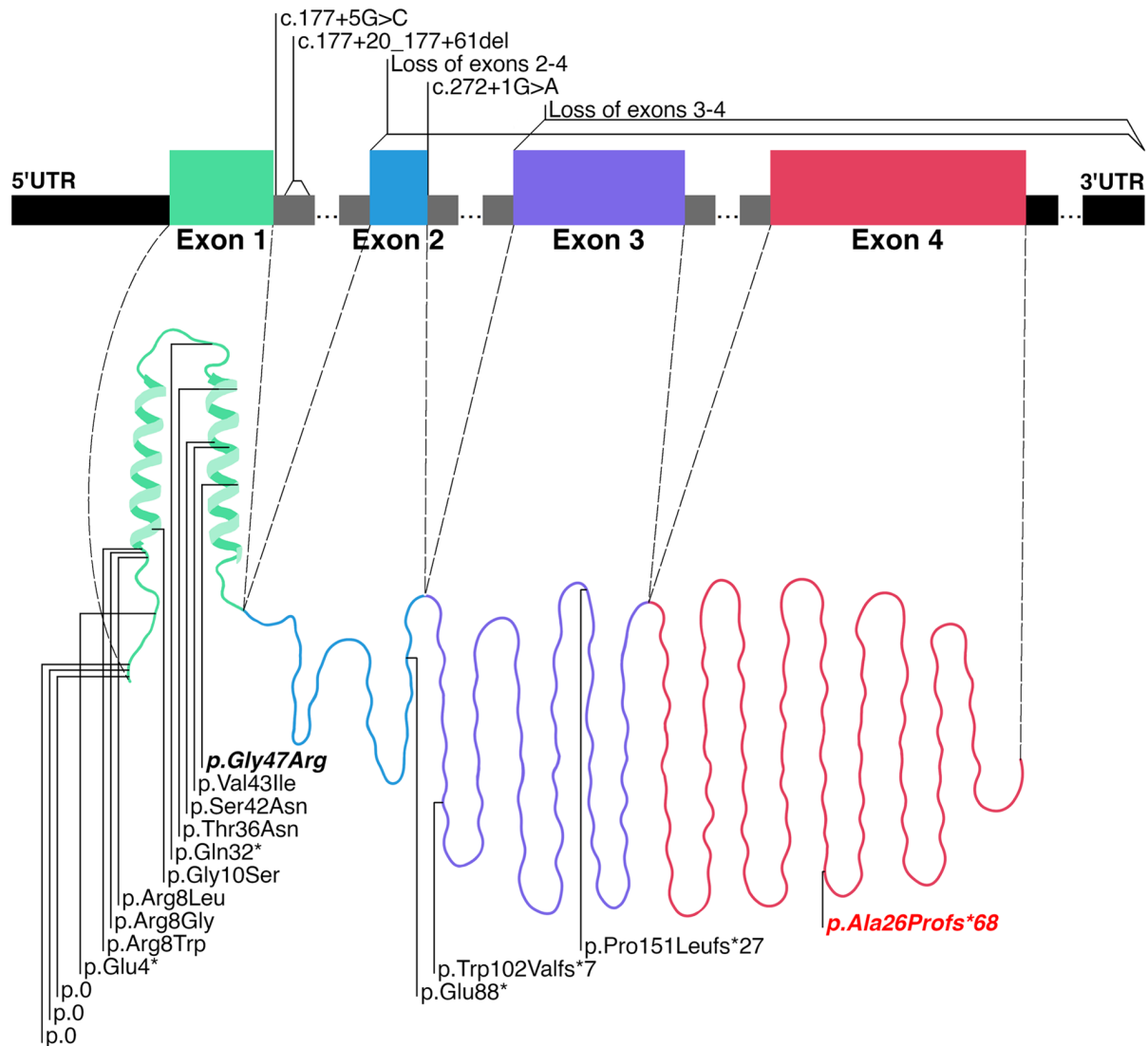


Figure 2. Bartter type IVa causing variants in the *BSND* gene and Barttin protein. Schematic of the position of the known variants and their position in the protein (missense and nonsense SNPs, and frameshifts) and coding sequence (loss of exons and splicing variants).

discussed (28). A naturally occurring pathogenic truncation in Glu88 was described in 2006 (36), and further investigations of this variant showed that even if expression and function were maintained, proteolysis was increased and membrane distribution of ClC-K channels was affected, reducing transepithelial transport (51). This suggests some trafficking regulation function beyond Glu88. Since truncation studies rely solely on electrophysiology observations, they would not note the effects of improper epithelial sorting. This could explain the pathogeny of c.784delG;p. (Ala262Profs*68), if it is assumed that the trafficking regulation region lies beyond Ala262. Another possibility is the impact of the stretch of 68 residues produced by the frameshift that affect proper protein folding, leading to increased proteolysis. For more robust conclusions, these hypotheses must be thoroughly tested in further studies involving the expression of the mutated protein in order to evaluate expression levels, proteolysis and channel function. The lack of western blot experiments is a limitation of the present study.

The current patient appeared with a typical clinical presentation, but late diagnosis despite her clinical history. The diagnosis of BS usually occurs before early childhood, especially in antenatal BS cases (28,36,38,41,56,57). Some cases of late diagnosis of BS type IV have been reported (30-32,43). However in those cases, clinical presentation was markedly milder than in our patient, who had moderately inadequate growth and an epilepsy diagnosis at the age of 7. Interestingly, in all the other late diagnosis cases aforementioned, patients were homozygous for the c.139G>A;p.Gly47Arg variant.

This variant was shown to decrease conductance of ClC-Ka when co-expressed in *Xenopus* oocytes in a similar manner to other missense variants reported as pathogenic. There was no analysis of co-expression with ClC-Kb (49), which Brum *et al* (31) pointed out as possibly retaining some function in relation to ClC-Kb, allowing for a milder presentation. In the sole study in which the c.139G>A;p.Gly47Arg variant was found as a heterozygous compound variant with c.94C>T (a nonsense variant), the patient had a mild presentation in the

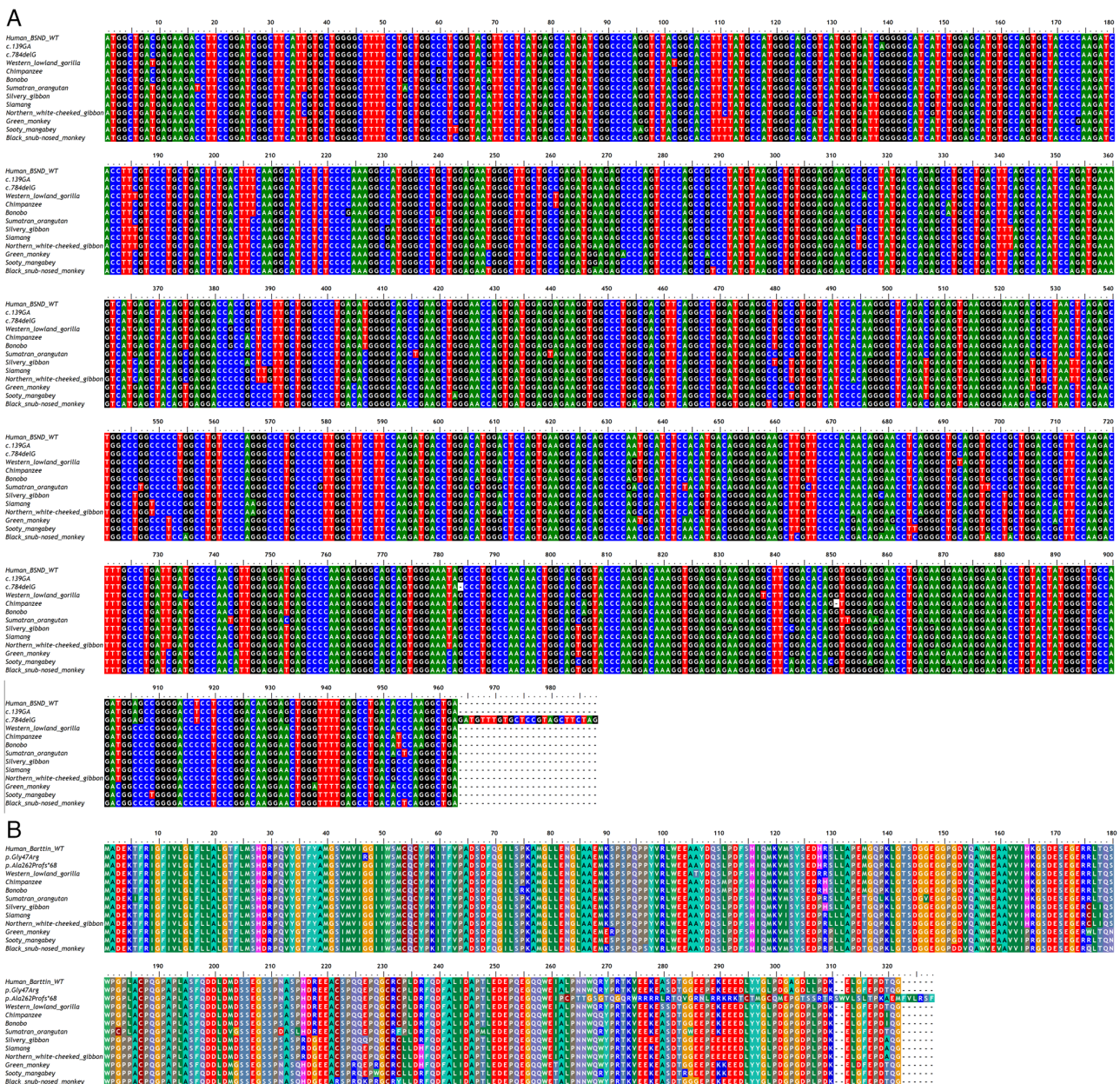


Figure 3. Sequence homology analysis of (A) *BSND* coding sequence and (B) Barttin amino acid sequence. Analysis was performed with c.139G>A;p.Gly47Arg, c.784delG;p.(Ala262Profs*68), wild type and primates with >90% protein identity with wild-type human Barttin obtained from BLAST.

perinatal period but had severe and rapid kidney deterioration in early adolescence. In that case, c.139G>A;p.Gly47Arg was compound heterozygous with c.94C>T; (p.Gln32*), which results in a significant truncation (42). Therefore, the possible function retention of c.139G>A;p.Gly47Arg may not be enough to present as a mild version of BS type IV when in heterozygosity.

In conclusion, the novel c.784delG;p.(Ala262Profs*68) variant in the cytoplasmatic C-terminal region was presented. While caution must be taken as this is a single patient study, the presence of a variant expected to elicit lesser clinical manifestations, combined with the novel variant in the C-terminal region in a patient with typical clinical presentation, suggests important functional features in this region. There is also evidence that the variant c.139G>A;p.Gly47Arg, known to lead to a milder phenotype in homozygosity, may

not have the same lessening effect when in heterozygosity. The current lack of functional evidence for the novel variant is a limitation of the present study. Further studies are being performed to better understand the pathological mechanism of this novel variation.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

JHB performed the PCR amplifications, analyzed the sequencing results and performed bioinformatic analysis. LCDO and MDCSDS provided patient information and samples. AT collected the patient's data and clinical information. AFDC and AT were involved in the design of the study and acquired funding. JHB, AFC and AT contributed in the analysis and interpretation of data and wrote the manuscript. JHB and AFC confirm the authenticity of all the raw data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The present study was approved by the Federal University of São Carlos Ethics Committee (approval no. 81191617.3.0000.5504; opinion no. 2.550.807; São Paulo, Brazil).

Patient consent for publication

The patient and parent were informed and signed a consent form (parent) or assent form (child).

Competing interests

The authors declare that they have no competing interests.

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