Inheritance of an Autosomal Recessive Disorder, Gitelman’s Syndrome, Across Two Generations in One Family

Hiroki Yagi, Kensei Yahata, Takeshi Usui, Chinatsu Hasegawa, Koichi Seta and Akira Sugawara

Abstract

Gitelman’s syndrome (GS) is an autosomal recessive disorder; it is rarely inherited over several generations. A 16-year-old boy showed hypokalemia and hypocalciuria. Clinically, he was diagnosed as GS because of diuretic responsiveness to furosemide but not thiazide. Genetic testing disclosed he was a compound heterozygote (T180K/V677M) for the SLC12A3 gene. Unexpectedly, the patient’s father also showed hypokalemia and hypocalciuria. The genetic analysis showed he had an L849H mutation in addition to T180K. The present pedigree showed an extremely rare case. Diuretic tests are useful diagnostic methods, and genetic testing is necessary for precise evaluation of complicated cases as in this family.

Key words: Gitelman’s syndrome, thiazide, diuretic test, two generations


Introduction

Gitelman’s syndrome (GS) is an autosomal recessive renal tubular disorder characterized by hypokalemia and metabolic alkalosis (1). Because the phenotype of GS exhibits electrolyte abnormalities similar to those observed in patients taking thiazide diuretics, it has been suggested that the loss of function of the distal thiazide-sensitive sodium-chloride (NaCl) cotransporter is responsible for GS (2-5). This hypothesis has been substantiated by the demonstration that GS is caused by mutations in the SLC12A3 gene, which encodes the thiazide-sensitive NaCl cotransporter (NCCT; also known as TSC) (6). To date, numerous mutations in the SLC12A3 gene have been reported, including Japanese GS patients (7-11). While the SLC12A3 gene is the only gene known to be involved in GS, at least five genes are involved in Bartter’s syndrome (BS) (12-18), which indicates the genetic heterogeneity of this syndrome.

As described above, the mode of inheritance of GS is autosomal recessive. Therefore, it is extremely rare for GS to occur over two generations within a family. Here we describe a family in which the father and son had GS, but both had different mutations.

Case Report

A 16-year-old boy was referred for evaluation of hypokalemia. The patient had noticed fatigue and muscle weakness on getting up in the morning. The patient’s muscle weakness worsened after exercise. The patient was 170.8 cm tall and weighted 55 kg. The patient’s blood pressure was 112/65 mmHg and pulse rate was 69 beats/minute. The serum creatinine was 0.9 mg/dL. The patient showed hypocalciuria and normomagnesemia (Fig. 1). Arterial blood gas was pH 7.463, Pco2 48.9 mmHg, Po2 108.5 mmHg, and HCO3- 34.2 mmol/L. Plasma renin activity and the plasma aldosterone concentration were both elevated (Fig. 1). The patient’s father also showed hypokalemia and hypocalciuria, but the patient’s mother showed normokalemia and normocalciuria (Fig. 1), and the patient’s sister showed normokalemia (Fig. 1). The patient’s parents are not consanguineous.

Diuretic Tests

Furosemide and thiazide tests were performed according to the protocols described previously, with some modifica-
Inform consent for subsequent genetic analysis was obtained from the patient and the patient’s parents. Genomic DNAs were extracted from the peripheral blood obtained from the patient and the patient’s parents using a DNA Blood Kit (Qiagen, Hilden, Germany). All 26 exons of the SLC12A3 gene were amplified by polymerase chain reaction (PCR), using primers described elsewhere (6). PCR was performed in a final volume of 25 μL containing 10 mM Tris-HCl pH8.3, 50 mM KCl, 1.5 mM MgCl2, 200 nM of each dNTP, 1 mM of each primer, 100 ng of genomic DNA, and 5 units of LA Taq polymerase (Takara, Shiga, Japan). Amplifications were performed for 30 cycles of 95°C for 30 seconds, 60°C for 30 seconds, and 72°C for 1 minute. The PCR products were purified using Montage PCR Centrifugal Filter Devices (Millipore, Billerica, MA, USA) and then sequenced using a BigDye Terminator Cycle Sequencing Kit and an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

In the proband, a heterozygous missense mutation (T180 K ACG>AAG) was observed in exon 4 of the SLC12A3 gene (Fig. 2). In addition, the proband had a heterozygous missense mutation (V677M GTG>ATG) in exon 16. As shown in Fig. 2, the father harbors the former mutation, and the mother harbors the latter mutation. Therefore, the proband was recognized as a compound heterozygote (T180K/ V677M). As the father also showed hypokalemia and hypocalciuria, all exons of the SLC12A3 gene were analyzed for the father. In addition to the T180K mutation, the father had an L849H (CTC>CAC) mutation, which was not observed in the proband (Fig. 2). Therefore, the father was also recognized as a compound heterozygote (T180K/ L849M) and was also genetically diagnosed as having GS.

**Discussion**

This patient visited our hospital because of fatigue and muscle weakness during exercise, and was found to be hypokalemic. Because urinary potassium excretion was increased despite hypokalemia, renal potassium loss was suspected. Plasma renin activity and the plasma aldosterone concentration were both elevated. Metabolic alkalosis was found and the patient’s blood pressure was within the normal range. These findings suggested BS or GS. Hypomagnesemia and hypocalciuria are found in most cases of GS. However, some cases with mutations in the NCCT do not show hypomagnesemia or hypocalciuria (19, 20). The present patient had hypocalciuria, but not hypomagnesemia. Hypercalciumia is usually found in BS, but many cases of BS type III do not show increased calcium excretion (21-23). Meanwhile, some cases of BS type III have been reported to show hypocalciuria or hypomagnesemia (21-23). As these reports indicated that it is difficult to distinguish GS and BS using only basal data, renal clearance studies are necessary for further diagnosis.
Figure 2. Nucleotide sequences of codon 180 in exon 4 (left), codon 677 in exon 16 (middle), and codon 849 in exon 22 (right). The sequences of the proband (upper panel), the proband’s father (middle panel), and the proband’s mother (lower panel) are shown. At codon 180, a heterozygous T180K (ACG>AAG) missense mutation was detected in the proband and the father. On the other hand, at codon 677, a heterozygous V677M (GTG>ATG) missense mutation was detected in the proband and the mother. In addition to these two distinct mutations, the father also showed a heterozygous L849H (CTC>CAC) missense mutation at codon 849. The mutated nucleotides are indicated by arrows.

Recently, Colussi et al. reported that a thiazide test enables the differentiation of GS from BS or pseudo BS (24). The present patient responded well to furosemide, but not to thiazide, which suggested the diagnosis of GS before genetic testing was performed. These features indicate that clearance studies might be useful in such cases. However, a recent report described BS type III patients who showed no response to thiazide and a normal response to furosemide (25). The authors suggested that the position of the mutations within SLC12A3 gene may explain these contradictory results. In some cases, genetic testing may be needed to distinguish between BS type III and GS.

Despite the autosomal recessive pattern of transmission of GS, the father also had hypokalemia. Genetic testing revealed that the father was also a compound heterozygote for the SLC12A3 gene, indicating that the father also had GS. When there are consanguineous marriages in the family, homozygous GS can occur over two generations (7). However, to our knowledge, only two other families have been reported with compound heterozygous GS occurring over two generations (6, 11), although one family was not sampled for genetic testing of the parents (6).

What is the probability that an unrelated spouse of a GS subject is heterozygous for an independent mutation in the SLC12A3 gene? The estimated prevalence of the SLC12A3 gene mutations in unrelated members of the Framingham Heart Study was 0.48%, convergent to the estimated population frequency of GS, of approximately 1 per 40,000 (26). Half of the above couple’s offspring would be affected.

All three mutations identified in this family have been re-

### Table 1. Result of Diuretic Tests

<table>
<thead>
<tr>
<th></th>
<th>FENa</th>
<th>FENa</th>
<th>ΔFENa</th>
<th>FECi</th>
<th>FECi</th>
<th>ΔFECi</th>
<th>FEK</th>
<th>FEK</th>
<th>ΔFEK</th>
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<td>11.7</td>
<td>11.2</td>
<td>0.82</td>
<td>16.9</td>
<td>16.1</td>
<td>32.2</td>
<td>66.9</td>
<td>34.7</td>
</tr>
<tr>
<td><strong>Thiazide Test</strong></td>
<td>0.21</td>
<td>0.22</td>
<td>0.01</td>
<td>0.41</td>
<td>0.33</td>
<td>0.08</td>
<td>22.2</td>
<td>23.4</td>
<td>1.20</td>
</tr>
</tbody>
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‘basal’ is the mean of two basal data. ‘peak’ indicates peak excretion after administration of diuretics. ‘Δ’ indicates the difference between peak and basal. ‘FE’ is an abbreviation for ‘fractional excretion’.

Table 1. Result of Diuretic Tests
ported before (9-11, 27), and the T180K and L849H mutations have been reported in Japanese patients (9-11). Overall, T180K mutations have been reported in three unrelated families, and the L849H mutation in five unrelated families. Therefore, it is possible that the T180K and L849H mutations are common mutations in Japanese patients with GS.

It is important to consider how the function of the gene product is affected by a particular mutation. Using a mammalian cell expression system, the L849H mutation, but not T180K mutation, was confirmed to be a loss-of-function mutation that appears to be responsible for GS (28). We must be cautious in concluding that T180K mutation is responsible for GS; however, the phenotypic characteristics presented here suggest that T180K mutation is indeed responsible for GS in the present family.

In conclusion, we have reported a rare family in which mutations causing GS occurred in two generations. Renal clearance studies are useful for distinguishing GS from BS. However, genetic testing is also needed for the precise evaluation of complicated cases as in the present family.

The authors state that they have no Conflict of Interest (COI).

References