Abstract

Introduction: Gitelman syndrome (GS) is a tubulopathy caused by SLC12A3 gene mutations, which lead to hypokalaemic alkalosis, secondary hyperaldosteronism, hypomagnesaemia and hypocalciuria. Aim: The aim of this study was to assess the prevalence of SLC12A3 gene mutations in adult hypokalaemic patients; to compare the phenotype of homozygous, heterozygous and non-mutated patients; and to determine the efficiency of treatment.

Methods: Clinical, biological and genetic data were recorded in 26 patients.

Results: Screening for the SLC12A3 gene detected two mutations in 15 patients (six homozygous and nine compound heterozygous), one mutation in six patients and no mutation in five patients. There was no statistical difference in clinical symptoms at diagnosis between the three groups. Systolic blood pressure tended to be lower in patients with two mutations ($P=0.16$). Hypertension was unexpectedly detected in four patients. Five patients with two mutated alleles and two with heterozygosity had severe manifestations of GS. Significant differences were observed between the three groups in blood potassium, chloride, magnesium, supine aldosterone, 24 h urine chloride and magnesium levels and in modification of the diet in renal disease. Mean blood potassium levels increased from 2.8 ± 0.3, 3.5 ± 0.5 and 3.2 ± 0.3 before treatment to 3.2 ± 0.5, 3.7 ± 0.6 and 3.7 ± 0.3 mmol/l with treatment in groups with two ($P=0.003$), one and no mutated alleles respectively.

Conclusion: In adult patients referred for renal hypokalaemia, we confirmed the presence of mutations of the SLC12A3 gene in 80% of cases. GS was more severe in patients with two mutated alleles than in those with one or no mutated alleles. High blood pressure should not rule out the diagnosis, especially in older patients.

European Journal of Endocrinology 165 665–673
of the CLCNKB gene, diuretic abuse, vomiting (when urinary chloride is not available) and rare cases of acquired autoimmune tubulopathies such as Sjögren’s syndrome. Type III Bartter syndrome often presents as dehydration in the first year of life and is associated with hypomagnesaemia in 20% of cases and normal or increased calciuria, in contrast to GS. Most asymptomatic patients with GS remain untreated except for a high-sodium and high-potassium diet with lifelong magnesium supplementation. A cardiac work-up should be offered to screen for risk factors of cardiac arrhythmias, but the treatment remains difficult and not well standardised. The long-term prognosis of GS is considered to be good, but indeed very little data is available in the literature, especially on GS patients confirmed by genotyping (7).

The aim of this retrospective study was i) to assess the prevalence of SLC12A3 mutations in adult patients exhibiting chronic renal hypokalaemia after ruling out iatrogenic and adrenal causes, ii) to compare the phenotype of homozygous, heterozygous and non-mutated patients and iii) to determine the efficiency of treatment on blood potassium levels.

**Patient and methods**

**Patient recruitment**

Twenty-six patients from 24 unrelated families were recruited from the three largest general hospitals and the University Hospital of Northwestern France, an area including 4 million inhabitants (Nord–Pas de Calais region), over a nine-year period (2000–2008). The probands had all been referred for low blood potassium level (K⁺ < 3.5 mmol/l) associated with renal potassium wasting (kaliuresis > 40 mmol/24 h). They did not take laxatives or diuretics and claimed no alcohol or drug abuse. Other extrarenal and renal causes of hypokalaemia, such as primary hyperaldosteronism, Cushing’s syndrome, stenosis of the renal artery, history of nephrotoxic drugs or liquorice intake, tubular acidosis, severe hypomagnesaemia and transcellular potassium shift, such as with the use of bronchodilators and thyrotoxic or familial periodic paralysis, were also excluded. Two patients (#19 and #20), belonging to the same family, were carriers but were included since they had a borderline low blood potassium of 3.6 mmol/l. Seventeen patients came from an Endocrinology Department, eight from a Nephrology Department and one from a Cardiology Department. Data were retrospectively collected by file review.

**Clinical evaluation**

The following clinical data were recorded for each patient:

- Age at the first complete laboratory examination for hypokalaemia.
- Personal and familial history.
- Symptoms: cramps, salt craving, dizziness and palpitations.
- Weight in kilogram and height in meter.
- Blood pressure after 2 h supine by conventional manual sphygmomanometry at diagnosis and during follow-up. Hypertension was diagnosed when blood pressure was above 140/80 mmHg on at least two occasions.
- Number of years of follow-up.
- Complications of GS: renal function alterations as assessed by blood creatinine level increase and glomerular filtration rate evaluation; cardiac events such as syncope or severe palpitations requiring admission to an intensive care unit; chondrocalcinosis.
- Diabetes.
- Treatment.

**Laboratory assessment**

The following laboratory data were collected without tourniquet for each patient i) at the time of diagnosis, without any treatment other than a small dose of potassium chloride when the blood potassium level was below 2.8 mmol/l (which explains why some blood potassium values were above 3.5 when the patients were investigated) and ii) after treatment when available:

- Blood sample to assess levels of sodium, potassium, chloride, magnesium, calcium, bicarbonate, creatinine, aldosterone and plasma renin activity (PRA) or active renin measured at 0800 h after 2 h in recumbent position between 0600 and 0800 h and at 0100 h after 2 h of upright posture.
- 24 h urine collection to evaluate urine levels of sodium, potassium, chloride, magnesium, creatinine, calcium and aldosterone.
- The glomerular filtration rate evaluation was calculated with the modification of the diet in renal disease (MDRD) simplified formula.

The hormone measurement techniques were as follows:

- PRA was assessed by RIA (Renctk, DiaSorin, Inc., Stillwater, MN, USA), with within-assay variability between 5.4 and 9.9% for values between 1.6 and 17.9 ng/ml per h, and between-assay variability of 5.6–11.5% for values between 1.6 and 15.2 ng/ml per h. In two cases, active renin was measured by chemiluminescence assay instead of PRA.
- Plasma aldosterone was measured by RIA aldosterone (Immunotech–Beckman Coulter, Marseille, France), with within-assay variability between 2.5 and 8.3% for values between 140 and 1120 pmol/l. and between-assay variability between 3.9 and 10.4% for values between 70 and 700 pmol/l.
- Urine aldosterone was measured by RIA aldosterone (Immunotech–Beckman Coulter), with within-assay variability between 3 and 6.8% and between-assay variability of around 9%.

**Molecular analysis of the SLC12A3 and CLCNKB genes**

The molecular genetic analysis of most patients in this study was recently published (9). Shortly after, patients’ written informed consents were obtained, DNA was extracted from blood leucocytes and used for PCR amplification and direct sequencing of exons and flanking intronic sequences of the SLC12A3 gene on an ABI 3100 capillary sequencer (Perkin Elmer Applied Biosystems, Foster City, CA, USA) in the Molecular Genetic Department of Hôpital Européen Georges Pompidou, Paris, France.

An investigation for large rearrangements by multiplex ligation-dependent probe amplification was also done in the four probands of the six patients harbouring only one SLC12A3 mutation, since a second mutation in the same gene was more probable than a mutation of another gene. A complementary investigation for mutations in the CLCNKB gene was also performed in the ‘no SLC12A3 mutation’ group according to the same technique as for SLC12A3 gene.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Mutation 1</th>
<th>Mutation 2</th>
<th>References</th>
<th>Mutation 1</th>
<th>Mutation 2</th>
<th>References</th>
<th>Mutation*</th>
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</thead>
<tbody>
<tr>
<td>Two mutations</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Exon 12</td>
<td>c.1484T&gt;G, p.Phe495Ser</td>
<td>(9)</td>
<td>Exon 18</td>
<td>c.2221G&gt;A, p.Gly741Arg</td>
<td>(2)</td>
<td>Yes</td>
</tr>
<tr>
<td>2</td>
<td>Exon 11</td>
<td>c.1440T&gt;C, p.Ser473Pro</td>
<td>(38)</td>
<td>Exon 11</td>
<td>c.1390G&gt;A, p.Ala464Thr</td>
<td>(38)</td>
<td>No</td>
</tr>
<tr>
<td>4</td>
<td>Exon 15</td>
<td>c.1390G&gt;T, p.Ala464Thr</td>
<td>(9)</td>
<td>Exon 18</td>
<td>c.2221G&gt;A, p.Gly741Arg</td>
<td>(2)</td>
<td>No</td>
</tr>
<tr>
<td>5</td>
<td>Exon 1</td>
<td>c.160G&gt;C, p.Arg54Cys</td>
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<td>Exon 24</td>
<td>c.2782C&gt;T, p.Arg928Cys</td>
<td>(40)</td>
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</tr>
<tr>
<td>6</td>
<td>Intron 9</td>
<td>c.1180+1G&gt;T</td>
<td>(39)</td>
<td>Intron 9</td>
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<tr>
<td>7</td>
<td>Intron 23</td>
<td>c.2747+1G&gt;C</td>
<td>(9)</td>
<td>Intron 24</td>
<td>c.2883+1G&gt;T</td>
<td>(2)</td>
<td>No</td>
</tr>
<tr>
<td>8</td>
<td>Exon 22</td>
<td>c.2576T&gt;C, p.Leu859Pro</td>
<td>(2)</td>
<td>Exon 22</td>
<td>c.2581G&gt;T, p.Arg861Cys</td>
<td>(40)</td>
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</tr>
<tr>
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<td>Exon 16</td>
<td>c.1928C&gt;T, p.Pro643Leu</td>
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<td>Intron 7</td>
<td>c.964+1G&gt;T</td>
<td>(9)</td>
<td>No</td>
</tr>
<tr>
<td>10</td>
<td>Exon 7</td>
<td>c.1145C&gt;T, p.Thr382Met</td>
<td>(42)</td>
<td>Exon 24</td>
<td>c.2807_2810dup, p.Thr936GlyfsX17</td>
<td>(9)</td>
<td>No</td>
</tr>
<tr>
<td>11</td>
<td>Intron 24</td>
<td>c.2883+1G&gt;T</td>
<td>(39)</td>
<td>Exon 26</td>
<td>c.2981G&gt;A, p.Cys944Tyr</td>
<td>(38)</td>
<td>No</td>
</tr>
<tr>
<td>12</td>
<td>Intron 9</td>
<td>c.1180+1G&gt;T</td>
<td>(39)</td>
<td>Intron 9</td>
<td>c.1180+1G&gt;T</td>
<td>(39)</td>
<td>Yes</td>
</tr>
<tr>
<td>14</td>
<td>Exon 17</td>
<td>c.2120G&gt;T, p.Ala707Val</td>
<td>(9)</td>
<td>Exon 17</td>
<td>c.1145C&gt;T, p.Thr382Met</td>
<td>(9)</td>
<td>No</td>
</tr>
</tbody>
</table>

| One mutation | | | | | | | |
| 16 | Exon 11 | c.1390G>A, p.Ala464Thr | (38) | | | | Yes |
| 17 | Exon 24 | c.2782C>T, p.Arg928Cys | (40) | | | | No |
| 18 | Exon 6 | c.791G>C, p.Gly264Ala | (41) | | | | Yes |
| 19 | Exon 6 | c.791G>C, p.Gly264Ala | (41) | | | | Yes |
| 20 | Exon 6 | c.791G>C, p.Gly264Ala | (41) | | | | Yes |
| 21 | Exon 6 | c.791G>C, p.Gly264Ala | (41) | | | | No |


**Statistical analysis**

Values were expressed as median and interquartile values and were compared by the non-parametric Kruskal–Wallis test using commercial software (Statview SAS, Cary, NC, USA). Mean ± s.d. values were also calculated and are given in tables and figures for easier comparison with previous data. When data to be compared were nominal, the Fisher’s exact test was performed. Correlations between laboratory data were studied by the non-parametric Spearman correlation test. The Wilcoxon non-parametric test was used to compare biological data before and after treatment. P values < 0.05 were considered significant.

**Results**

**SLC12A3 and CLCNKB gene analysis**

Twenty-two potentially pathogenic mutations of SLC12A3 were found in 21 patients from 19 different families. Seven mutations were recently reported in a larger study (9). The patients were classified into three groups according to the number of pathogenic mutations: two, one or none. A summary of these data is shown in Table 1.

Fifteen patients (57%), from 15 different families, had two mutations of the SLC12A3 gene, defining GS (six...
patients had homozygous mutations and nine showed composite heterozygosity).

Only one heterozygous mutation of the SLC12A3 gene was detected in six hypokalaemic patients belonging to four families, patient #19 being the father of patients #18 (the proband) and #20 (her sister). No large genomic rearrangements of the SLC12A3 gene were identified in this ‘one mutation’ group.

Neither SLC12A3 nor CLCNKB gene mutations were detected in five other patients belonging to five families.

Comparison of phenotypic characteristics in the three groups of patients at first assessment

Clinical symptoms
There were no statistical differences in clinical symptoms at the time of diagnosis between patients with two, one or no mutations (Table 2). Systolic blood pressure (SBP) tended to be lower in patients with two mutations ($P = 0.16$), whereas patients with no mutation tended to be older ($P = 0.14$).

In patients with two mutations, the diagnosis was made in a fortuitous way in most cases, but when patients were questioned, they complained of symptoms possibly linked to GS in 60% of cases (cramps in 84%, salt craving in 90%, dizziness in 80% and palpitations in 62%).

Hypertension was present in one patient at the time of GS diagnosis (patient #5 in Table 1, aged 36 years, who had no familial history of hypertension but had morbid obesity and sleep apnoea). Three other patients (# 2, #9 and #11 in Table 1) developed hypertension after GS diagnosis (3, 6 and 13 years later, at the age of 62, 71 and 47 years respectively). They had no familial history of hypertension, but two of them were obese (body mass index (BMI) 26.8 and 28.1 kg/m$^2$).

Diabetes was present in three patients with GS: one had autoimmune type 1 diabetes (patient #4 in Table 1), one had genetic haemochromatosis complicated by cirrhosis and secondary diabetes (patient #2 in Table 1) and the last one had type 2 diabetes linked to obesity (patient #5 in Table 1). These last two patients also had hypertension that appeared, respectively 4 years after and 16 years before the diabetes.

Five patients with two mutations had severe manifestations: two had symptomatic chondrocalcinosis (patients #2 and #10 in Table 1), two had growth retardation (patients #7 and #13 in Table 1, 24.6 kg for 1.26 m and 23 kg for 1.26 m, respectively, at 10 years, $-1.5$ s.d.), and one had cardiac arrhythmia related to hypokalaemia with several hospitalisations in the cardiology intensive care unit (patient #15 in Table 1). It should be also noted that the brother of another patient died suddenly at the age of 23 years with the same biological abnormalities as his sister, but genetic analysis could not be performed before his premature death. Two patients complaining of palpitations had normal cardiac exploration results by echocardiography and 24 h electrocardiographic ambulatory monitoring (patients #3 and #5 in Table 1), and another patient had multiple atrial and ventricular extrasystoles (patient #12 on Table 1). This last patient was homozygous, while the two others were compound heterozygous.

Among heterozygous patients, one patient (#16) had hypertension and two also had complications: one suffered from syncope and the other had chondrocalcinosis.

No complications occurred in patients without mutations.

Laboratory data
Significant differences were observed between the three groups with regard to blood potassium, chloride, magnesium and supine aldosterone; 24 h urine chloride and magnesium levels; as well as glomerular filtration rate assessed with MDRD (Table 3). Blood levels of upright PRA were borderline

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Clinical characteristics at first assessment.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with</td>
<td>Two mutations ($n=15$)</td>
</tr>
<tr>
<td>Sex ratio (M/F)</td>
<td>0.6</td>
</tr>
<tr>
<td>Age (years)</td>
<td>35±15 (9–65)</td>
</tr>
<tr>
<td>Fortuitous diagnosis</td>
<td>54% (8/15)</td>
</tr>
<tr>
<td>Symptoms at clinical examination</td>
<td>60% (9/15)</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>24.3±6.7 (19.2–46)</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>113±20 (80–160)</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>68±11 (50–90)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>26% (4/15)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>20% (3/15)</td>
</tr>
<tr>
<td>Complications</td>
<td>33% (5/15)</td>
</tr>
<tr>
<td>Cardiac arrhythmia</td>
<td>1</td>
</tr>
<tr>
<td>Growth retardation</td>
<td>2</td>
</tr>
<tr>
<td>Chondrocalcinosis</td>
<td>2</td>
</tr>
</tbody>
</table>
significant. In patients with two mutations, upright PRA levels were above 5 mmol/l in more than 90% of cases, while hypocalciuria was present in 83% of cases and hypomagnesaemia in 50% of cases.

Otherwise, the pairwise comparison of the three groups confirmed significantly lower blood potassium and magnesium levels in the ‘two mutations’ group compared with the ‘one mutation’ and ‘no mutation’ groups. The chloride level was also significantly lower in blood and higher in urine in the ‘two mutations’ group compared with the ‘one mutation’ group. There were no differences observed between the ‘one mutation’ and ‘no mutation’ groups with regard to the chloride level.

**Comparison of phenotypic characteristics of the ‘two mutations’ group according to follow-up**

The duration of follow-up varied from 3 to 25 years, with a mean of 7.0 ± 4.7 years. SBP and diastolic blood pressure (DBP) did not increase significantly, in contrast to blood potassium (P < 0.01) and creatinine (P < 0.05) levels, whereas MDRD decreased significantly (P < 0.01) between the first and the last available evaluation (especially in patients #5 and #9), both with high blood pressure (patient #5 being obese in addition), and patient #14 (Fig. 1). Laboratory parameters not shown in this figure did not vary over time. One patient had chondrocalcinosis diagnosed 10 years after the diagnosis of hypokalaemia, while other patients with GS complications developed them before diagnosis.

**Treatment**

Patients with two pathogenic mutations received potassium supplementation to improve hypokalaemia in all cases. It was associated with anti-aldosterone treatment in seven of 15 patients, anti-aldosterone and non-steroidal anti-inflammatory drugs (NSAIDs) in five of 15 patients and anti-aldosterone, NSAIDs and ACE inhibitors or angiotensin II receptor antagonists in two patients as shown in Fig. 2. Treatment led to normalisation of blood potassium in only seven of the 15 patients, but correction of blood potassium above 3 mmol/l was obtained in 73% of patients (11 of 15 patients). Patients with one pathogenic mutation either received no treatment (three of six cases), oral potassium supplementation (one case) or anti-aldosterone, NSAIDs and ACE inhibitors or angiotensin II receptor antagonists in two patients. Patients with no mutation either received oral potassium supplementation (3/5 cases), anti-aldosterone treatment alone (1/5) or combined with NSAIDs (1/5). Mean blood potassium levels increased from 2.8 ± 0.3, 3.5 ± 0.5 and 3.2 ± 0.3 before treatment to 3.2 ± 0.5, 3.7 ± 0.6 and 3.7 ± 0.3 mmol/l with treatment in groups with two, one and no mutations respectively (P = 0.003 for the ‘two mutations’ group). Blood magnesium levels, however, did not differ before and after treatment.
value was not different from the median potassium level (3.65 mmol/l) of the four remaining patients when investigated, and we chose to include ‘carriers’ in the heterozygous group.

Clinically, the diagnosis was made fortuitously in half of the patients with two mutated alleles, and in nearly 3/4 of the other patients. However, symptoms of GS were present at the clinical examination in about 2/3 cases, as previously described (7). Nonetheless, no differences were found between the clinical symptoms of patients according to the different groups, emphasizing the lack of clinical specificity for the diagnosis of GS. It should be noted, however, that the homozygous or compound heterozygous patients tended to be slightly younger, which is not surprising for an inherited disease, with a tendency toward a lower blood pressure. Indeed, a significantly lower blood pressure in heterozygous patients has already been shown, but this was in population and case-control studies (12–14). GS is usually described as a clinical model of hypotension or normotension (15, 16). However, mild hypertension was present in four of 15 patients with GS in our study, particularly after the age of 40 years and in obese and/or diabetic patients. Moreover, SBP tended to increase over time in the mutated patients included in this study. The involvement of NSAID treatment in this development does not appear pertinent due to the fact that patients without NSAID treatment also had increased SBP over the years. High angiotensin II levels, through the activation of inflammation-related processes (15) and potassium depletion, have also been implicated in hypertension, as well as in insulin resistance mechanisms (16–18). This point means that the presence of mild hypertension should not rule out the diagnosis of GS and might sometimes challenge the diagnosis of primary hyperaldosteronism. Also despite the recruitment from an Endocrinology Department, the frequency of diabetes mellitus did not differ among the groups in spite of the increased susceptibility to glucose intolerance recently highlighted in GS heterozygous patients (14) and in patients treated with thiazide diuretics (19, 20). Therefore, in this small study, hyperaldosteronism, promoting insulin

Discussion

This retrospective study compared three groups of patients with two, one or no mutations in the SLC12A3 gene. The prevalence of these mutations in the population of hypokalaemic patients was recently evaluated in a recruitment that was mainly national and nephrological (9). Our results provided here were found in a recruitment that was regional and mainly endocrinological with phenotype and follow-up assessment. Indeed, few studies have focused on the treatment of GS (10, 11), and none have been done on the clinical and biological progression of GS.

Therefore, the first aim of this study was to determine the prevalence of genetically proven GS in a population of patients referred for chronic low blood potassium levels with renal potassium wasting. In this study, more than 50% of the patients harboured two pathogenic mutations of the SLC12A3 gene and 80% had at least one pathogenic mutation, which is in line with a previous national study (9). No large genomic rearrangements were identified in the patients tested from the ‘one mutation’ group, but a second mutation in non-studied region or in other genes cannot, however, be excluded in these groups, even if the complementary analysis did not support CLCNKB gene mutations in the ‘no mutation’ group.

The second aim of the study was to compare the phenotype of homozygous, heterozygous and non-mutated patients. Interestingly, despite the small size of our groups, and even though we were not able to completely exclude the presence of a second mutation for the group of heterozygous patients, we observed a mild phenotype for some biological features in this group. The inclusion of three patients belonging to the same family (patients #19, #20 and #18, the last one being the proband) in the ‘one mutation’ group, might, however, raise questions about a possible difference between ‘obligatory carriers’ and ‘heterozygous’ patients. Nevertheless, patients #19 and #20 showed low blood potassium levels (3.6 mmol/l for both). This

Figure 1 Follow-up of patients with two mutated alleles of the SLC12A3 gene (n = 15 patients). Mean time between the two evaluations was 7.0 ± 4.7 years. *P < 0.05, **P < 0.01 versus evaluation at the time of the diagnosis. Mean ± s.d.: K, blood potassium level; MDRD, estimated creatinine clearance; BP, blood pressure.

Figure 2 Distribution of treatment according to the presence of two, one or no SLC12A3 gene mutation.

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resistance (18, 21) or potassium depletion favouring insulin secretion defect (22) did not seem to play a major role in the genesis of metabolic syndrome, but rather other factors were involved, such as obesity or haemochromatosis.

Biologically, GS is usually defined by the combination of metabolic alkalosis with renal potassium and sodium wasting, hypomagnesaemia and hypocalciuria, with a molar urinary calcium/creatinine ratio <0.2 mmol/mmol (23). Indeed, despite the small size of our study, significant differences were observed between the three groups concerning blood potassium, chloride, magnesium, supine aldosterone and 24 h urine chloride and magnesium levels. However, hypomagnesaemia and hypocalciuria were not constant, which is in line with other previous studies in which the absence of hypomagnesaemia reached 20–40% and the absence of hypocalciuria 26% (24–28). Indeed, numerous factors can interfere with urinary calcium excretion, such as calcium, salt and protein intake; age; vitamin D level and other gene polymorphisms such as the CaSR gene (29).

In our study, cardiac complications were mainly found in patients with two or one mutated alleles: more severe cases were found in those with two mutations, who also showed the lowest level of blood potassium, without obvious differences between genders. A few case reports have emphasized the possibility of prolonged QTc intervals with increased risk of syncope and cardiac arrest, perhaps promoted by myocardial perfusion abnormalities (30, 31). According to these results, it is difficult to determine the exact risk of cardiac complications in GS patients, but cardiac assessment seems to be recommended. Although blood potassium normalisation is difficult to achieve, most severe cardiac events in this short study were observed before diagnosis and/or in cases of severe hypokalaemia, supporting the favourable effect of treatment.

Renal injury has rarely been reported in GS, with only a few cases of acute renal failure (27, 32). Chronic hypokalaemia may be the cause of tubulo-interstitial nephropathy and renal tubular vacuolisation (33). An explanation for the scarcity of renal injury reported in GS patients could be their young age at the time of diagnosis. However, our study shows mild renal function alteration within years in patients with GS, with an elevation of creatinine levels and slight decrease in the glomerular filtration rate measured with the MDRD clearance formula. The interpretation of these data should, however, take into account the fact that MDRD was evaluated under treatment at the last assessment during follow-up.

A possible explanation for the lower frequency of complications found in the ‘no mutation’ group compared with the other groups could be related either to genetic background or more probably to less severe potassium and salt wasting, which induces less activation of the renin–aldosterone axis and fewer deleterious consequences on the GH–IGF1 axis and the metabolic pathways (18, 34, 35).

Treatment of GS is not standardised in the literature. Most authors recommend anti-aldosterone treatment, such as spironolactone or amiloride, in combination with oral potassium and magnesium supplementation (36). In our 15 patients with proven GS, potassium supplementation was always prescribed, most often in combination with amiloride or spironolactone. NSAID treatment was mainly given in Nephrology Departments and was not necessarily related to an early age of diagnosis (which could indicate a more severe form: a mean age of 36 years in patients treated with NSAIDs versus 34 years in patients not treated with NSAIDs). The treatment, regardless of type, resulted in blood potassium normalisation in only half of the patients with two mutated alleles, even though the mean blood potassium improved significantly, in contrast with the ‘one and no’ mutation groups. The absence of significant increase in these last two groups could be due to different factors: a lack of power in the comparison related to the small size of the groups; a less offensive treatment proposed by the physicians; or lower compliance by the patients, in reason of the milder degree of hypokalaemia.

Nevertheless, since the long-term prognosis of GS is known to be poor without any trial comparing treatment versus placebo, the benefits and the therapeutic strategies remain unknown. Blood magnesium levels did not differ before or after treatment, perhaps due to poor oral tolerance of magnesium treatment and possible lack of compliance, as previously described (37). Overall, no cardiac complications occurred in treated patients.

### Conclusion

In this study of 26 patients referred for hypokalaemia with renal potassium wasting, we confirmed the presence of mutations of the SLC12A3 gene in 80% of patients (two pathogenic mutations in more than 50% (n=15) of the 26 patients, heterozygosity in 23% (n=6)). Although we cannot completely rule out the presence of a second mutation in the last group, our data raises the question of the modulating role of heterozygous mutations in the genesis of borderline or low blood potassium. These data need confirmation in a large population of heterozygous carriers. Patients with two mutations were characterized by more severe hypokalaemia and upright PRA levels >5mmol/l in more than 90% of cases, while hypocalciuria was present in only 83% of cases and hypomagnesaemia in 50% of cases. A non-negligible frequency of cardiac symptoms was found, in contrast to the patients with only one heterozygous mutation who showed a milder phenotype. Surprisingly, mild hypertension occurred over time in some cases with two mutations, indicating that GS patients could develop hypertension when other risk factors are present. The benefit/risk ratio of treatment, especially with regard to renal function.
is not yet well known and should prompt prospective studies in this rare disease.

**Declaration of interest**

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

**Funding**

This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

**Acknowledgements**

The authors would like to extend their deep gratitude to Dr Vargas-Pousou and Prof X Jeunemaitre for genetic studies and the discussion on patient results.

**References**


Phenotype–genotype correlations in Gitelman syndrome


Received 10 March 2011
Revised version received 1 July 2011
Accepted 13 July 2011