


A novel mutation of *SLC12A3* gene causing Gitelman syndrome

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Abstract

A 48-year-old patient with a history of diabetes mellitus, presented to a surgical ward with abdominal pain. She was found to have hypokalemia. Her younger sister had passed away due to sudden cardiac death at the age of 25 years. Further evaluation revealed an elevated trans-tubular potassium gradient suggestive of renal potassium loss, normal blood pressure, hypomagnesemia, hypocalciuria, and alkalosis. Moreover, there was evidence of secondary hyperaldosteronism. Genetic studies revealed two heterozygous mutations of the *SLC12A3* gene, including a novel mutation which has not been reported before anywhere in the world. She was treated with intravenous potassium supplementation and was later converted to oral potassium and oral magnesium supplementation with spironolactone. Her potassium and magnesium levels normalized and glycaemic control also improved. Hypokalemia and hypomagnesemia found in Gitelman syndrome may be associated with insulin resistance and correction of electrolytes can lead to better glycaemic control.

Keywords

Gitelman syndrome, hypokalemia

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Introduction

Hypokalemia is a common problem encountered in day-to-day clinical practice. While it is mild and asymptomatic in the majority of patients, it can lead to life-threatening arrhythmias when severe. A thorough history and examination coupled with focused and systematic evaluation can unveil the diagnosis in most instances. Gitelman syndrome is an important cause of hypokalemia.

Gitelman syndrome is characterized by hypokalemic alkalosis, hypomagnesemia, and low urinary calcium excretion.¹ It has a population prevalence of approximately 1:40,000, and the prevalence of heterozygotes in Caucasian populations is estimated to be 1%. Therefore, it is one of the most common inherited renal tubular disorders.² Prevalence may be higher in Asians.³ Most of the patients present in adolescence or adulthood.

Gitelman syndrome has an autosomal recessive inheritance pattern and it is due to a defect in Solute Carrier Family 12 Member 3 (*SLC12A3*) gene, which encodes the renal thiazide-sensitive sodium chloride co-transporter (NCC) that

is expressed in the apical membrane of cells in the first part of the distal convoluted tubule (DCT). NaCl co-transporter (NCC) is a polypeptide of 1021 amino acids, and it has 12 trans-membrane domains. More than 400 different mutations of *SLC12A3* gene have been reported to date. These mutations include missense-, nonsense-, frame-shift-, and splice-site mutations and are distributed throughout the whole protein.² Many new mutations of the *SLC12A3* gene have been reported recently.^{4–11} We are presenting a patient who was evaluated for hypokalemia and was found to have a novel mutation of the *SLC12A3* gene.

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Table 1. Baseline investigations of the patient.

Investigation	Value	Reference range
Serum potassium	2.4 mmol/L	3.5–5.1
Serum sodium	135 mmol/L	135–148
Serum creatinine	0.92 mg/dL	0.5–1.1
Arterial blood gas analysis		
pH	7.46	7.36–7.44
pCO ₂	45 mm Hg	35–45
HCO ₃ ⁻	32 mmol/L	22–26
Total albumin corrected calcium	9.2 mg/dL	8.2–10.3
Serum magnesium	0.9 mg/dL	1.6–2.6
Serum osmolality	287 mOsm/kgH ₂ O	285–295
Urine osmolality	385 mOsm/kgH ₂ O	
Urine spot sodium	114 mmol/L	
Urine spot potassium	15 mmol/L	
Trans-tubular potassium gradient (TTKG)	5	More than three suggests renal potassium wasting
Urine spot magnesium	2.8 mmol/L	
Urine spot magnesium/creatinine ratio	0.05 mol/mol	<0.16
24-h urinary calcium excretion	21.6 mg/24 h	100–300
Plasma aldosterone concentration (PAC)	15.6 ng/dL	2.21–35.3
Plasma direct renin concentration	212.2 µU/mL	4.4–46.1
Plasma renin activity (PRA)	17.68 ng/mL/h	
TSH	2.798 mIU/L	0.55–4.78
ft ₄	1.22 ng/dL	0.89–1.76
Anti-TPO antibody	64.25 IU/mL	<5.61
HbA1c	9.0%	<7%

TSH: thyroid stimulating hormone; ft₄: free thyroxine; TPO: thyroid peroxidase; HbA1c: haemoglobin A1C.

Case presentation

A 48-year-old female patient presented with right-sided lower abdominal pain. She did not have any recent history of loose stools or vomiting and she was not on any diuretics, insulin, beta agonists, or laxatives. She did not complain of alteration of bowel habits or urinary symptoms. She did not complain of recent onset weight gain, purple striae, or any other features of Cushing's syndrome. She was not a diagnosed patient with hypertension. There was no history of recurrent episodes of paralysis. She had been diagnosed to have type 2 diabetes 1 year back and was on oral hypoglycaemic agents (metformin 1 g twice daily) with poor control. Her younger sister had been investigated for hypokalemia and had passed away at the age of 25 years due to sudden cardiac death.

On examination, there were no signs of Cushing's syndrome and her blood pressure was 100/70 mmHg and pulse rate was 80 beats per minute. Her neurological examination was completely normal. Abdominal examination elicited mild tenderness in the right lower quadrant.

Her investigations showed evidence of hypokalemia (2.4 mmol/L) and hypomagnesemia (0.9 mg/dL) (Table 1). These reports were confirmed by repeating multiple times. Her electrocardiogram (ECG) showed evidence of hypokalemia such as U waves and flattening of T waves. She had a haemoglobin A1C (HbA1c) of 9% suggestive of

poor glycaemic control. She had normal thyroid-stimulating hormone (TSH) and free thyroxine (ft₄), but her anti-thyroid peroxidase (TPO) antibody level was elevated. Her ultrasound scan showed evidence of grade II fatty liver, but there was no nephrocalcinosis and the cause for abdominal pain was not ascertained. A contrast-enhanced CT scan of the abdomen and a colonoscopy was planned by the surgical team which did not reveal any new findings.

With the above background, the main diagnoses remained were diuretic abuse, Gitelman syndrome, and Bartter syndrome. Diuretic abuse was excluded by a thorough history. Low serum magnesium levels and low 24-h urinary calcium excretion favoured a diagnosis of Gitelman syndrome over Bartter syndrome.

Whole exome sequencing detected that the proband is compound heterozygous for the autosomal recessive Gitelman syndrome. The variant details are as shown in Table 2.

The second frameshift variant is a novel variant that has neither been reported in population frequency databases nor in the scientific literature.

Management

Liberal salt intake to counteract the effect of NCC inhibition along with lifelong potassium and magnesium supplementation remains the first-line treatment. Intravenous

Table 2. Variants of *SLC12A3* gene identified.

Gene	Variant	Zygoty	Variant classification
1. <i>SLC12A3</i>	c.911C>A[p.Thr304Lys] NM_000339.3 ENST00000438926 Exon 07	Heterozygous	Likely pathogenic
2. <i>SLC12A3</i>	c.2099delT[p.Leu700fs] ENST00000438926 Exon 17	Heterozygous	Likely pathogenic

supplementation of magnesium and potassium should be considered in those with very low levels of these electrolytes to reduce the risk of life-threatening cardiac arrhythmias. Potassium level of 3.0 mmol/L and magnesium level of 1.46 mg/dL (0.6 mmol/L) have been suggested as reasonable targets. Only those who remain hypokalemic despite supplementation should be considered for treatment with mineralocorticoid receptor agonists like spironolactone or eplerenone due to possible side effects.¹² An open-label randomized crossover study, which compared indomethacin, eplerenone, and amiloride, showed that all three drugs increased serum potassium by about 0.3 mmol/L.¹³ Indomethacin and other nonsteroidal anti-inflammatory drugs should be used with caution due to the risks of gastro intestinal and renal toxicity with long-term use.

Our patient was initially treated with intravenous potassium chloride 40 mmol daily over 4 h due to ECG changes to minimize the risk of life-threatening arrhythmias. She was also started on spironolactone at a dose of 50 mg daily, indomethacin at a dose of 50 mg thrice daily, and oral magnesium supplementation (magnesium oxide sachet 400 mg daily). Potassium supplementation was later converted to oral potassium chloride 600 mg thrice daily. She was advised on liberal salt intake. Her blood glucose level was initially managed with premixed insulin twice daily and oral metformin. Abdominal pain was managed symptomatically as the cause was not found. By discharge (2 weeks after initial presentation), her potassium level had improved to 3.7 mmol/L and serum magnesium level had improved to 1.6 mmol/L. Her abdominal pain completely resolved and did not recur. Since her glycaemic control had improved, insulin was omitted and she was discharged on metformin 1 g twice daily. At 6 months follow-up, her potassium level was 4 mmol/L on spironolactone alone. Her glycaemic control had markedly improved and HbA1c was 6.4% only with oral metformin 500 mg twice daily. Her blood pressure was 90/60 mm Hg during follow-up visit. Usually, normokalemia is difficult to be achieved even with high-dose potassium replacement in patients with Gitelman syndrome. However, the phenotype in Gitelman syndrome can have a wide spectrum from normokalemia¹⁴ to severe hypokalemia resistant to treatment. This phenotypic variability is the possible explanation for the achievement of normokalemia only with spironolactone in this patient.

Our patient had positive anti-TPO antibodies. Autoimmune thyroid disease has been reported in patients with Gitelman syndrome.⁵ However, since her thyroid functions were normal, we planned to monitor her thyroid function tests to detect the onset of hypothyroidism.

Method

Extraction of genomic DNA was done using QIAamp DNA Mini Kit according to the manufacturer's protocol. Whole exome sequencing was performed in Illumina® NovaSeq® 6000 Next Generation Sequencer using the SureSelectXT® Human (Mouse) Exon V6 5190-8864 kit.

Genetic analysis was performed using the in-house bioinformatics pipeline. Generated paired end sequences were mapped to the GrCh37 human reference using the BWA-MEM algorithm. SAMtools were used to convert the generated SAM files to BAM files. Thereafter, sorting of the BAM file was done. Picard tools were used to remove duplicated reads and indexing of the generated BAM file was done. Finally, variant calling was performed using the Genome Analysis Tool Kit (GATK). Annotation of the generated variants at the end of the process was done using SnpEff. Based on population frequency data, data in clinical databases, and in silico prediction tool results, benign variants were filtered out according to the standard ACMG guidelines (<https://www.acgs.uk.com/media/11631/uk-practice-guidelines-for-variant-classification-v4-01-2020.pdf>). During the process of further scrutinization, a novel frameshift variant (NM_000339:c.2099delT|Leu700Argfs*34) was discovered in the Exon 17 of the *SLC12A3* gene.

Discussion

Gitelman syndrome can present as salt craving, muscle weakness, tetany, abdominal pain, vomiting, carpopedal spasms, and tingling sensation of the face.¹⁵ Our patient also presented with abdominal pain and an alternative cause was not found. Sudden cardiac death has been reported in Gitelman's syndrome presumably due to hypokalemia-induced cardiac arrhythmia.¹⁶ This could be the explanation for the sudden cardiac death of her sister.

Novel variant found in our patient, denoted as c.2099delT at the cDNA level, which is located in Exon 17 of the

SLC12A3 gene. At the protein level, it substitutes the leucine resulting in a shifted reading frame. This frameshift can cause nonsense-mediated decay of mRNA leading to loss of SLC12A3 protein. Loss-of-function mutations of the NCC lead to impaired absorption of sodium at the DCT. Therefore, more sodium will reach the collecting ducts and the loss of excess sodium and water in urine causes a mild degree of volume contraction. This activates the renin–angiotensin–aldosterone mechanism leading to increased production of renin and aldosterone. Aldosterone acts on cortical collecting ducts to increase sodium reabsorption through epithelial sodium channels (ENaCs), thus maintaining salt homeostasis at the expense of increased excretion of potassium and hydrogen ions. This causes hypokalemia and metabolic alkalosis.² Hypomagnesemia is probably due to defects in transient receptor potential cation channel subfamily M member 6 (TRPM6) channel which is responsible for magnesium reabsorption in the DCT.¹⁷ There are two possible mechanisms for hypocalciuria in Gitelman syndrome. Mild volume contraction may cause increased sodium and calcium reabsorption in the proximal convoluted tubule. Inhibition of the NCCs in the distal tubule leads to increased uptake of sodium in exchange for increased calcium extrusion through the sodium–calcium exchanger 1 (NCX1) channel in the basolateral membrane. This leads to increased calcium reabsorption through transient receptor potential cation channel subfamily V member 5 (TRPV5) channel. Inhibition of TRPM6 channel also upregulates the TRPV5 channel.¹⁸

In a Chinese study, patients with Gitelman syndrome were more likely to have type 2 diabetes mellitus when compared with the general population.¹⁹ Hypokalemia can cause impaired insulin secretion and reduce glucose uptake and utilization by peripheral tissues.²⁰ Chronic hypomagnesemia also can impair insulin secretion, insulin–insulin receptor interaction, and post-receptor signalling.²¹ Our patient also had a history of diabetes and her glycaemic control improved with correction of electrolyte abnormalities.

Conclusion

Hypokalemia is an important clinical problem which can even lead to cardiac death when severe. Gitelman syndrome is a rare but important cause of hypokalemia. We report a novel mutation of *SLC12A3* gene causing Gitelman syndrome. Hypokalemia and hypomagnesemia found in Gitelman syndrome may be associated with insulin resistance and correction of electrolytes can lead to better glycaemic control.

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Ethical approval

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Informed consent

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