



Short Original

A new SLC12A3 founder mutation (p.Val647Met) in Gitelman's syndrome patients of Roma ancestry

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ABSTRACT

Background: Gitelman's syndrome (GS) is an autosomal recessive disorder caused by mutations in the SLC12A3 gene. GS is characterized by hypokalaemic metabolic alkalosis, hypomagnesemia and hypocalciuria. Most of the reported patients of Roma ancestry are homozygous for an SLC12A3 intron 9 frameshifting mutation (c.1180+1G>T). Some forms of Bartter's syndrome result from mutations in the CLNCKB gene and clinically overlap with GS.

Objectives: To characterize a second SLC12A3 mutation in Roma patients negative for the intron 9 variant.

Methods: SLC12A3 and CLNCKB genes were analyzed by next-generation sequencing in two Spanish and Greek gypsy patients who were negative for the intron 9 splicing mutation. Sanger sequencing was performed to confirm the putative mutations in patients and family members.

Results: We identified a missense variant (p.Val647Met, c.1939G>A) in both cases, and both were homozygous for Met. This mutation was also found in three additional patients; two homozygous and one heterozygous compound with the intron 9 splicing mutation. This new SLC12A3 mutation seems to be characteristic of gipsy GS patients and was linked to the same

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haplotype in all cases, supporting a founder origin. All the patients showed biochemical features characteristic of GS.

Conclusion: We report a second founder mutation among GS patients of Roma ethnic background. The direct screening of this mutation would facilitate the characterization of patients who are negative for the more common intron 9 +1G>T mutation.

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Una nueva mutación fundadora en SLC12A3 (p.Val647Met) en pacientes con síndrome de Gitelman de etnia gitana

R E S U M E N

Palabras clave:

Síndrome de Gitelman
Gen SLC12A3
Pacientes de etnia gitana
Antecedentes genéticos gitanos

Antecedentes: El síndrome de Gitelman (SG) es un trastorno autosómico recesivo causado por las mutaciones en el gen SLC12A3. El SG se caracteriza por una alcalosis metabólica hipopotasémica, hipomagnesemia e hipocalciuria. La mayoría de los pacientes de etnia gitana notificados son homocigotos para la mutación con desplazamiento del marco de lectura del intrón 9 de SLC12A3 (c.1180+1G>T). Algunas formas del síndrome de Bartter proceden de las mutaciones del gen CLNCKB y se solapan clínicamente con el SG.

Objetivos: Determinar las características de una segunda mutación en SLC12A3 en pacientes de etnia gitana con resultados negativos en la variante intrón 9.

Métodos: Se analizaron los genes SLC12A3 y CLNCKB mediante secuenciación de nueva generación en 2 pacientes –uno español y otro griego– de etnia gitana con resultados negativos en la mutación de empalme del intrón 9. Se llevó a cabo una secuenciación de Sanger para confirmar las supuestas mutaciones en los pacientes y sus familiares.

Resultados: Se identificó una variante con cambio de sentido (p.Val647Met, c.1939G>A) en ambos casos, y ambos eran homocigotos con respecto a Met. También se observó esta mutación en 3 pacientes adicionales, 2 homocigotos y uno heterocigoto compuesto con la mutación del intrón 9. Esta nueva mutación del SLC12A3 parece ser característica de los pacientes con SG de etnia gitana y se relacionó con el mismo haplotipo en todos los casos, lo que indica un origen fundador. Todos los pacientes presentaron rasgos bioquímicos propios del SG.

Conclusión: Informamos de una segunda mutación fundadora en los pacientes con SG de etnia gitana. El cribado genético directo de esta mutación facilitará la determinación de las características de los pacientes con resultados negativos en la mutación del intrón 9 + 1G>T, que es más frecuente.

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Introduction

Gitelman's Syndrome (GS) is the most common primary renal tubular disorder, affecting approximately 1/40,000 Caucasians. GS is inherited as an autosomal recessive disease mainly linked to mutations in the SLC12A3 gene (solute carrier family 12 sodium/chloride transporters, member 3), that encodes a thiazide-sensitive sodium-chloride co-transporter (NCC) protein that locates at the luminal membrane of the distal convoluted tubule in the kidney.¹ GS is characterized by hypokalemic metabolic alkalosis with hypomagnesemia and hypocalciuria.^{2,3} The loss of function of the NCC channel leads to decreased sodium re-absorption and subsequently secondary volume depletion. The hypovolemia stimulates sodium re-absorption at the expense of increased potassium and hydrogen secretion, which results in hypokalemia and

metabolic alkalosis.⁴ Hypocalciuria and hypomagnesaemia are also characteristic of GS, the latter being a likely consequence of the elevation of the excretion of magnesium in urine.^{5,6}

Most of the GS patients are homozygous or heterozygous compound for rare private mutations. Due to the absence of common mutations, the sequencing of the full SLC12A3 is required to uncover the genetic background of GS patients.⁷ Moreover, some cases presenting as GS might carry mutations at the CLNCKB gene, a fact that increases the technical effort of the genetic study.⁷

The Roma or Gypsies represent the largest and most widespread ethnic minority in Europe. Anthropological and genetic data support their origin in the Indian subcontinent.⁸ Although there was some mixture with their hosting populations, consanguinity is common among Gypsies and results

Table 1 – Serum biochemistry and genetic data of the affected members in the five families.

Kindred	Sex	Age at diagnosis	pH	HCO ₃ ⁻ (mEq/L)	K ⁺ (mEq/L)	Mg ²⁺ (mg/dL)	Mutations in SLC12A3	
Family I	PI.1	Male	14 years	7.61	30	2.1	1.40	p.Val647Met/p.Val647Met
	PI.1.1	Female	11 months	7.50	28	3.9	1.50	p.Val647Met/p.Val647Met
	PI.1.2	Female	7 months	7.50	NA	NA	1.83	p.Val647Met/p.Val647Met
	PI.1.3	Male	11 months	7.44	24.5	3.72	2.10	p.Val647Met/p.Val647Met
Family II	PII.1	Female	29 months	7.48	28	2.5	1.80	p.Val647Met/p.Val647Met
Family III	PIII.1	Male	7 years	7.51	NA	1.8	1.00	p.Val647Met/p.Val647Met
Family IV	PIV.1	Male	49 years	NA	30.6	2.4	1.58	p.Val647Met/p.Val647Met
Family V	PV.1	Male	33 years	NA	34	2.6	0.96	p.Val647Met/Intron 9 +1

K⁺: potassium; Mg²⁺: magnesium; HCO₃⁻: bicarbonate; Val: valine; Met: methionine. NA: data not available.

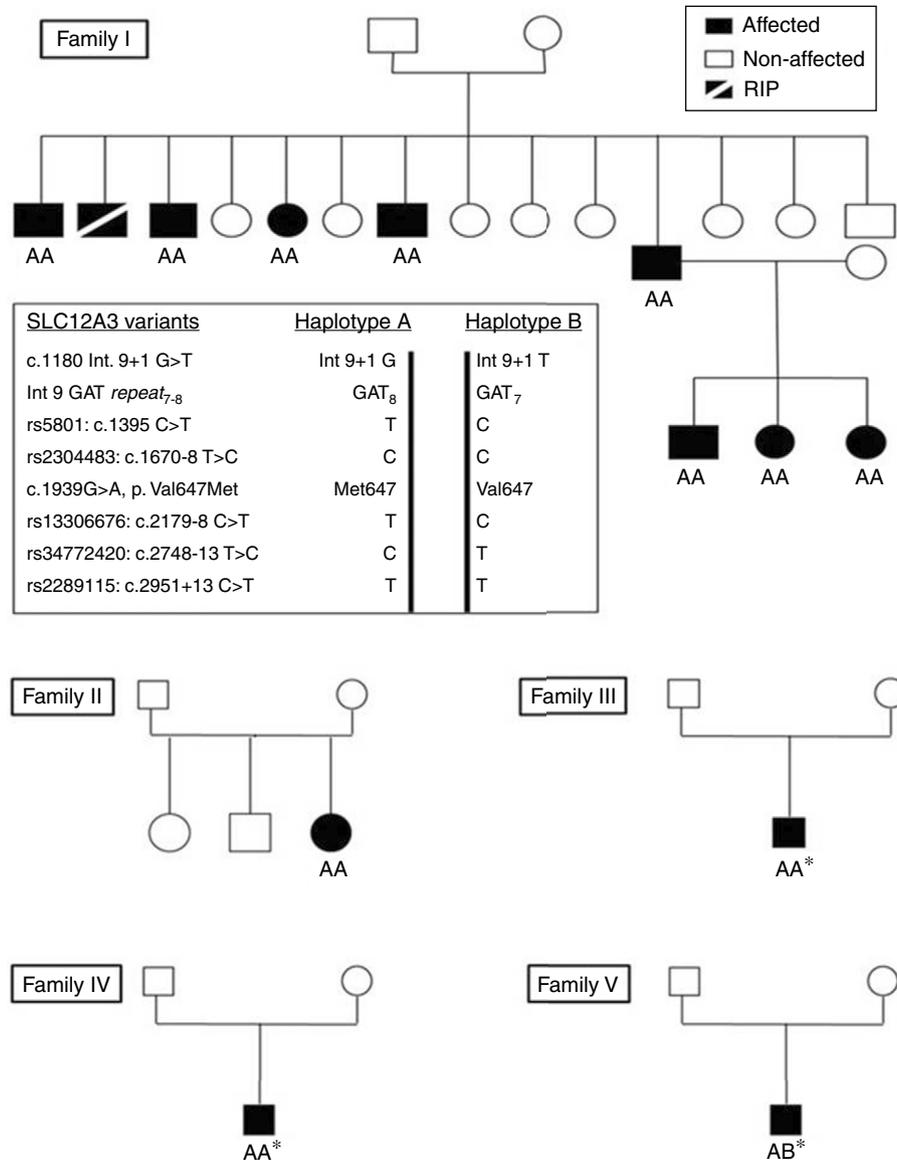


Fig. 1 – Pedigrees of the five families. A and B represents the haplotypes containing the p.647 Met. *Intron 9 GAT-repeats not available.

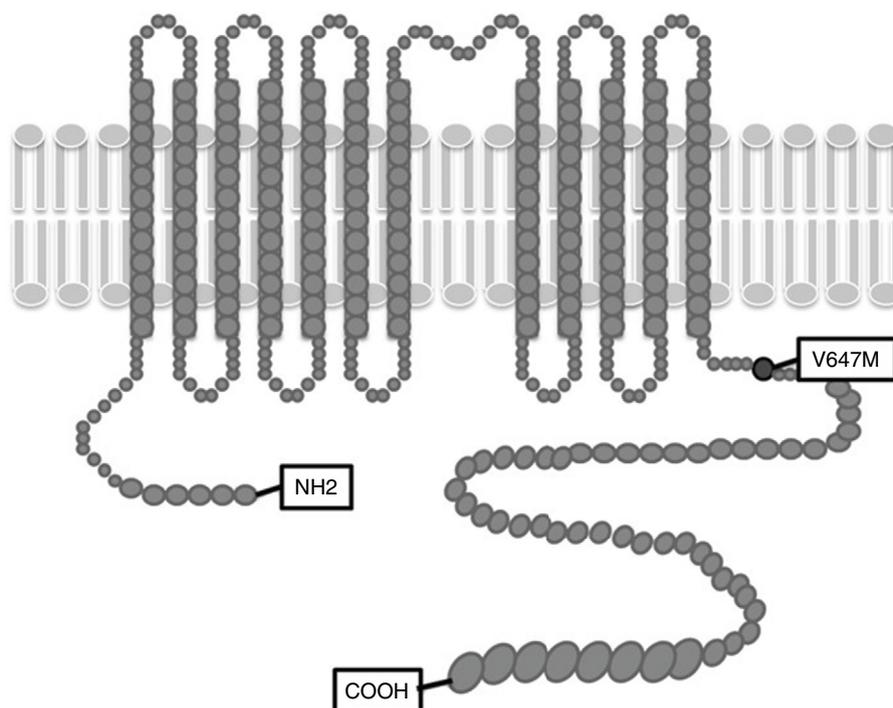


Fig. 2 – Map of the SLC12A3 showing the position of amino acid 647.

in the presence of founder-mutations for several inherited disorders.⁹ To date, most of the reported Gypsy patients having GS are homozygous for the intron 9 +1G>T mutation (c.1180+1G>T).¹⁰⁻¹² Here, we describe a new SLC12A3 mutation characteristic of Roma SG patients.

Methods

Patients

We studied eight patients from five families diagnosed of GS (Table 1). The five families had a Roma ethnic background and traced their ancestry to the South-East European region, including the Spanish family whose ancestors emigrated from Romania two generations ago. The Spanish and Greek families were recruited through the Renaltube Consortium (www.renaltube.com).¹³

Genetic study

The search for SLC12A3 and CLCNKB mutations was first performed in the index cases from families I and II. The two were negative for the intron 9 splicing mutation. The whole coding plus at least 5 intronic flanking nucleotides were sequenced through semiconductor Next Generation Sequencing (NGS) with the Ion Torrent Personal Genome Machine (PGM, Life Technologies), following a previously reported procedure.¹⁴

To confirm the putative mutations identified by NGS, the corresponding exons were amplified and sequenced by the Sanger method. This procedure was also used to determine the presence of the mutation in all the family members.

To characterize the haplotypes linked to the SLC12A3 mutation, five common single nucleotide polymorphisms were determined by Sanger sequencing of PCR fragments: rs5801 (c.1395C>T), rs2304483 (c.1670-8T>C), rs13306676 (c.2179-8C>T), rs34772420 (c.2748-13T>C), and rs2289115 (c.2951+13C>T). In families I and II we also genotyped an intron 9 repeat polymorphism.

Results

Clinical data

Family I had several GS patients in two generations. The index case (PI.1) was the father of three children with mild clinical features of GS. The father was diagnosed in the adolescence whereas the three siblings were diagnosed before the age of two years. Three brothers and one sister of the index case presented also GS findings such as hypokalemia, hypomagnesaemia and elevated bicarbonate serum values with a blood pH > 7.35 (data not showed).

Genotype

The NGS of SLC12A3 and CLCNKB in the index cases from families I and II revealed the presence of a nucleotide variant in exon 16 of SLC12A3 (c.1939G>A) that would result in a missense amino acid change (p.Val647Met). This variant is predicted to be pathogenic/deleterious by the bioinformatic programs SIFT (<http://sift.jcvi.org>) and Polyphen (<http://genetics.bwh.harvard.edu/pph2>). The Sanger sequencing of exon 16 fragments showed that these patients were

homozygous for the mutation, and we also determined the mutation genotype in all the family members (Fig. 1).

The analysis of the five *SLC12A3* SNPs showed that the p.647 Val mutation was in the same haplotype in the five families (Fig. 1).

Discussion

More than 180 mutations in *SLC12A3* gene have been reported in GS patients.⁷ There is not a prevalent mutation, and this means that the sequencing of the 26 *SLC12A3* exons is required to search for mutations in most of the GS patients.^{15,16} 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. Thus, the discovery of mutations common in an ethnic group will facilitate the genetic analysis by limiting the sequencing to one or few exons.¹⁰

In this article we present Gypsy cases from Spain and Greece with the same mutation in the *SLC12A3* gene. Patients were recruited through the Renaltube Consortium¹³ which collects data from patients worldwide in an attempt to better define the clinical profile and the genetic background of renal tubulopathies. To date, >50 Gypsy GS patients are included in the Renaltube database and most of these patients are homozygous for the intron 9 splicing mutation. The index cases of Families I and II, were subjected to NGS to search for new *SLC12A3* mutations, resulting in the identification of p.Val647Met. The p.Val647Met variant was predicted to be pathogenic with at least two bioinformatic programs (SIFT and Polyphen). This missense change locates at the intracellular carboxy-end of the protein (Fig. 2), where other GS mutations have been found and aminoacids at this region are conserved not only among species but also in other human channels such as bumetanide-sensitive cotransporter (*SLC12A2* gene).¹⁷

The p.Val647Met mutation had been previously reported by Vargas-Poussou et al. in one GS patient (PV.1) compound heterozygous for this variant and the intron 9 mutation.⁷ Thus, the two families we studied and the three previously reported by Vargas et al.⁷ had a common Gypsy genetic background and traced their ancestry to South-Eastern Europe. The p.Val647Met was linked to the same *SLC12A3* polymorphisms in the 5 families, a fact that strongly supports a common origin for this new Gypsy SG mutation.

In conclusion, we report a new *SLC12A3* founder mutation among GS patients of Gypsy background, p.Val647Met. This mutation was associated with typical clinical features of GS, either in homozygosity or in combination with the intron 9+1G>A mutation. Our finding facilitates the diagnosis of GS in Gypsy patients.

Compliance with ethical standards

All the patients/participants in the study gave their informed consent, approved by the Ethical Committee of Hospital Universitario Central Asturias.

Conflict of interest

The authors declare no conflict of interest.

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