Application of molecular biology at the approach of Bartter's syndrome: case report

Aplicação da biologia molecular na abordagem da síndrome de Bartter: relato de caso

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Abstract

This study shows the usefulness of a molecular biology approach to the diagnosis of Bartter`s syndrome (BS), through a report of two cases. A flow chart for the molecular diagnosis of BS is also proposed. The two cases (two sisters) featured polyhydramnios-complicated pregnancy, prematurity and low birth weight. During the first year of life, the younger sibling exhibited polyuria, polydipsia and failure to thrive, leading to the investigation of renal tubular diseases and innate errors of metabolism. Laboratory work-up suggested BS, but the definitive diagnostic was only obtained after the detection of a homozygous mutation of the exon 5 of the KCNJ1 gene, resulting in a substitution of valine for alanine at codon 214 (A214V) in both DNA strands of the two sisters and a heterozygous mutation in their parents. The definitive diagnostic of BS is frequently very difficult to be obtained. Consequently, considering the reported cases, we showed the utility of molecular techniques for the definitive diagnostic of BS, and proposed a diagram for the rational use of these techniques.

Keywords: Molecular Biology. Renal Tubular Transport Inborn Errors. Bartter`s Syndrome.

Resumo

O presente trabalho teve como objetivo mostrar a utilidade da biologia molecular para o diagnóstico da síndrome de Bartter (SB) por meio do relato de caso de duas irmãs e propor um algoritmo para abordagem molecular dessa síndrome. Os dois casos relatados apresentaram prematuridade, gestação complicada com poli-hidrâmnio e baixo peso ao nascer. Durante o primeiro ano de vida, as crianças apresentaram poliúria, polidipsia e atraso no crescimento, o que levou à investigação de doenças tubulares renais e erros inatos do metabolismo. Os exames laboratoriais sugeriram SB, mas a confirmação diagnóstica só foi obtida pela detecção de mutação em homozigose no exon 5 do gene KCNJ1, resultando em substituição do aminoácido alanina por valina no códon 214 (A214V) nas duas fitas de DNA nas duas irmãs e de mutação em heterozigose em seus pais. O diagnóstico de certeza da SB muitas vezes é difícil de ser obtido. Dessa forma, por meio dos casos relatados, mostrou-se a utilidade de métodos moleculares para o diagnóstico de certeza da SB, e foi proposto um algoritmo para a utilização racional dessas técnicas.

Palavras-chave: Biologia Molecular. Erros Inatos do Transporte Tubular Renal. Síndrome de Bartter.

Introduction

Bartter`s syndrome (BS), a heterogeneous group of tubulopathies with recessive and dominant autosomal inheritance, is due to impairment of sodium and chloride resorption in the thick ascending limb of Henle`s loop.[1-3] Molecular studies allowed identification of at least five different subtypes of the syndrome[1-6] (Table 1).

BS type I may present with hypercalciuria, nephrocalcinosis, metabolic alkalosis and hypocalcemia, due to mutations of the SLC12A1 gene, which encodes the bumetanide-sensitive co-transporter NKCC2.[3,6] BS type II,

Table 1 Bartter`s syndrome types according to the usual nomenclature, affected genes and clinical denomination

BS type	Genetic mutation	Localization	Clinical denomination
BS type I	SLC12A1	12q15-21	Neonatal, prenatal
BS type II	KCNJ1	11q24	Neonatal
BS type III	CLCNKB	1p36	Classic
BS type IV	BSND	1p32.1	Neonatal with deafness
BS type V	CASR	3q13	Neonatal with deafness

BS: Bartter`s syndrome.

which is characterized by polyhydramnios, prematurity, severe polyuria and increased prostaglandin E, is caused by mutations in the potassium inwardly-rectifying channel, subfamily J, member 1(KCNJ1) gene, which encodes the renal outer medullary K (ROMK) potassium channel.[2,7] Changes in the chloride channel Kb (CLCNKB) gene, which encodes CLC-Kb, reduce channel activity, producing BS type III, which is associated with significant salt loss and hypokalemia.[8,9] BS type IV has a prenatal presentation consisting of sensorineural deafness and early renal failure.[10] It is chiefly caused by mutations of the Bartter syndrome, infantile, with sensorineural deafness (BSND) gene, which encodes bartin, a protein modulating stability, superficial cellular location and function of the CIC-Ka and CIC-Kb channels.[10] BS type V consists of a gain-of-function mutation of the CASR gene encoding the calcium ion-sensitive receptor (CaR).[2,3] Mutations of the Calcium Sensing Receptor (CaSR) may cause autosomal dominant forms of BS.[2-5]

Because their diagnosis is frequently delayed, BS patients are inadequately managed for long periods, with the development of nephrocalcinosis and even end-stage chronic kidney disease (CKD).[9] We report two sisters diagnosed with BS, highlighting the importance of genetic characterization for a definitive diagnosis. We also propose a flow chart for the rational use of molecular biology techniques for BS diagnosis.

Case report

Case 1

The index case was a 1-year-old girl, born after a polyhydramnios-complicated 34-week gestation, with low birth weight (1,940 g). During the first year of life she had recurrent fever episodes, vomiting, polyuria, polydipsia and failure to thrive. Extensive work-up and several treatment regimens were to no avail. The girl`s parents were first-degree cousins. BS was initially diagnosed on clinical and laboratory grounds, after urinary salt loss (fractional excretion of Na^+ = 3.5%) associated with intermittent hypokalemia (K^+ = 3.2 to 3.7 mEq/L), signififcant hypochloremia (Cl^- = 93 mmol/L), metabolic alkalosis ($HCO3^-$ = 30), hyperfiltration (220 mL/min/1.73 m^2), hypercalciuria (urinary Ca^{+2} = 6,5 mg/kg/day) and increased aldosterone concentration (65 pg/mL) and plasma renin activity (4,3 ngAngI/mL/h) were detected. Renal ultrasound showed mild medullary nephrocalcinosis.

Case 2

Because of the index case and the parents` consanguinity, we raised the possibility of BS in the girl`s older and only sister. This second girl had also been prematurely born, after a polyhydramnios-complicated 34-week gestation, with low birth weight (2,235 g). During the first years of life there were no symptoms. At the age of 3 years, she developed moderate polyuria and polydipsia, with mild failure to thrive. Laboratory work-up revealed hypochloremia (Cl^- = 96 mmol/L), metabolic alkalosis ($HCO3^-$ = 28 mEq/L) with low-normal serum potassium levels, urinary salt loss (fractional excretion of Na^+ = 2%), hyperfiltration (150 mL/min/1.73 m^2) and high-normal urinary calcium levels (3.8 mg/Kg/day). The plasma renin activity (2.2 ngAng I/mL/h) and aldosterone (47 pg/mL) were increased. Renal ultrasound showed mild medullary nephrocalcinosis.

DNA was extracted from the whole blood of the two patients and their parents, according to a standard protocol. Details about the PCR reaction and the oligonucleotides used are available on request. Automated sequencing (ABI 3130, Applied Biosystems, Foster City, CA) identified a homozygous mutation of the exon 5 of the KCNJ1 gene,

resulting in a substitution of valine for alanine at the codon 214 (A214V) of the two strands of the children and a heterozygous mutation in the parents (Figure 1).

Initial treatment consisted basically of sodium chloride supplementation, indometacin, hydrochlothiazide for hypercalciuria control and oral potassium supplementation. This approach led to improvement of the clinical and laboratory parameters, with normalization of the metabolic imbalance and growth resumption. Nephrocalcinosis regressed and renal function has remained preserved.

Discussion

Diagnosis of BS in reference centers is normally achieved through teamwork experience.[11] Some findings, such as the presence of nephocalcinosis (frequent with mutations of the KCNJ1 and SLC12A1 genes) may suggest the diagnosis. Other signs and symptoms, such as polyhydramnios-complicated pregnancy, prematurity and low birth weight, are common to the several types of BS. Parental consanguinity and/or a history of similar cases in the family may arouse clinical suspicion. In our cases, consanguinity motivated an investigation of BS in the older sister, in spite of the absence of significant symptoms. Although BS can be diagnosed on clinical and laboratory grounds, only the finding of genetic mutations allows a definitive diagnosis and subsequent genetic counseling.

In the early 1990`s, it was difficult to distinguish BS from other tubulopathies, such as the Gitelman`s syndrome. Therefore, although not widely available in Brazil, molecular tools have become increasingly important for the diagnosis of BS.[2,5,6,9] The rational use of molecular biology techniques will certainly improve our understanding of the genetic aspects of BS, allowing an individualized approach to our patients.

There are few studies on the genetic diagnosis of BS.[2,5,6,9,12] The recent study by Brochard *et al.*,[2] who investigated mutations in 42 children with BS, is worth mentioning. Most of those children had heterozygous mutations of the KCNJ1 gene (45%). Transient neonatal hyperkalemia, which is generally little diagnosed, was detected in 63% of the children with a mutation of the KCNJ1 gene, not being observed in children with mutations of other genes.[2] Nozu *et al.*[12] showed that analysis of the genetic material of urinary cells may obviate the need of more invasive procedures, such as blood sampling and renal biopsy. The method proposed by those authors allowed detection of mutations in the SLC12A1 gene of BS type I.[12]

In Brazil, even in reference centers, the main difficulty in the diagnosis of BS lies in the precise identification of the mutation involved. Accordingly, the use of molecular biology techniques in the approach to BS diagnosis may speed diagnosis and treatment, allowing genetic counseling to be provided. We thus propose a flow chart as a rationale for the molecular diagnosis of BS in reference centers (Figure 2). As shown in Figure 2, the clinical and laboratory presentations should guide the initial molecular investigation. It should be pointed out that cases of combined mutations of the chloride channels, with a clinical picture resembling that of the bartin mutations have been described.[13]Although these rare cases may confound diagnosis, the proposed flow chart can still be used.

We reported two cases of BS, in which the investigation of mutations allowed a definitive diagnosis. We also proposed a flow chart to rationalize the molecular investigation of such cases. Molecular studies with a large number of patients are necessary to better understand the genetic expression of the disease in Brazil.

Figure 1. Result of the sequencing performed in the patients (C-Patient 2 and D-Patient 1) and their parents (A-Father and B-Mother), showing the presence of recessive homozygous alteration in the patients and its absence in the parents.

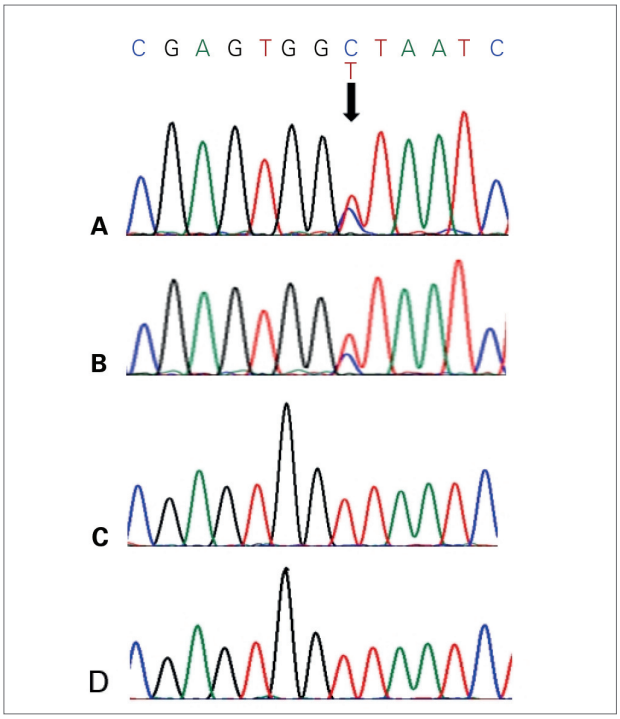
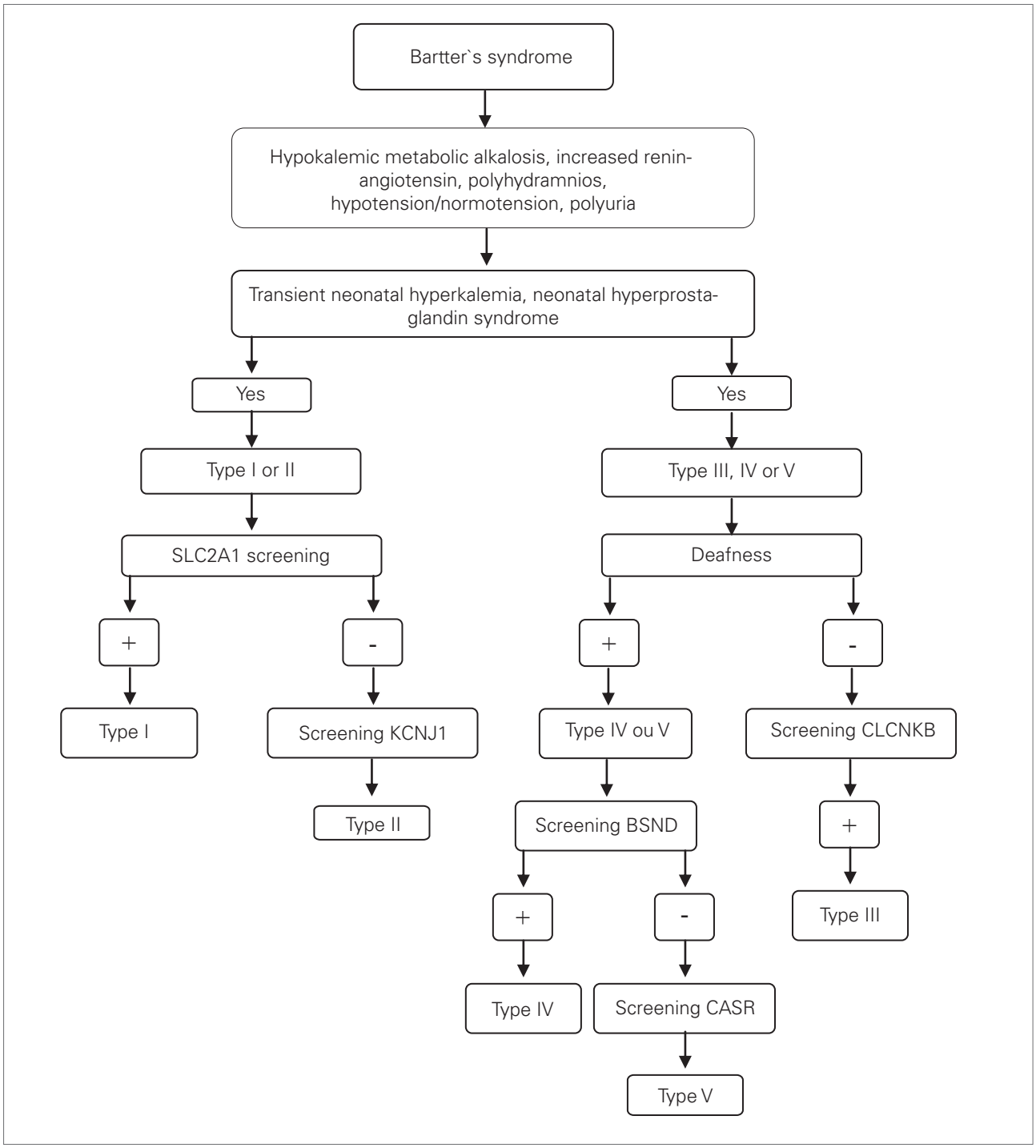


Figure available in color at the website: www.jbn.org.br

Figure 2. Flow chart for diagnostic investigation of the Bartter`s syndrome, including genetic analyses.



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