Exceptional Case

Late-onset Bartter syndrome type II

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Abstract

Mutations in the ROMK1 potassium channel gene (KCNJ1) cause antenatal/neonatal Bartter syndrome type II (aBS II), a renal disorder that begins in utero, accounting for the polyhydramnios and premature delivery that is typical in affected infants, who develop massive renal salt wasting, hypokalaemic metabolic alkalosis, secondary hyperreninaemic hyperaldosteronism, hypercalciuria and nephrocalcinosis. This BS type is believed to represent a disorder of the infancy, but not in adulthood. We herein describe a female patient with a remarkably late-onset and mild clinical manifestation of BS II with compound heterozygous KCNJ1 missense mutations, consisting of a novel c.197T > A (p.I66N) and a previously reported c.875G > A (p.R292Q) KCNJ1 mutation. We implemented and evaluated the performance of two different bioinformatics-based approaches of targeted massively parallel sequencing [next generation sequencing (NGS)] in defining the molecular diagnosis. Our results demonstrate that aBS II may be suspected in patients with a late-onset phenotype. Our experimental approach of NGS-based mutation screening combined with Sanger sequencing proved to be a reliable molecular approach for defining the clinical diagnosis in our patient, and results in important differential diagnostic and therapeutic implications for patients with BS. Our results could have a significant impact on the diagnosis and methodological approaches of genetic testing in other patients with clinical unclassified phenotypes of nephrocalcinosis and congenital renal electrolyte abnormalities.

Key words: Bartter syndrome, hypokalaemia, KCNJ1, nephrocalcinosis, ROMK

Introduction

Inactivating, i.e. loss-of-function, mutations in the KCNJ1 gene encoding the apical potassium inwardly-rectifying channel (ROMK) in the thick ascending limb (TAL) of the Henle’s loop cause autosomal recessive antenatal/neonatal Bartter syndrome type II (aBS II) [1]. aBS II begins in utero, accounting for polyhydramnios, which may in turn contribute to premature delivery that is typical in affected infants. aBS II presents with severe renal salt wasting resulting in life-threatening volume depletion in the neonatal period. Additional features are secondary hyperreninaemic hyperaldosteronism, metabolic alkalosis, and hypercalciuria and nephrocalcinosis. It is unique in often presenting with hyperkalaemia,
rather than hypokalaemia, at birth, sometimes making the diagnosis challenging. This hyperkalaemia later evolves into hypokalaemia commonly seen in the postnatal period [1–3]. More than 40 KCNJ1 mutations have been described so far [3]. Most of these mutations are missense/nonsense mutations substituting conserved amino acids residues, predominantly within the coding exon 2, the most influential putative functional domain of ROMK [3]. Interestingly, all reported patients with KCNJ1 mutations presented a severe BS phenotype at infancy. Only one adult man has been reported with a phenotype resembling a late-onset BS due to a homozygous KCNJ1 missense mutation (c.656C>T, p.L220F). This patient initially presented with an incidental finding of nephrocalcinosis. He had modest hypokalaemic metabolic alkalosis and mild-to-moderate chronic kidney disease (CKD Grade 3A, 2012 KDIGO guidelines) [4]. Since this is the only report on BS II with clinical presentation in adulthood, it remains unclear whether aBS II can be considered also as adult-onset disease. aBS II might have evaded detection in human adulthood because Sanger sequential sequencing of multiple genes is time-consuming and costly, and subjects with incomplete or non-penetrant phenotypes might not have been investigated.

Case presentation

A 43-year-old German woman was examined after incidental findings of bilateral nephrocalcinosis by ultrasound during her second pregnancy. Figure 1 shows an ultrasound image demonstrating renal medullary nephrocalcinosis in her left kidney. Even though her recent medical history was unremarkable, the patient had claimed strong thirst and polyuria in childhood. Even though her recent medical history was unremarkable, the patient may have had evaded detection in human adulthood because Sanger sequential sequencing of multiple genes is time-consuming and costly, and subjects with incomplete or non-penetrant phenotypes might not have been investigated.

![Ultrasound image demonstrating renal medullary nephrocalcinosis in the patient’s left kidney. This ultrasound image shows increased echogenicity of the renal medulla. Kidney size, 11.5 cm (blue dotted line).](image)

Molecular diagnosis

Following informed consent, genomic DNA of the patient and of their healthy two daughters was extracted from peripheral blood leukocytes. Parents and other family members were not available for genetic testing. We performed DNA sequencing by a two-step approach: (i) massively parallel sequencing (next generation sequencing [NGS]-based mutation screening combined (ii) with Sanger sequencing. High-coverage NGS-based mutation screening combined with oral supplementation of potassium (80 mmol/day) and ramipril (10 mg/day). In the follow-up period, her serum potassium improved to a level of 3.5 mmol/L. Her serum creatinine stabilized at the level of 1.13 mg/dL (eGFR 59 mL/min) over the next 3 years of follow-up.
The heterozygous c.875G > A mutation was detected (in absence of the c.197T > A mutation) in both healthy daughters. One of the mutations, c.875G > A (p.R292Q), has already been described as pathogenic mutation in a patient with BS II, although not in this allelic combination [7]. The other mutation, causing a T-to-A transition, c.197T > A (p.I66N) (Figure 2), is novel and most likely pathogenic based on the clinical phenotype. A number of other diseases have feature that can overlap with aspects of aBS II (Supplementary Table S1). However, there were no mutations in SLC12A1 (NKCC2), CLCNKB/CLCNKA (ClC-Kb, ClC-Ka and ClC-Kb), BSND (barttin), CASR (CaSR), MAGED2 (MAGE-D2) and SLC12A3 (NCCT), which cause other forms of BS or Gitelman syndrome (Supplementary Table S1) [2, 8, 9]. Moreover, there were no mutations in CLDN16 (claudin 16) and CLDN19 (claudin 19), which cause nephrocalcinosis and renal failure, due to renal magnesium wasting with hypomagnesaemia and hypercalciuria (Supplementary Table S1) [10].
We validated the results of the NGS approaches by Sanger sequencing, which was performed on PCR amplicons from genomic DNA covering that variant position (Figure 3). We sequenced the protein coding sequence of KCNJ1, which is composed of three different splice variants of one to two exons and comprising a region of nearly 31.3 kb. The sequenced region included the neighbouring splice sites, a total of 1.905 bp. The adjustment of the found sequences data was analysed in the Ensembl transcript gene database under ENST00000392665 available sequences.

KCNJ1 mutations

The following KCNJ1 mutations were identified:
- c.197T > A, heterozygote, exon 2, codon 66, protein p.I66N (ATC > AAC); Ile66Asn
- c.875G > A, heterozygote, exon 2, codon 292, protein p.R292Q (CGG > CAG); Arg292Gln

Discussion

aBS II is also called the neonatal variant of BS because it presents with severe symptoms in the neonatal period. In this study, we identified a novel KCNJ1 missense mutation (p.I66N) in a compound heterozygote setting with a previously recognized pathogenic mutation (p.R292Q) [7] in an adult woman leading to an unusual mild clinical presentation of aBS II. Based on the autosomal recessive pattern of aBS II, both missense mutations are expected to disrupt KCNJ1 gene function. However, the KCNJ1 mutation p.I66N could represent a mutation causing only a mild phenotype. Our experimental approach of NGS-based mutation screening combined with Sanger sequencing proved to be a reliable approach for molecular diagnosis in our patient with a clinical unclassified phenotype resembling a late-onset BS. Two different in silico NGS software approaches were employed for mapping, base calling and variant detection. Both approaches detected the two mutations. These approaches included (i) Exomiser [11] and (ii) customized SeqNext (JSI Medical Systems) approaches for data analyses.

Our findings confirm a recent observation that aBS II can present as late-onset disease in adulthood [4]. Interestingly, this case and our case presented with nephrocalcinosis as incidental presentation of BS. Although different KCNJ1 mutations were found, both patients exhibited unusual mild clinical manifestations of aBS II, i.e. hypokalaemia, modest or tendency towards metabolic alkalosis, mild hypercalciuria and modest
hyperreninaemic hyperaldosteronism due to renal salt wasting. Therefore, oral potassium replacement (80 mmol/day) was sufficient for improvement of serum potassium levels to near normal values. Both patients maintained normal serum Mg²⁺ levels, which is consistent with previous observations demonstrating that Bartter’s patients with hypomagnesaemia rarely reach the levels seen in patients with Gitelman syndrome (Supplementary Table S1) [2]. Uprogulation of NCCT via aldosterone may contribute to the increase in expression of TRPM6 Mg²⁺ channels in the distal convoluted tubule (DCT) and be protective against hypomagnesaemia in BS [2]. Although patients with salt-losing nephropathy have an increased salt intake (salt craving) while they consume their normal ad-lib diet [8, 12], oral salt supplementation has no or little effects on potassium homeostasis in adult BS. Klaus et al. examined a 20-year-old female with BS (although not genetically tested) and found that neither low-salt diet (30 mval or 10 mval Na+) nor salt-rich diet (240 mval Na+/day) over 5 days improved hypokalaemia and potassium excretion in the urine. These manoeuvres also did not affect plasma renin levels, indicating insignificant effects on volume regulation [13]. However, it is noteworthy that the addition of potassium-sparing diuretics or an aldosterone antagonist may improve hypokalaemia in late-onset aBS II [4].

Our patient initially presented with hypercalciciuric nephrocalcinosis associated with chronic renal impairment. BS patients commonly develop nephrocalcinosis. This is presumed to be due to their hypercalciuria, however the mechanisms are poorly understood. For example, nephrocalcinosis is less prevalent among Bartter’s patients with CLCNKB and BSND mutations (Supplementary Table S1), who have less hypercalciuria [2, 14]. It is possible that bone reabsorption from elevated PTH levels, which is consistent with previous observations demonstrating that Bartter’s patients with hypomagnesaemia rarely reach the levels seen in patients with Gitelman syndrome (Supplementary Table S1) [2]. Uprogulation of NCCT via aldosterone may contribute to the increase in expression of TRPM6 Mg²⁺ channels in the distal convoluted tubule (DCT) and be protective against hypomagnesaemia in BS [2]. Although patients with salt-losing nephropathy have an increased salt intake (salt craving) while they consume their normal ad-lib diet [8, 12], oral salt supplementation has no or little effects on potassium homeostasis in adult BS. Klaus et al. examined a 20-year-old female with BS (although not genetically tested) and found that neither low-salt diet (30 mval or 10 mval Na+) nor salt-rich diet (240 mval Na+/day) over 5 days improved hypokalaemia and potassium excretion in the urine. These manoeuvres also did not affect plasma renin levels, indicating insignificant effects on volume regulation [13]. However, it is noteworthy that the addition of potassium-sparing diuretics or an aldosterone antagonist may improve hypokalaemia in late-onset aBS II [4].

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Our study suggests a high degree of variability of aBS II regarding severity of disease as shown in another report in adolescence [23]. Mutations in KCNJ1 should thus be considered even beyond the neonatal period in patients who present with nephrocalcinosis, hypokalaemia, metabolic alkalosis or symptoms of renal salt wasting (Supplementary Table S1).

Conclusions
We report an unusual, in severity mild, variant of BS II associated in a female patient with a remarkably late onset of the disease. This phenotype is associated with compound heterozygous KCNJ1 mutations, consisting of a novel c.197T > A (p.I66N) and a previously reported c.875G > A (p.R292Q) KCNJ1 mutation. Our results demonstrate that aBS II may be suspected in patients with a late-onset phenotype resembling BS. Our experimental approach of NGS-based mutation screening combined with Sanger sequencing proved to be a reliable approach for molecular diagnosis in our patient and can be implemented in genetic testing approaches in various clinical conditions. Our methodological approach and results may help to diagnose and classify other subjects with clinical unclassified renal phenotypes of nephrocalcinosis and electrolyte disorders.

Supplementary data
Supplementary data [24-27] are available online at http://ckj.oxfordjournals.org.

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**Conflict of interest statement**

None declared.

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