Case Report

Gitelman syndrome: genetic and expression analysis of the thiazide-sensitive sodium-chloride transporter in blood cells

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Abstract
Gitelman syndrome is caused by mutations of the SLC12A3 gene, which encodes the thiazide-sensitive NaCl transporter NCCT. Although several mutations causing Gitelman syndrome have been described, their molecular consequences have been rarely studied. We report a patient with Gitelman syndrome due to a mutation in the GT donor splicing site of intron 9. The analysis of RNA from peripheral blood cells showed a complete deletion of exon 9. This case report confirms the feasibility of using readily accessible blood cells to study the expression of the SLC12A3 gene, a procedure that may facilitate further studies of the functional genomics of Gitelman syndrome.

Keywords: gene expression; Gitelman syndrome; hypokalaemia

Introduction
Gitelman syndrome (GS, OMIM 263800) is an autosomal recessive disorder characterized by hypokalaemia, hypomagnesaemia, metabolic alkalosis, renal potassium and magnesium wasting, hypocalciuria and normal blood pressure. Most cases are due to mutations in the SLC12A3 gene, encoding the thiazide-sensitive sodium chloride transporter NCCT of the distal nephron [1,2]. The absence of a functional NCCT leads to decreased sodium and chloride reabsorption in the distal convoluted tubule and increased solute delivery to the collecting duct. This causes volume contraction, activation of the renin–angiotensin–aldosterone system, and increased reabsorption of sodium and secretion of potassium and hydrogen ions in the collecting duct.

More than 100 different mutations have been described in patients with GS. It has been suggested that most of them are inactivating mutations that impair gene transcription or translation. However, as a renal biopsy is not usually performed in these patients, only a few expression studies have been reported. Therefore, we decided to explore the feasibility of using peripheral blood mononuclear cells (PBMC) as an accessible cell source to study DNA mutations and their consequences on RNA expression in a patient with GS.

Case report
A 27-year-old man was sent to the outpatient clinic because of hypokalaemia, initially found during a routine analysis. Serum potassium was 2.2–2.8 mEq/l (normal 3.5–5) in several analyses performed over a 3 month period. He was asymptomatic, but reported some craving for salty foods, including pickles. He did not take any therapy. Blood pressure was 100/60 mm Hg; the physical examination was normal. Other common blood tests were normal.

The patient was of Roma (Gypsy) origin. He had four siblings; two of them had normal serum potassium, whereas in the other two potassium was decreased (2.9 and 3 mEq/l, respectively). Their parents were first cousins; both had normal serum potassium. A cousin of his father died of diabetes and hypokalaemia of unknown cause. There were no other family members affected.

Genetic analyses

Methods
Genomic DNA analysis. Genomic DNA was isolated from PBMC by using the Qiagen kit, according to the manufacturer’s instructions. Exon 9 and intron 9 were amplified as described by Coto et al. [3], who found a frequent mutation in that region among Gypsy patients.
with GS. Forward primer was CTCTCTCCCTCCC TCCTTCAG; reverse primer was CTGGCCTGGGC CGGCCAGTT. Two hundred nanograms of DNA were subjected to 40 PCR cycles (annealing 65°C). PCR products were digested with the restriction enzyme HpaI, separated by electrophoresis in a 3% agarose gel and stained with ethidium bromide. The reverse primer contained a mismatch that introduced a site for HpaI when there was a G→T mutation in the first position of intron 9.

RNA expression analysis. Total RNA was also isolated from PBMC. The buffy coat was obtained by centrifugation and RNA was isolated by using Trizol (Invitrogen). Then it was reverse-transcribed onto cDNA by using random hexamers and the Superscript III kit (Invitrogen). cDNA was amplified by PCR with a forward primer located in exon 7 (G1), and reverse primers complementary to exons 9 (G2) or 11 (G3), designed with GeneFisher software (http://bibiserv.techfak.uni-bielefeld.de/genefisher). Primer sequences were: G1, CCTCCAAAGGCTTCTTGCTA; G2, GCTGAGATGGCCAGGTAGGA; and G3, GAAGCCTGACACCATGCTCA. After 40 cycles of amplification (annealing at 60°C), PCR products were separated in 2% agarose. In addition, both strands of the PCR product obtained with the primer pair G1+G3 were sequenced with a dye-terminator method as previously reported [4].

Results

Restriction fragment analysis of genomic DNA after digestion with HpaI revealed that the patient was homozygous for the G→T mutation in the first position of intron 9. We also had the opportunity to study the patient’s mother, who was heterozygous (Figure 1).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Normal range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatinine, mg/dl</td>
<td>0.9</td>
<td>0.5–1.3</td>
</tr>
<tr>
<td>Urea, mg/dl</td>
<td>32</td>
<td>10–50</td>
</tr>
<tr>
<td>Sodium, mEq/l</td>
<td>139</td>
<td>135–145</td>
</tr>
<tr>
<td>Potassium, mEq/l</td>
<td>2.4</td>
<td>3.5–5</td>
</tr>
<tr>
<td>Chloride, mEq/l</td>
<td>96</td>
<td>95–105</td>
</tr>
<tr>
<td>Ionized calcium, mmol/l</td>
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<td>1.15–1.35</td>
</tr>
<tr>
<td>Magnesium, mg/dl</td>
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<td>1.6–2.4</td>
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<tr>
<td>Bicarbonate, mEq/l</td>
<td>27</td>
<td>24–26</td>
</tr>
<tr>
<td>pH</td>
<td>7.42</td>
<td>7.35–7.45</td>
</tr>
<tr>
<td>PTH, pg/ml</td>
<td>11</td>
<td>&lt;55</td>
</tr>
<tr>
<td>Renin, pg/ml</td>
<td>492</td>
<td>2–22</td>
</tr>
<tr>
<td>Aldosterone, pg/ml</td>
<td>213</td>
<td>10–310</td>
</tr>
<tr>
<td>Urine</td>
<td></td>
<td></td>
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<tr>
<td>Sodium, mEq/24 h</td>
<td>482</td>
<td>100–250</td>
</tr>
<tr>
<td>Potassium, mEq/24 h</td>
<td>136</td>
<td>25–100</td>
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<tr>
<td>Calcium, mg/24 h</td>
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<tr>
<td>Calcium/creatinine, mg/mg</td>
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<td>0.03–0.25</td>
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<tr>
<td>Chloride, mEq/24 h</td>
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<td>100–250</td>
</tr>
<tr>
<td>Magnesium, mg/24 h</td>
<td>147</td>
<td>70–120</td>
</tr>
</tbody>
</table>

RNA analysis. After several attempts we were not able to amplify the patient’s cDNA with a pair of primers (G1+G2) recognizing exons 7 and 9. However, cDNA from control subjects was consistently amplified with those primers (Figure 2). On the other hand, when cDNA from the patient with GS was used, the PCR product obtained by using primers G1+G3, located on exons 7 and 11, was about 80 bp shorter than that obtained with control cDNA (Figure 2). Those results suggested that the RNA expressed by cells from the GS patient lacked exon 9, which has 85 bp. The lack of exon 9 in mature RNA was further confirmed by direct

![Fig. 1. Electrophoresis of genomic DNA after PCR and HpaI digestion. Molecular weight markers are run in lane 1. Control sample of a healthy subject shows a single 127 bp band. A patient with Gitelman syndrome (GS) is homozygous for a mutation in intron 9, which introduces a HpaI restriction site, thus appearing as a single 106 bp band. Both bands are seen after amplification of his mother’s DNA, who was heterozygous.](https://academic.oup.com/ndt/article-abstract/21/1/217/1818924)

![Fig. 2. PCR products of cDNA from a patient with Gitelman syndrome (GS) and control subjects. Upper panel: PCR using primers G1+G2, located on exons 7 and 9. With control cDNA, the region is successfully amplified, whereas no band is seen when cDNA from the patient with GS is used (results from two separate RT-PCR). Lower panel: PCR using primers G1+G3, located on exons 7 and 11. The amplicon is about 80 bp shorter in the patient with GS than in the control.](https://academic.oup.com/ndt/article-abstract/21/1/217/1818924)
sequencing of both strands of the cDNA obtained by RT-PCR (Figures 3 and 4).

Comments

This patient had the typical characteristics of GS, including hypokalaemia, hypocalciuria, hypomagnesaemia and increased renin levels. Normal aldosterone levels are not unusual in GS, despite high renin, as hypokalaemia may have a direct inhibitory affect on the adrenal glands [5].

Both GS and Bartter syndrome (BS) are characterized by renal wasting of potassium [6,7]. GS is due to mutations in the NCCT gene, expressed in the distal convoluted tubule [1,2]. However, BS is due to mutations of ion transporters in the thick ascending limb of Henle (including NKCC2, ROMK and CLCNKB). Typical cases can be readily distinguished. Usually, GS is less severe than BS. Also, whereas patients with BS typically show hypercalciuria and increased prostaglandin excretion, patients with GS have hypocalciuria and prostaglandin secretion is not so clearly augmented. Nevertheless, clinical diagnosis may be difficult in some cases. First, some patients with GS do not show hypocalciuria, especially in cases of magnesium supplementation, low muscle mass or volume expansion [5]. Second, tubular abnormalities similar to GS may be caused by thiazides, cisplatin or Sjogren's syndrome [8,9]. Third, patients with clinical features resembling BS but with hypocalciuria have been described [10,11]. Therefore, genetic analysis may be important to characterize the underlying disorder in normotensive patients with renal potassium wasting [11].

The patient reported here was homozygous for the G→T mutation in intron 9, confirming that this mutation is common among Gypsy patients with GS, as reported by Coto et al. [3]. Since a renal biopsy is rarely performed in patients with GS, there are few studies about the transcriptional consequences of gene mutations. However, although the NCCT is typically expressed in the distal convoluted tubuli, there is increasing evidence for gene expression in extra-renal cells, including some of the digestive system [12,13]. Abuladze also reported NCCT expression in PBMC [14]. Therefore, we decided to explore the feasibility of using this accessible cell population to study gene transcripts.

SLC12A3, the gene encoding NCCT, is located in chromosome 16 and comprises 26 exons. NCCT is a 1021-aminoacid protein with 12 predicted transmembrane domains. The majority of mutations of SLC12A3 have been predicted to result in aminoacid substitutions [15]. Mutations causing the deletion of full exons, as it happened in the present case, seem to be infrequent. Two other cases were recently reported by Iida et al. [16] and Maki et al. [17]. In the latter report, blood cells were also used as a source of RNA. Exon 9, the region lost in our patient, codes for aminoacids 366–395, which correspond to a part of an intracellular loop and the majority of the seventh transmembrane region (Swissprot database, P55017: http://au.expasy.org).

Fig. 3. Fragment of the sequence of the sense strand of cDNA from patient with Gitelman syndrome. Exon 8 is followed by exon 10, skipping exon 9. The same result was found when the antisense strand was sequenced.

Fig. 4. (Top) Schematic diagram of the SLC12A3 gene, with some exons (numbered boxes) and introns (lines). The localization of primers used in the amplification of genomic DNA (thick arrows) and cDNA (thin arrows) are shown. The asterisk marks the mutated site in intron 9. (Bottom) Diagram of normally assembled exons after RNA splicing and RNA with exon 9 skipping in patient with Gitelman syndrome.
Although we were not able to perform further protein studies, omitting this whole intermediate exon probably results in a nonfunctional protein. Thus, although we did not search for mutations in other regions of the gene, such a defect in a homozygous state is likely to account for the Gitelman genotype. In fact, this type of mutation was the only gene sequence aberration found by Coto in his series of GS patients, whereas it was not found in 200 healthy Spanish individuals [3].

In summary, we report a new patient with GS due to a mutation in the NCCT gene. The mutation abolishes a consensus GT donor splicing site and results in defective RNA lacking exon 9. Most important, this study confirms the feasibility of using peripheral blood cells to analyse the expression of this gene, which may facilitate further studies of the functional genomics of GS.

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Conflict of interest statement. None declared.

References


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