Three Cases of Gitelman’s Syndrome Possibly Caused by Different Mutations in the Thiazide-Sensitive Na-Cl Cotransporter

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Three adult Japanese cases of Gitelman’s syndrome were characterized by secondary aldosteronism, hypokalemic alkalosis, hypomagnesemia, and hypocalciuria. Two were revealed to be familial cases. A mutation in the thiazide-sensitive Na-Cl cotransporter gene, which had already been confirmed in one family (Takeuchi et al. J Clin Endocrinol Metab 81: 4496, 1996), was not detected in the other two cases. These observations may possibly support the previous report (Simon et al. Nature Genet 12: 24, 1996) that Gitelman’s syndrome is caused by a variety of mutations in the thiazide-sensitive Na-Cl cotransporter.

Key words: Bartter’s syndrome, secondary aldosteronism, missense mutation, restriction fragment length polymorphism

Introduction

“Bartter’s syndrome” has recently been divided into two subsets, true Bartter’s syndrome and Gitelman’s syndrome (1, 2). Bartter’s syndrome is characterized by hypokalemic alkalosis, and secondary aldosteronism without hypertension, and with blunted pressor responsiveness to angiotensin (Ang) II (3). True Bartter’s syndrome also refers to patients with normal or hypercalciuria, and typically normal magnesium levels. Gitelman’s syndrome (4), however, refers to patients with hypocalciuria and hypomagnesemia. Genetic analyses have shown that Bartter’s syndrome is due to mutations in Na-K-2Cl cotransporter NKCC2 (5) or K* channel ROMK (6), and that Gitelman’s syndrome is attributable to mutations in thiazide-sensitive Na-Cl cotransporter (TSC) (7). We have recently reported the close association of a mutation in TSC with familial Gitelman’s syndrome (8), which further supports the report by Simon et al (7) that a mutation in TSC gene (probably leading to inhibition of the transporter activity) causes Gitelman’s syndrome. Here we report three cases of Gitelman’s syndrome, and suggest a possible variety of mutations in TSC in Gitelman’s syndrome.

Case Reports

Case 1

A 45-year-old Japanese female had noticed mild periodic paralysis and tetany since the age of 28. In March 1994, she felt discomfort in her lower abdomen, and a tumor was felt palpable in the region. Uterine myoma was identified by a gynecologist, and she was admitted to her neighboring hospital for resection. Laboratory examination disclosed her hypokalemia (2.4 mmol/L), metabolic alkalosis, secondary aldosteronism and normotension. She had no history of chronic diarrhea, vomiting, or diuretics abuse. Bartter’s syndrome was suspected, and the operation was postponed. For the therapy, either potassium 32 mmol/day, non-steroidal anti-inflammatory drug (diclofenac sodium 100 mg/day), or mineralocorticoid receptor antagonist (spironolactone 50 mg/day) was administered. Hypokalemia was however not completely corrected. In February 1995, the patient was referred to our department in Tohoku University Hospital for further investigation of hypokalemia and resection of uterine myoma.

Her parents and grandparents are consanguineous. Individuals in her family had a tendency towards hypotension. Her blood pressure was 106/70 mmHg; she was 155 cm tall and weighed 65 kg. Laboratory data are listed in Table 1. Pressor response to...
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Table 1. Representative Laboratory Data in Three Cases of Gitelman’s Syndrome

<table>
<thead>
<tr>
<th></th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
<th>Normal range</th>
</tr>
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<tbody>
<tr>
<td>Serum electrolyte levels</td>
<td></td>
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<tr>
<td>Na</td>
<td>143</td>
<td>140</td>
<td>135</td>
<td>135–147 mmol</td>
</tr>
<tr>
<td>K</td>
<td>2.6</td>
<td>2.7</td>
<td>3.4</td>
<td>3.4–5.0 mmol</td>
</tr>
<tr>
<td>Cl</td>
<td>101</td>
<td>105</td>
<td>96</td>
<td>98–108 mmol</td>
</tr>
<tr>
<td>Ca</td>
<td>8.9</td>
<td>9.2</td>
<td>9.1</td>
<td>8.6–10.5 mg/dl</td>
</tr>
<tr>
<td>Mg</td>
<td>1.8</td>
<td>1.1</td>
<td>1.3</td>
<td>1.8–2.4 mg/dl</td>
</tr>
<tr>
<td>Urinary electrolyte excretions</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na</td>
<td>181</td>
<td>112</td>
<td>92</td>
<td></td>
</tr>
<tr>
<td>K</td>
<td>59</td>
<td>61</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td>Cl</td>
<td>170</td>
<td>117</td>
<td>108</td>
<td></td>
</tr>
<tr>
<td>Ca</td>
<td>2.3</td>
<td>16</td>
<td>6.0</td>
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<tr>
<td>Plasma renin activity</td>
<td>83.6</td>
<td>48.6</td>
<td>78.4</td>
<td>&gt;50 mg/day</td>
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<tr>
<td>Plasma aldosterone concentration</td>
<td>26.9</td>
<td>41.4</td>
<td>25.7</td>
<td>2–12 ng/dl</td>
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<tr>
<td>Serum pH</td>
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<td>7.48</td>
<td>7.45</td>
<td>7.35–7.45</td>
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<tr>
<td>Serum bicarbonate levels</td>
<td>32.5</td>
<td>30.3</td>
<td>28.5</td>
<td>21–25 mmol/l</td>
</tr>
</tbody>
</table>

Figure 1. Pedigrees of kindred of the three patients with Gitelman’s syndrome. Probands are indicated by arrows.

Ang II (9, 10) was normal, and hypomagnesemia and marked hypocalciuria were confirmed. Based on these clinical findings, Gitelman’s syndrome was diagnosed. Familial relationships are shown in Fig. 1.

Case 2
A 51-year-old Japanese female had suffered from diabetes mellitus since the age of 40. When diabetes mellitus was diagnosed, hypokalemia (2.4 mmol/l) was also observed, and she was admitted to our department. By intense examinations, hypotension, hypokalemia, metabolic alkalosis, and hyperreninemic hyperaldosteronism were identified. Pressor response to Ang II was normal. Hypomagnesemia (1.6–1.8 mg/dl) was also observed. A variant of Bartter’s syndrome was diagnosed because of the lack of blunted pressor response to Ang II. Potassium (32 mmol/day) has been administered, and she has been followed up in the outpatient clinic. Hypokalemia (2.4–3.1 mmol/l) and hypomagnesemia (1.6–1.8 mg/dl) however persisted. She had no history of chronic diarrhea, vomiting, and diuretics abuse. Her height was 146.7 cm, and weight, 47.4 kg. Blood pressure levels were less than 110/70 mmHg.

In February 1996, urinary calcium levels were determined, and marked hypocalciuria was confirmed. Gitelman’s syndrome was therefore diagnosed. Laboratory data during the admission are listed in Table 1. Pressor response to Ang II was normal. Family relationships are shown in Fig. 1. Her father suffered from cerebral infarction in August 1994. During his admission in a hospital, persistent hypokalemia (2.4–2.7 mmol/l), hyponatremia (124–134 mmol/l), hypomagnesemia (1.6 mg/dl), and hypocalciuria (12 mg/day) were observed. A diagnosis of Gitelman’s syndrome was also likely for him.
Case 3
A 66-year-old Japanese female had suffered from recurrent pyelonephritis. She was first admitted to a neighboring hospital in 1983 because of pyelonephritis. Mild muscle paralysis was felt, and laboratory examination disclosed low serum potassium levels (2.0-3.0 mmol/l). Although pyelonephritis improved, hypokalemia and secondary aldosteronism were persistently observed. Potassium 32 mmol/day and spironolactone 50 mg/day were then administered, and she was followed up in the outpatient clinic. She had no history of chronic diarrhea, vomiting, and diuretics abuse. Blood pressure levels were within the normal range. In July 1995, hypokalemia worsened (serum potassium, 1.9 mmol/l), and she was referred to our department for further examinations. Representative laboratory data during admission are listed in Table 1. Pressor response to AngII was normal. Based on these findings, Gitelman’s syndrome was diagnosed. Familial relationships are shown in Fig. 1.

DNA restriction fragment length polymorphism
We have reported (8) a mutation in TSC gene (T to C change at 1,868 nucleic acid position) in the Gitelman’s syndrome kindred including Case 1, which causes substitution of leucine for proline at the 623 amino acid position, and creates Nci I restriction site in the exon 15. We performed Nci I digestion of the DNA fragment (~250 base pairs) of PCR-amplified exon 15 of TSC gene based on the previously reported methods (8) in order to determine if the same mutation as found in Case 1 would be identified in the other cases with Gitelman’s syndrome (Case 2 and 3). Genome DNA was obtained and analyzed after informed consent was obtained. Figure 2 shows that Case 1a or 1b is homozygous or heterozygous for the Nci I digestion fragment length polymorphism, respectively, as shown previously (8). This mutation was not detected in Cases 2 and 3.

Figure 2. Nci I digestion of exon 15 of thiazide-sensitive Na-Cl cotransporter gene in cases of Gitelman’s syndrome. ~250 base pairs of exon 15 is amplified, and the fragment is digested with Nci I when the mutation (thymine to cytosine) at 1,868 base position in exon 15 is occurred (8). Case 1a or 1b is a homozygote or heterozygote of the previously reported mutation, respectively. Cases 2 and 3 lack this mutation.

Discussion
Gitelman’s syndrome was diagnosed in three patients with hypokalemia, secondary aldosteronism, and normotension in conjunction with hypomagnesemia and hypocalciuria. Although Gitelman’s syndrome is similar to Bartter’s syndrome, both are distinguished on the basis of urinary calcium excretion because true Bartter’s syndrome shows a tendency towards hypercalciuria whereas Gitelman’s syndrome shows marked hypocalciuria. Mild secondary aldosteronism was observed in the patients, and pressor response to Ang II was not impaired in the three subjects. On the other hand, true Bartter’s syndrome is known to show the blunted pressor response. In the three patients, secondary aldosteronism was not persistent, and the plasma renin activity was normal in the experimental periods. In Gitelman’s syndrome, secondary reninism due to salt wasting is not so severe, and therefore desensitization of Ang II receptor may not necessarily occur. Congenital disorder of Gitelman’s syndrome was first diagnosed in adults in these patients. Since the clinical findings of Gitelman’s syndrome are so mild compared to those of true Bartter’s syndrome, Gitelman’s abnormalities may possibly be discovered by chance in adults such as in the present cases, in which the disorder was unmasked in the course of examinations for other diseases such as uterine myoma, diabetes mellitus, and pyelonephritis. Early and careful determinations of urinary calcium excretion as well as plasma magnesium levels are recommended for the diagnosis of Gitelman’s syndrome in normotensive patients of secondary aldosteronism with hypokalemic alkalosis (1).

Sodium metabolism at the renal distal convoluted tubules (DCT) is known to be mediated by a Na-Cl cotransporter, which stimulates absorption of NaCl, and is inhibited by thiazide diuretics (11, 12). Actually, the thiazide-sensitive Na-Cl cotransporter has been shown to be localized to DCT (13). In the administration of thiazide diuretics, hypocalciuria as well as hypokalemia is frequently observed (14). Hypokalemia may likely be due to an increase in the sodium delivery to distal collecting ducts, where potassium secretion occurs in exchange for sodium absorption. Hypocalciuria may possibly be due to a decrease in the cytosolic chloride level by inactivation of the Na-Cl cotransporter in conjunction with chloride channel activity, which activates the calcium channel causing calcium reabsorption at DCT (15). Inactivation of this transporter caused by any mutation may therefore lead to the hypokalemia and hypocalciuria observed in Gitelman’s syndrome similar to those observed with administration of thiazide diuretics. Previously, Simon et al showed by linkage analysis with genetic markers that the locus responsible for Gitelman’s syndrome encodes TSC, and a wide variety of mutations in TSC gene were identified in patients with Gitelman’s syndrome (7). We have also recently reported that a missense mutation in TSC at 623 amino acid position is associated with the clinical findings (especially, hypokalemia, and hypocalciuria) observed in the kindred including Case 1 (8). However, the mutation detected by Nci I digestion of the exon 15 DNA fragment of TSC gene...
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was not identified in either Case 2 or 3. The results suggest that the Gitelman’s syndrome is not only attributed to the same mutation as identified in Case 1, and may possibly further support the report by Simon et al (7) that Gitelman’s syndrome is possibly derived by a wide variety of mutations in TSC gene, although abnormalities in the other functional proteins in Cases 2 and 3 could not be excluded.

Genetic heterogeneity in Gitelman’s syndrome has recently been reported: one type is that with autosomal recessive inheritance presenting frequent tetanic episodes and lower plasma potassium and magnesium levels, and the other is that with autosomal dominant inheritance presenting mild muscular weakness and normal potassium levels (16). Although it is not determined whether these two types of Gitelman’s syndrome are caused by the same genetic disorder, the clinical findings observed in the present cases are compatible with the autosomal recessive inheritance type of Gitelman’s syndrome, as shown previously (8). Considering the family relationship of Case 2, her father, who has suffered from hyponatremia, hypokalemia and cerebral infarction probably due to salt wasting, is possibly a homozygote for a mutation.

Gitelman’s syndrome shows normotension or hypotension probably due to a defect in the Na-Cl cotransporter. Bartter’s syndrome is also characterized by normotension/hypotension, hypokalemic alkalosis, and salt wasting, etc which has recently been shown to be due to mutations of Na-K-2Cl cotransporter NKCC2 (5) or potassium channel ROMK (6) by inhibiting these activities. In contrast, mutations of the epithelial Na channel at the distal tubules have been identified in patients with Liddle’s syndrome (17), and the mutations have been suggested to cause constitutive stimulation of this channel leading to excessive sodium absorption and thereby excessive potassium secretion (18) resulting in Liddle’s syndrome that shows clinical findings such as hypertension, hypokalemic alkalosis, and hyporeninemic hypoaldosteronism. Thus, these channels and Na-Cl transporters located in the distal tubules are suggested to be important for blood pressure regulation in terms of renal sodium, chloride, and potassium homeostasis.

Acknowledgements: The present study was supported in part by Grants-in-Aid (Nos. 09470236 and 09877216) from the Ministry of Education, Science, and Culture, Japan, and a grant for the Promotion of High Grade Medical Science, Tohoku University School of Medicine. We wish to thank Drs. S. Kure and K. Narisawa, Department of Biochemical Genetics, Tohoku University, for technical assistance and discussion in the mutation study.

References