Gitelman syndrome in Gypsy paediatric patients carrying the same intron 9 + 1 G>T mutation. Clinical features and impact on quality of life

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Abstract

Background. Gitelman syndrome is a primary tubular disorder causing hypokalaemic metabolic alkalosis with hypocalciuria. Its prevalence is high in Gypsies, who harbour an identical mutation, intron 9 + 1 G>T, in the SLC12A3 gene.

Methods. To better define the Gitelman syndrome in Gypsies, the clinical and biochemical features of 34 Spanish paediatric Gypsy patients were analysed. At diagnosis, symptoms, height and weight as well as serum and urinary biochemical data were collected. During a follow-up of 4.5 ± 2.4 years [X ± standard deviation (SD)], therapy, treatment compliance, symptoms, frequency of hospital admissions and, at the last visit, growth and biochemical work-up of 29 patients followed for at least 6 months were analysed. Quality of life items were also assessed by a questionnaire.

Results. Muscle cramps (41%) and asthenia (35%) were the most frequent presenting symptoms. Biochemical data at diagnosis were serum K 2.76 ± 0.46 mEq/L, serum Mg 1.32 ± 0.28 mg/dL, blood pH 7.45 ± 0.06, serum bicarbonate 28.2 ± 2.9 mEq/L, urinary calcium/creatinine ratio 0.03 ± 0.04 mg/mg, fractional K excretion 24.4 ± 17.1% and fractional Mg excretion 8.9 ± 8.3%. During follow-up, Mg and K supplements were prescribed to 79 and 86% of patients, respectively; compliance with treatment was good in 35%. Hospital admission rate was 0.03/patient/month. Muscle cramps were the symptom most often referred by the patients (45%) during the follow-up, and 71% of patients considered their health status as excellent or good. Twenty-one patients stated that their disease did not adversely interfere with their mood or social relationships. Height and weight of patients at diagnosis were −0.60 ± 1.17 and −0.49 ± 1.32 SD, respectively, and improved to −0.44 ± 1.28 (P < 0.05) and 0.18 ± 1.79 SD (P < 0.01) at the last visit.

Conclusions. Gypsy children with Gitelman syndrome mostly exhibit muscle symptoms and asthenia although the disease is not particularly severe in this ethnic group. Body growth improves with treatment and close follow-up.

Keywords: Gitelman syndrome; hypokalaemic tubulopathy; magnesium deficiency; quality of life; sodium–chloride cotransporter

Introduction

Gitelman syndrome (OMIM 263800) is a primary tubular disorder first described by Gitelman, Graham and Welt in 1966 [1]. It is a rare disease transmitted following an autosomal recessive inheritance [2–5] with overall prevalence of patients of ∼1 case/50,000 and of heterozygous carriers of 1/100 [5–9]. Gitelman syndrome is caused by a wide variety of loss-of-function mutations in the SLC12A3 gene, sited in the chromosomal region 16q13, responsible for the synthesis of the thiazide-sensitive Na–Cl cotransporter (NCCT) located in the luminal membrane of the distal convoluted tubule, which is believed to be the principal mediator of sodium and chloride reabsorption in this segment of the nephron [2,7,9–13]. Some patients with mutations in the CLCNKB gene, encoding the renal chloride channel CIC-Kb, located in the basolateral membrane of cells of the thick ascending limb of Henle’s loop (TAL) and the distal convoluted tubules may present with manifestations resembling Gitelman syndrome [12,14–17].

The defective function of NCCT causes hypokalaemic metabolic alkalosis secondary to sodium depletion and subsequent stimulation of the renin-angiotensin-aldosterone axis [1,3,4,6,9,10,18–20]. Passive calcium reabsorption in the proximal tubule and reduced abundance of the epithelial Mg2+ channel TRPM6, located in the distal convo-
luted tubules, are likely involved in the pathogenesis of hypokalaemia and hypomagnesaemia, respectively, characteristically found in the syndrome [12].Clinically, the syndrome may give rise to a broad spectrum of symptoms, from asymptomatic subjects [2,4,10,13,16,20–23] to severe cases of rhabdomyolysis, tetany and paralysis [5,6,10,13,22–25]. It has been reported that the syndrome’s manifestations are more severe in males than females [5,24,26], and significant involvement of life quality has been found in adult patients addressed by a specific questionnaire. No phenotype–genotype correlation has been demonstrated [3,5,12,24,27,28].

Gitelman syndrome is particularly frequent in Gypsies, the prevalence of carriers being 1/50 in this ethnic group [29]. We reported a single point mutation intron 9 + 1 G>T leading to the lack of exon 9 in mature RNA in Gypsies with Gitelman syndrome and suggested a founder effect in this population [29–31].

To characterize the Gitelman syndrome in a particular population of patients having both the same underlying SLC12A3 gene mutation and identical ethnical origin, as well as to analyse the manifestations of the syndrome in the largest series of paediatric patients ever reported, we describe here the biochemical and clinical features of Gitelman syndrome in Spanish Gypsy children as well as the impact of the disease on the patients’ quality of life.

Materials and methods

To find out the peculiarities of the Gitelman syndrome phenotype in patients sharing identical underlying SLC12A3 mutation, a questionnaire asking for the clinical and biochemical manifestations of Gypsy patients with Gitelman syndrome at diagnosis and follow-up was sent to the Paediatric Nephrology units of Spanish hospitals. The questionnaire included information on the following issues: patient identification (sex, date of birth and race), presenting manifestations at diagnosis (age, symptoms, height and weight, serum and urinary biochemical data) and information on follow-up (treatments, treatment compliance, symptoms, frequency of hospital admissions, height and weight as well as biochemical work-up at the last visit).

Gitelman syndrome was diagnosed on the basis of primary hypokalaemic metabolic alkalosis and hypomagnesaemia in the presence of high urinary losses of potassium and magnesium and hypocalciuria. Mutational analysis of the SLC12A3 gene was performed in our laboratory as follows: genomic DNA of each patient was amplified by polymerase chain reaction in a final volume of 20 µL with primers 9F = CTCTTCCTCCTCCCTCC- TCCAG and 9R = CTGGCTGGGCGGCCCCAGTT (68°C required for the annealing process). The reverse primer contains a mismatch that introduces a site for the restriction enzyme Hpal when T (the mutation) was present. After digestion with this enzyme, electrophoresis on a 3% agarose gel and staining with ethidium bromide alleles were visualized as 151 bp (G) and 131 bp (T) [31].

A quality of life questionnaire based on that used by Cruz et al. [22], translated and adapted to Spanish children, was posted to the families of Gitelman syndrome children. Patients older than 12 years were asked to answer the questionnaire. For those children younger than 12 years, one of the parents was asked to fulfill the questionnaire’s items. Families who did not return the questionnaire were kindly requested to answer it by phone to one of the investigators (D.H.).

Quantitative variables are expressed as mean ± standard deviation (SD) and were compared between groups by using the Student’s t-test or Mann–Whitney U-test. Qualitative variables are expressed as percentage, as otherwise described, and were compared by the chi-square or Fisher’s exact test. Growth measurements were expressed as SD of reference values of Spanish children of same age and sex [32]. Growth data at diagnosis and at the end of follow-up were graphically represented as box plots and compared by the Student’s t-test for paired values. Values of P below 0.05 were considered as statistically significant.

Written informed consent was obtained from all study participants or from their parents.

Results

Diagnosis

Thirty-four Gipsy children, 53% females, from 17 Spanish Paediatric Nephrology units were enrolled in the study. Their age at diagnosis was 8.8 ± 3.9 years (median 8.1 years, range 2.9–18.8 years). Eight patients were diagnosed as a result of a family study and another six because of casual detection of hypokalaemia. Twenty-four patients had manifestations likely related to the Gitelman syndrome at diagnosis, although in four of them these symptoms were not the motive that led to the diagnosis. The most frequent symptoms were muscular cramps in 14 cases (41%), asthenia in 12 (35%), muscle weakness in 6 (18%), myalgias in 5 (15%), vomiting in 4 (12%), abdominal pain in 4 (12%), arthralgias in 3 (9%) and dizziness in 2 (6%). Height and weight Z scores were −0.62 ± 1.35 SD and −0.44 ± 1.25 SD, respectively. Relevant biochemical data at the moment of diagnosis were as follows: serum potassium 2.76 ± 0.46 mEq/L, serum magnesium 1.32 ± 0.28 mg/dL (correction factor to transform values to international units: mg/dL × 0.4114 = mmol/L), blood pH 7.45 ± 0.06, serum bicarbonate 28.2 ± 2.9 mEq/L, serum sodium 137.8 ± 3.1 mEq/L, serum chloride 96.6 ± 5.9 mEq/L, serum calcium 9.8 ± 0.5 mg/dL (correction factor to transform values to international units: mg/dL divided by 4 = mmol/L), serum creatinine 0.57 ± 0.18 mg/dL, urinary calcium/creatinine ratio 0.03 ± 0.04 mg/mg, fractional excretion of potassium 24.4 ± 17.1%, fractional excretion of magnesium 8.9 ± 8.3% and spontaneous urinary osmolality 551 ± 233 mOsm/Kg H2O.

The mutational analysis of SLC12A3 gene disclosed the intron 9 + 1 G-T mutation in homozygosity in all Gypsy patients, their parents being heterozygous carriers for this mutation. This mutation affects the consensus donor splice site and abolishes the correct RNA splicing, giving rise to an aberrant mRNA [30].

Follow-up

Twenty-nine patients were followed for 4.5 ± 2.4 years (median 4.8 years, range 0.6–9.1 years). Patients with a follow-up below 6 months were excluded. According to the information provided by their physicians, 79% of these patients were prescribed chronic treatment with magnesium and 86% with potassium supplements; compliance with this treatment was considered good in 35% of the patients. Rate of hospital admissions due to metabolic imbalance was 0.03/patient/month. Muscle cramps were the symptom most often referred by the patients (45% of them) to their physicians during the follow-up outpatient visits. Height and weight Z scores at the last visit were −0.44 ± 1.28 SD and 0.18 ± 1.79 SD, respectively, significantly different (P < 0.05 and P < 0.01) from those found at diagnosis. The lab work-up at the last visit was as follows: serum potassium 3.26 ± 0.43 mEq/L, serum magnesium
1.40 ± 0.29 mg/dL, blood pH 7.42 ± 0.05, serum bicarbonate 29.3 ± 2.7 mEq/L, serum sodium 139.1 ± 2.4 mEq/L, serum calcium 9.9 ± 0.6 mg/dL, serum creatinine 0.67 ± 0.18 mg/dL, urinary calcium/creatinine ratio 0.04 ± 0.06 mg/mg, fractional excretion of potassium 20.2 ± 10.3%, fractional excretion of magnesium 11.6 ± 10.2% and spontaneous urinary osmolality 689 ± 187 mOsm/Kg H2O.

The questionnaire was answered by 24 of the patients or their families (only cases with a follow-up ≥ 6 months).

Asthenia was the clinical manifestation most frequently reported. Myalgias, muscle weakness, muscular cramps, dizziness and arthralgias were also present (Figure 1). Subjective overall assessment of their health status was excellent in 5 cases, good in 12, intermediate in 6 and poor in 1 case. Twenty-one patients stated that their disease did not adversely interfere with their mood or social relationships, but 4 out of 17 patients stated that it made their work or education more difficult or restricted their ability to work or to study. Three patients considered asthenia as a big problem and six as a moderate problem in their life. Likewise, two patients referred muscle weakness as a big problem and five as a moderate problem. Overall, 11 patients consider the whole array of symptoms as no problem, 8 as trivial, 3 as moderate and 2 as a big problem.

**Growth evolution**

Growth evolution was analysed only in patients with a follow-up of at least 6 months. Height and weight of these patients at diagnosis were −0.60 ± 1.17 SD and −0.49 ± 1.32 SD, respectively, and improved to −0.44 ± 1.28 SD (P < 0.05) and 0.18 ± 1.79 (P < 0.01) at the end of the follow-up period (Figures 2 and 3). Height < −2 SD was found in 2 out of 26 patients at diagnosis and 1 out of 23 patients at the last follow-up.
Height changed from $-1.02 \pm 0.42$ SD at diagnosis to $-0.54 \pm 1.03$ SD after follow-up (difference not significant) and weight from $-0.94 \pm 0.57$ SD at diagnosis to $-0.06 \pm 0.96$ SD after follow-up ($P < 0.05$), in patients who manifested good adherence to treatment. In patients with bad treatment compliance, height and weight SDs remained essentially unchanged (height from $-1.06 \pm 1.16$ to $-0.92 \pm 1.41$ and weight from $-0.77 \pm 0.75$ to $-0.31 \pm 1.36$).

**Gender effect**

Clinical, biochemical and growth data were compared between females and males in the series of 34 patients. Significant differences found between females and males were blood pH at diagnosis ($7.48 \pm 0.05$ versus $7.43 \pm 0.06$, $P < 0.05$), serum calcium at diagnosis ($9.7 \pm 0.4$ versus $10.1 \pm 0.5$, $P < 0.05$), serum potassium at the last visit ($3.11 \pm 0.43$ versus $3.45 \pm 0.44$, $P < 0.05$), serum calcium at the last visit ($9.7 \pm 0.7$ versus $10.2 \pm 0.4$, $P < 0.05$) and urinary calcium/creatinine ratio at last visit ($0.05 \pm 0.05$ versus $0.03 \pm 0.07$, $P < 0.05$).

**Discussion**

This study describes the clinical features of Gitelman syndrome in the largest paediatric series reported so far, characterizes the Gitelman syndrome in Gypsy paediatric patients who have identical underlying mutation, gives information on the manifestations of the disease such as recorded at diagnosis by the patients’ doctors or perceived by their own patients during the follow-up and provides the evolution of growth in these patients after the diagnosis.

It shows that asthenia and muscular symptoms, such as cramps, weakness and myalgias, were the most frequent manifestations at diagnosis and during the follow-up. Our study does not reveal what percentage of children with Gitelman syndrome remains asymptomatic but 41% of Gypsy patients (14 out of 34 cases) were diagnosed not because of developing clinical manifestations but from family study or casual detection of their hypokalaemia. Interestingly, the present study provides information on the impact of Gitelman syndrome on the quality of life in paediatric patients. According to the answers to the questionnaire, 71% of young children with Gitelman syndrome considered their health status as excellent or good and most of them felt that the disease would not interfere with their social relationships or mood. Quality of life was better in our study than that reported in adult individuals, 21% of our patients considering their symptoms as a moderate to big problem versus 45% in the Cruz study [22]. Hypothetical reasons for this difference are that our patients were younger, $13.1 \pm 4.3$ years versus $40.5 \pm 12.5$ years, the majority were students and not workers and the absence of concomitant toxic habits in children. Thus, our findings show that Gitelman syndrome leads to mild manifestations in the majority of symptomatic paediatric patients.

The variability in the clinical and biochemical features found among the Gypsy patients emphasizes the lack of genotype-phenotype correlation in Gitelman syndrome even in individuals of the same ethnic origin having the same loss-of-function mutation in the $SLC12A3$ gene. It has recently been proposed that patients of male gender who harbour compound heterozygous mutations in the $SLC12A3$ gene leading to splicing defects and intrinsic functional alterations in NCCT exhibit a severe form of the disease [5]. In our series of patients, there was not a more severe phenotype in males than in females despite the fact that the intron 9 + 1 G→T mutation has been shown to cause a loss of exon 9 of the $SLC12A3$ gene, region that codes for amino acids 366–395 corresponding to a part of an intracellular loop and the majority of the seventh transmembrane region of the transporter [30]. Our study does not confirm this adverse influence of male gender on the phenotype since the degree of alkalosis and hypokalaemia at diagnosis was greater in females than males, and no gender-related differences were appreciated in the impact of the disease on growth and quality of life.

There is very limited information on growth of children with Gitelman syndrome. Bettinelli et al. [33], in 16 children diagnosed with Gitelman syndrome on the basis of biochemical and clinical data, found that the mean height at diagnosis was $-0.5$ SD and it was below $-2$ SD in three patients. Riveira-Muñoz [5] reported growth retardation, identified by height below the third percentile, or height velocity below the tenth percentile, and/or final short stature (below the third percentile) in eight patients with severe forms of Gitelman syndrome from a total of 27 individuals with this syndrome. Our results indicate that 8% of children with Gitelman syndrome present a height $< -2$ SD at diagnosis. Although isolated cases of growth hormone deficiency have been reported in patients with Gitelman syndrome [34,35], electrolyte and fluid depletion likely play a role in the pathogenesis of growth retardation [36]. In the present study, improvement of height and weight of patients during follow-up might suggest a beneficial effect of potassium and magnesium supplementation on growth although the low percentage of good treatment compliance accounts for a limitation for this assumption. In this regard, it is of note that height and weight of patients who manifested good adherence to treatment changed from $-1.02 \pm 0.42$ SD and $-0.94 \pm 0.57$ SD at diagnosis to $-0.54 \pm 1.03$ SD and $-0.06 \pm 0.96$ SD, respectively, after follow-up, the difference being statistically significant ($P < 0.05$) for weight, whereas in patients with bad treatment compliance and height and weight SDs remained essentially unchanged.

**Conclusion**

In summary, the analysis of the large series of patients presented in this study confirms that Gitelman syndrome usually causes mild manifestations in children, discloses the characteristics of the syndrome in the Gypsy ethnic group, where the syndrome is highly prevalent but not particularly severe, provides novel information on the impact of the syndrome on quality of life parameters in paediatric population and shows the evolution of body growth in these patients.
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Conflict of interest statement. None declared.

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