Journal of Genetics (2019) 98:5
https://doi.org/10.1007/s12041-019-1056-7

RESEARCH NOTE

A novel heterozygous duplication of the *SLC12A3* gene in two Gitelman syndrome pedigrees: indicating a founder effect

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Received 17 August 2018; revised 17 September 2018; accepted 10 January 2019; published online 7 February 2019

Abstract. Gitelman syndrome is an autosomal recessive salt-wasting tubulopathy caused by mutations in the *SLC12A3* gene. A female and a male sibling from two unrelated Greek-Cypriot families presenting with a severe salt-wasting tubulopathy due to compound heterozygous mutations of a novel duplication and a previously reported missense mutation in the *SLC12A3* gene are described. Sanger sequencing was used to identify possible mutations in the *SLC12A3* gene. For the detection of duplications/conversions and deletions in the same gene, Multiplex ligation probe amplification (MLPA) analysis was performed. Direct sequencing and MLPA analysis of the *SLC12A3* gene identified two compound heterozygous mutations in both unrelated probands. Both probands were identified to carry in compound heterozygosity the known p.Met581Lys and a novel heterozygous duplication of exons 9-14 (E9_E14dup). The diagnosis of Gitelman syndrome was made through clinical assessment, biochemical screening and genetic analysis. The identification of the novel *SLC12A3* duplication seems to be characteristic of Greek-Cypriot patients and suggests a possible ancestral mutational event that has spread in Cyprus due to a possible founder effect. Testing for Gitelman syndrome probable variants can be performed before proceeding to a full gene sequencing dropping the diagnostic cost. In addition, this report adds to the mutational spectrum observed.

Keywords. Gitelman syndrome; salt-wasting tubulopathy; *SLC12A3* gene; hypokalaemia; tubular disorders; novel mutation.

Introduction

Gitelman syndrome (GS) is a rare autosomal recessive salt-wasting tubulopathy characterized by hypokalaemic metabolic alkalosis, hypomagnesaemia and hypocalciuria (Blanchart *et al.* 2017). GS is considered the most common renal tubular disorder and its prevalence in the Caucasian population was reported to be 1:40,000 (Blanchart *et al.* 2017). Most cases of GS are diagnosed in adulthood, but the first signs of the disorder appear around the age of six years (Cruz *et al.* 2001). Inactivating mutations in the *SLC12A3* gene, which encodes the thiazide-sensitive NaCl cotransporter (NCC) in the renal distal convoluted tubule is usually the cause of GS (Simon *et al.* 1996). Currently, in the *SLC12A3* gene >240 mutations have been reported and about 70% of which being missense (Vargas-Poussou *et al.* 2011). About one half of the patients suspected to have GS with only one mutated allele are estimated to carry a large genomic rearrangement on the other allele (Vargas-Poussou *et al.* 2011; Nakhoul *et al.* 2012).

Genetic diagnosis of GS is often difficult in the *SLC12A3* due to the fact that 5–15% are large genomic rearrangements (Vargas-Poussou *et al.* 2011). Moreover, several tubulopathies can be caused by several genes or have phenotypic overlap with other disorders, such as Bartter and Gitelman syndromes or distal renal tubular acidosis (Ashton *et al.* 2018). Usually, GS is distinguished from Bartter syndrome by a low rate of calcium excretion (urine calcium/creatinine ratio ≤0.1 mmol/mmol for GS vs >0.1 mmol/mmol for Bartter syndrome).

In this study, we describe the clinical, biochemical and genetic findings of two unrelated Greek-Cypriot families with GS. The phenotypes of the two probands from the two unrelated families were similar and strongly suggested GS. The identification of a novel *SLC12A3* duplication in both families seems to be characteristic of Greek-Cypriot patients with GS and suggests a possible ancestral mutational event.

Materials and methods

Patients

We studied two patients with suspected GS from two unrelated Greek-Cypriot families. The two families traced their ancestry to the Ayios Theodoros village in the south-east side of the island of Cyprus. Blood samples were obtained from patients, parents and siblings. Informed consent was obtained from the parents of the minors after appropriate genetic counselling. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants in the study.

Genetic testing of the SLC12A3 gene

PCR amplification and direct sequencing of the *SLC12A3* gene was performed as previously described (Simon *et al.* 1996). Multiplex ligation probe amplification (MLPA) technique was used (P136-Gitelman, Lot: B3-0617) for the detection of large gene rearrangements (MRC Holland, Amsterdam, The Netherlands).

Results

Clinical findings of patient I

Patient I is an 11-year-old girl who was admitted to the hospital due to one-day history of vomiting and mild fever. She is the first child of healthy nonrelated parents. Her medical history was unremarkable and she was not taking any medication.

On physical examination, patient was alert, mildly dehydrated, afebrile, heart rate 120 beats per minute, respiratory rate 18 per min, blood pressure 90/60 mmHg (95th percentile for age and height is 114/74 mmHg). Cardiovascular examination was suggestive of sinus tachycardia and no cardiac murmur was appreciated. The remainder clinical examination was normal. At the time of presentation, the weight was 21 kg (below 3rd percentile) and height was 133 cm (10th percentile). The last arthropometric assessment was performed when the child was five years old and at that time the weight was 15 kg (3rd percentile). The initial biochemistry tests (table 1) were normal apart from hypokalaemia (potassium = 2.28 mmol/L).

Patient I was initially diagnosed with gastritis and was treated with administration of fluids intravenously plus KCL 10%. Despite the fact that her hydration status had improved, hypokalaemia was persisting.

Upon further questioning, the patient reported that she had experienced symptoms of fatigue, generalised weakness, constipation and muscle cramps. The patients mother reported that the child had a history of a preference for salt-containing foods since childhood. These findings were further investigated by 24-h urine analyses which showed high potassium and low calcium levels (table 1). Further biochemical investigations revealed hypomagnesaemia and arterial blood gas analyses showed metabolic alkalosis. Renin and aldosterone levels were elevated (table 1). Renal ultrasonography indicated nephrocalcinosis. Audiometry assessment was normal. Electrocardiography showed mild ST-T elevations with normal QTc intervals and echocardiography had no abnormal findings.

The patient was diagnosed as GS based on clinical and biochemical results. She was initiated with magnesium (1000 mg/day) and chloride potassium (3300 mg/day) oral supplementation. She was also encouraged to maintain a high potassium, sodium and magnesium diet. A month later the pateint had normokalaemia, normomgnesemia and she gained four kilograms of weight.

Clinical findings of patient II

This patient is a 3.5-year-old boy who was admitted to the hospital with one-day history of vomiting and diarrhoea. A review of previous medical history did not reveale anything remarkable. He is the younger of two siblings from healthy, nonconsanguineous parents.

On physical examination, the patient was mildly dehydrated, afebrile, heart rate 107 beats per min, respiratory rate 20 per min, blood pressure 80/56 mmHg (95th percentile for age and height is 113/64 mmHg). Cardiovascular examination showed sinus tachycardia with normal rhythm. The remainder clinical examination was normal. The child was developmentally appropriate for his age. At the time of presentation, the weight was 15 kg (50th percentile) and her height was 105 cm (90th percentile). The initial biochemistry tests (table 1) were normal apart from hypokalaemia (potassium = 1.82 mmol/L).

The patient was initially diagnosed with viral gastroenteritis and was treated with administration of fluids intravenously plus KCL 10%. His hydration status was improved but hypokalaemia was enduring.

Further biochemical investigations revealed metabolic alkalosis, hypomagnesaemia with hypocalciuria as depicted on table 1. Renin and aldosterone levels were elevated. The electrocardiogram exposed ST segment change

Table 1. Laboratory results of the two patients.

Variable	Patient I test value	Patient II test value	Reference value
Results of blood tests			
Potassium	2.28	1.82	3.5–5 mmol/L
Sodium	136	135	135–145 mmol/L
Chloride	98	100	90–110 mmol/L
Calcium	9.4	10	8.8–10.5 mg/dL
Phosphate	3.4	3.8	3.5–6.3 mg/dL
Magnesium	0.46	0.64	0.7–0.85 mg/dL
Creatinine	0.37	0.32	0.26–0.77 mg/dL
Renin	610	>350	1.1–20.2 pg/mL (supine)
Aldosterone	139	>350	13–145 pg/mL (supine)
Arterial blood gas analyses			
pH	7.49	7.47	7.35–7.45
Bicarbonate	32.6	24.9	21–29 mmol/L
Result of 24-h urine tests			
Calcium	19	12	42–353 mg/24 h
Potassium	157.5	135	25–125 mmol/24 h
Magnesium	4.18	3.3	3–5 mmol/24 h
Sodium	122	114	40–220 mmol/24 h
Chloride	193.8	110	150–250 mmol/24 h

of the anterolateral wall whereas the echocardiogram was normal. Renal ultrasonography and audiometry assessment revealed normal findings.

GS was suspected based on clinical and biochemical results. He had been treated with magnesium (350 mg/day) oral supplementation and chloride potassium (600 mg/day). The mother was advised to maintain a high potassium, sodium and magnesium diet for the child. A month later the patient II had normokalemia and normomgnesemia.

Genetic findings

Genetic analyses of the two nonrelated probands and relatives by direct sequencing and MLPA of the *SLC12A3* identified two heterozygous mutations. Both probands carried in compound heterozygosity and in *trans* the known p.Met581Lys and the novel duplication of exons 9-14 (E9_E14dup) (figure 1). These mutations would be expected to modify the protein structure and implicated in the loss of function of the NaCl cotransporter (NCC) of the distal convoluted tubule. Moreover the p.Met581Lys is located between the 11 and 12 transmembrane domain of the NCC protein. Alterations of amino acids at the transmembrane domains might impair cotransporter function by interfering with protein processing or by partially insertion of the cotransporter into the plasma membrane (Gamba 2005). None of these variants have been found in the ExAC database (http://exac.broadinstitute.org/) and to our knowledge the duplication E9_E14dup has also not been previously described. The known p.Met581Lys was originally reported in patients with GS by Colussi *et al.* (2007).

Discussion

Most of GS cases are associated to inactivating mutations in the *SLC12A3* gene with about 70% being missense (Nakhoul *et al.* 2012). In the present study, the previously described missense p.Met581Lys was identified in two unrelated probands (Colussi *et al.* 2007) in compound heterozygosity with novel heterozygous duplication of exons 9-14 (E9_E14dup). Both of these mutations were present in heterozygosity in the parents and other close relatives of the two probands (figure 1). This denotes that compound heterozygosity causes structural alterations leading to protein dysfunction and which is accountable for the phenotype observed in both probands. About half of patients suspected to have GS with only one mutated allele by direct sequencing have a large genomic rearrangement on the other allele (Vargas-Poussou *et al.* 2011). The large-scale genomic rearrangements on the *SLC12A3* gene can be detected by MLPA, therefore this same technique was used in the present study for the identification of E9_E14dup in the two probands. These large scale genomic rearrangements that are usually observed in the *SLC12A3* gene correspond to a high frequency of *Alu* repetitive sequences within the gene (Vargas-Poussou *et al.* 2011). It is well documented that *Alu* sequences are distributed throughout the genome and that regions with high *Alu* repeat content such as intron 14 are prone to nonallelic homologous recombination, which may cause inherited disorders (Belancio *et al.* 2008).

Both patients were accidentally diagnosed with GS during their hospitalization due to a viral infection. Only after questioning the history of the 11-year-old, she reported experiencing typical symptoms of hypokalaemia (Blanchart *et al.* 2017). Failure to thrive was also a key

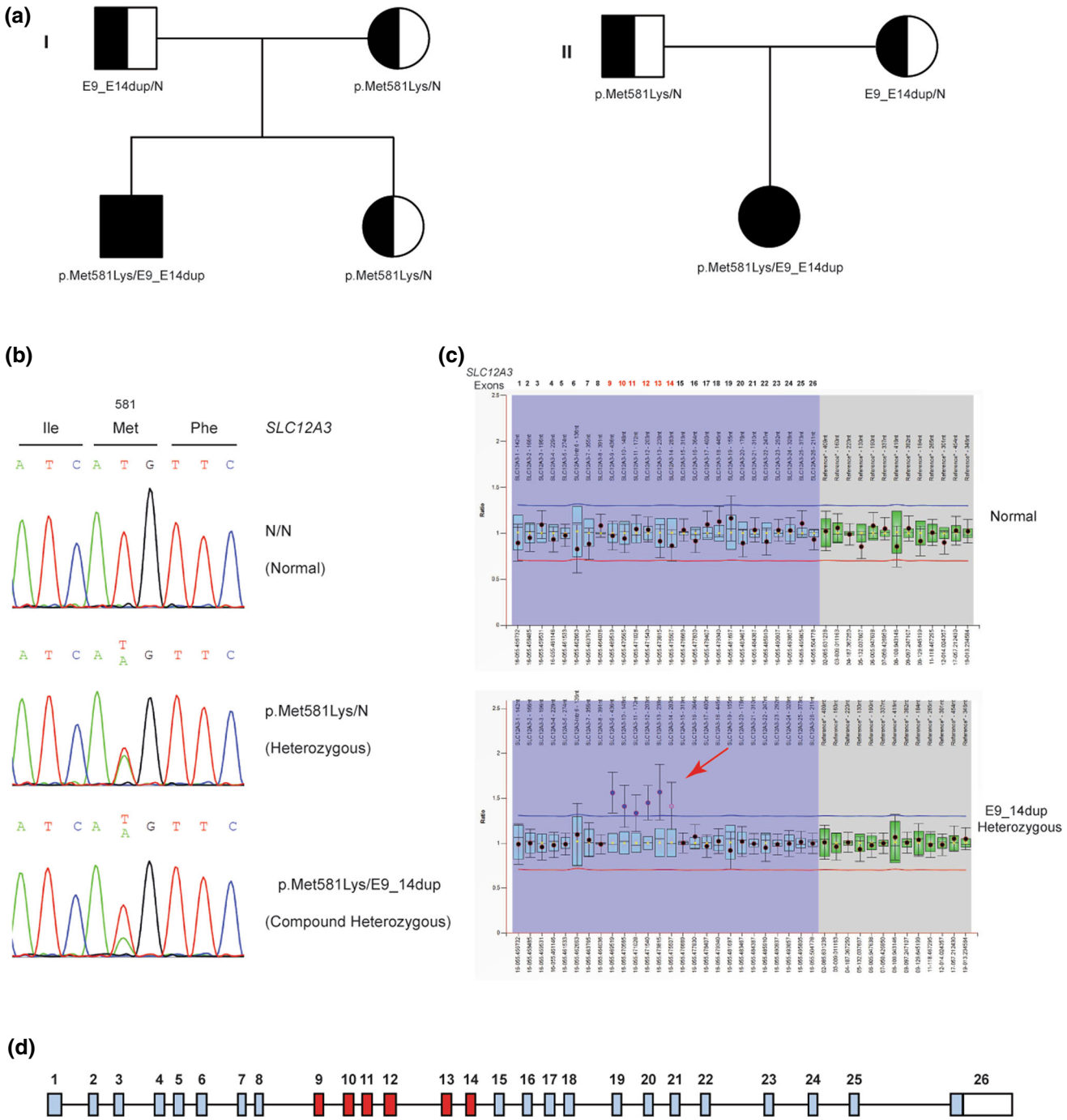

Figure 1. (a) Pedigrees of the two Greek-Cypriot families (I and II) with Gitelman syndrome. Affected individuals are represented by dark shaded symbols. (b) Sequence electropherograms of the heterozygous p.Met581Lys mutation and the compound heterozygous p.Met581Lys mutation with the novel E9_E14 duplication. Note the difference in peak height at the site of the p.Met581Lys mutation between the heterozygous and the compound heterozygous electropherograms. (c) MLPA results of *SLC12A3* gene analyses. Relative probe ratio values from comparative analysis overview chart obtained by Coffalyzer analyses. Red arrow indicates the *SLC12A3* exon 9-14 duplication (E9_E14dup). (d) Schematic representation of the *SLC12A3* exon 9-14 duplication (E9_E14dup). *SLC12A3* exons are numbered and depicted with square symbols. The duplication of exons 9-14 (E9_E14dup) is indicated with red colour.

feature that was found in this patient. On the other hand, the three-year-old patient was free of symptoms. This is in line with the reported age of manifestation of the disease (Blanchart *et al.* 2017). The diagnosis of GS is mostly made during late childhood or adulthood due to the delay of clinical manifestations. Notably, the phenotype variability of GS has been described within families with identical molecular defects (Riveira-Munoz *et al.* 2007).

Early diagnosis of GS is essential considering that undetected hypokalaemia could cause death due to cardiac arrest or respiratory muscles paralysis (Blanchart *et al.* 2017).

Biochemical findings were in both patients typical for GS. One of the two patients had nephrocalcinosis which is more compatible with Bartter syndrome although she had persisted hypomagnesaemia, one of the typical features that distinguishes GS from other tubulopathies (Blanchart *et al.* 2017). Even if biochemical and clinical features may be suggestive for GS disease overlapping with other renal tubulopathies may be observed. Consequently, molecular studies are useful in accurately diagnosing the disease.

Considering that the novel heterozygous duplication E9_E14dup has never been reported before and was exclusively found in the two unrelated Greek-Cypriot GS families, speculates that it is the result of an ancestral mutation that has spread in the island of Cyprus due to a possible founder effect. Similar founder effect phenomena have been projected for a number of other mutations or diseases among the population of Cyprus such as the predominance of the IVS1-2A>G mutation in the steroid 5α-reductase type 2 (*SRD5A2*) gene in patients with 5α-reductase deficiency (Skordis *et al.* 2010), the prevalent p.Cys618Arg missense mutation of the *RET* proto-oncogene in patients with multiple endocrine neoplasia 2A (MEN2A) (Fanis *et al.* 2018) and the reported novel *COL4A4* gene mutation p.Gly871Cys in a cohort of Greek-Cypriot families with thin membrane nephropathy and focal segmental glomerulosclerosis (Voskarides *et al.* 2008).

To our knowledge, no studies have been reported in Cyprus regarding the genetic mutations in GS. Given the small population of the country, the study may be abridging the genetic diagnostic protocol when GS is suspected. Testing for GS probable variants can be performed before proceeding to a full gene sequencing dropping the diagnostic cost of the procedure. In addition, this report adds to the mutational spectrum observed in GS.

Acknowledgement

We would like to thank the A. G. Leventis Foundation for funding this project.

Corresponding editor: INDRAJIT NANDA

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