Novel nonsense mutation in the *SLC12A3* gene in a Spanish case of Gitelman syndrome

Nueva mutación finalizadora en el gen SLC12A3 en un caso de síndrome de Gitelman en España

Dear Editor:

Gitelman syndrome (GS) is a rare, salt-losing tubulopathy characterized by hypokalemic metabolic alkalosis with hypomagnesemia and hypocalciuria.[1] The disease is caused by inactivating mutations in the *SLC12A3* gene that encodes the thiazide-sensitive sodium-chloride cotransporter (NCC)[2,3] and is recessively inherited. The prevalence of GS is estimated to range around 25 cases per 1 million, making it one of the most frequent inherited renal tubular disorders.[4]

A 35-year-old male, with a history of seborrheic dermatitis treated with topical steroids and iron deficiency anemia was admitted with a first episode of biliary colic and hypokalemia (2.9 mEq/L). His electrolyte imbalance, once the vomiting settled, was challenging to manage with oral potassium supplements.

He complained of thirst, muscle weakness and fatigue with limited exertion. He had occasional and doubtful arthritis. At the time of the visit, the patient did not present any gastrointestinal symptoms, except reflux due to treatment with potassium, but that was well controlled with a gastric protector.

The exploration was normal but on his electrocardiogram (ECG) we found a prominent U wave. Blood tests showed normal renal function with serum creatinine 0.81 mg/dL, glomerular filtration rate (GFR) 116 mL/min per 1.73 m^2, potassium 2.9 mEq/L, sodium 141 mEq/L, chloride 98 mEq/L, phosphate 2.5 mg/dL, magnesium 1.57 mg/dL, calcium 9.6 mg/dL, mild elevation of white blood cell (WBC) count of 10,640 per mm^3 (normal range 3500–10,500 per mm^3), hemoglobin 15.2 g/dL, liver function was normal, plasma renin activity 1.56 ng/mL/h with normosodic diet and supine position (normal range 0.2–2.8 ng/mL/h), metabolic alkalosis with pH 7.50 and bicarbonate 30.4 mmol/L, urinary sediment with mild density of 1008 g/L and pH 8, excretion in 24-h urine collection (4200 mm^3) 48 h without supplementation and diet of salt 5 grams showed glucose 105 mg, uric acid 1210 mg, creatinine 1617 mg, potassium 232 mEq, sodium 512 mEq, chloride 326 mEq, phosphate 1427 mg, magnesium 227 mg, calcium 81 mg, protein excretion 0.15 g confirmed and normal aminoaciduria. Abdominal ultrasound showed kidneys with normal corticomedullary differentiation.

A Next Generation Sequencing (NGS) Ion AmpliSeq™ Custom Research Panel was used to study the coding regions and intronic flanking regions of the tubulopathy genes: *CLCNKB*, *CLCNKA*, *SCL12A3*, *SLC12A1*, *KCNJ1*, *BSND*, *CASR*, *CLDN16* and *CLDN19*. Libraries were prepared using Ion Ampliseq Library kit 2.0 (Life Technologies, CA, USA). Finally, DNA high-throughput sequencing was performed on a Personal Genome Machine (PGM) using the Ion PGM™ Hi-Q™ Sequencing Kit (Life Technologies) on the Ion 318™ sequencing chip (Life Technologies). Results evidenced a homozygous mutation c.361G>T (p.E121Ter; NM_000339) in the *SLC12A3* gene. This nucleotide change results in a premature stop codon, or a nonsense codon, in the transcribed mRNA, resulting in a truncated, incomplete, and usually nonfunctional protein. This variant has not been previously reported. Sanger sequencing of the exon 2 fragment confirmed that this patient was homozygous for the mutation.

In the *SLC12A3* gene, more than 180 mutations have been reported in GS patients.[5] Sequencing of the *SLC12A3* exons is required to search for mutations in most GS patients because there is not a prevalent mutation.[6,7] Moreover, CLCNKB mutations may be found in some cases with clinical manifestations of GS, a fact that increases the complexity of the genetic study of GS.[8]

GS is inherited as an autosomal recessive trait, and homozygous and combined heterozygous mutations are expected.[9] In our case, the single nucleotide polymorphism (SNP) detected along the *SLC12A3* gene was homozygous with the notion of consanguinity. The p.E121Ter variant was predicted to be likely pathogenic by at least three bioinformatic programs (SIFT, Polyphen and Mutation Taster). The patient came from an isolated and small village in Spain, and had a probable consanguinity between parents and a founder effect that has previously been reported in GS disease.[10]

The mutations impair the activity of thiazide-sensitive sodium-chloride cotransporter encoded by *SLC12A3* and could be classified in five different types according to their effect on the protein. Large deletions including one or more exons of *SLC12A3* have also been detected[10] and the majority of these mutations should be included in this type 1 classification.

The definition of the *SLC12A3* genotype through description of new mutations may be a useful tool in the clinical characterization of GS and in genetic counseling of salt-losing tubulopathies.

First, we suspected either GS or Bartter syndrome III the cause of hypokaliemia. Other causes such as vomiting or the use of diuretics were rejected due to history. The electrocardiographic alterations were probably secondary to

hypokalemia. The slight glucosuria present at the diagnosis reminds us that hypomagnesemia and hypokaliemia were speculated to cause impaired glucose tolerance in GS patients. Our patient was not overweight, had normal to low blood pressure, and a proteinuria of 150 mg/24 h was monitored because of the podocyte dysfunction link described.

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