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MINI REVIEW

Gitelman's syndrome: a pathophysiological and clinical update

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Abstract Gitelman's syndrome (GS), also known as familial hypokalemic hypomagnesemia, is a rare autosomal recessive hereditary salt-losing tubulopathy, characterized by hypokalemic metabolic alkalosis, hypomagnesemia, and hypocalciuria, which is usually caused by mutations in the *SLC12A3* gene encoding the thiazide-sensitive sodium chloride contrasporter. Because 18–40% of suspected GS patients carry only one *SLC12A3* mutant allele, large genomic rearrangements must account for unidentified mutations. The clinical manifestations of GS are highly variable in terms of age at presentation, severity of symptoms, and biochemical abnormalities. Molecular analysis in our sibling's patients revealed compound heterozygous mutations in the coding region of *SLC12A3* as underlying their disease. Such compound heterozygosity can result in disease phenotype for such loss of function mutations in the absence of homozygosis through consanguineous inheritance of mutant alleles, identical by descent. Missense mutations account for approximately 70% of the mutations in GS, and there is a predisposition to large rearrangements caused by the presence of repeated sequences within the *SLC12A3*. We report two adult male siblings of Jewish origin with late onset GS, who presented in their fifth decade of life with muscle weakness, hypokalemia, hypomagnesaemia, and metabolic alkalosis. Rapid clinical and biochemical improvement was achieved by replacement therapy with potassium and magnesium.

Keywords Gitelman's syndrome · Hypokalemia–hypomagnesemia

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Introduction

Gitelman's syndrome (GS) is an autosomal recessive salt-losing tubulopathy characterized by hypokalemic metabolic alkalosis, renal magnesium wasting, and low urinary calcium excretion, which is caused by defective salt reabsorption at the distal convoluted tubule (DCT) followed by secondary hyperaldosteronism [1–6]. The disease can be asymptomatic or associated with mild symptoms, most commonly manifested by muscle weakness, salt craving, thirst, nocturia, paresthesia, tetany, and abdominal pain. Severe manifestations, such as early onset, manifest with growth retardation, chondrocalcinosis, and seizures. The age at presentation ranges between late childhoods to young adulthood, with the majority of patients presenting during adolescence [7]. Because of clinical resemblance with the adverse effects of thiazide diuretics, GS is most associated with inactivating mutation in the *SLC12A3* gene encoding the thiazide-sensitive sodium chloride contrasporter (NCCT) on the apical membrane of the distal convoluted tubule [5–9]. The lack of functional NCCT leads to the metabolic disturbances described in GS. In adult

patients, the presence of mutations of the *SLC12A3* gene is present in 80% of cases [10, 11].

The estimated prevalence of GS is 1:40,000, with more than 180 reported mutations being distributed through the entire coding region of *SLC12A3* [10]. To date, a number of different *SLC12A3* mutations have been reported, including missense, frame shift, and nonsense mutations. Apparently negative genetic testing could involve large genomic rearrangements that are missed by direct sequencing that usually accounts for 5–15% of the molecular defects responsible by autosomal recessive disease [10–12].

We describe two brothers who presented in their fifth decade of life with symptomatic hypokalemia-hypomagnesaemia, in whom molecular analysis revealed compound heterozygous mutations in the NCCT.

Case report

Patient 1, a 48-year-old man from a non-consanguineous Jewish-Moroccan family was admitted to the hospital with recent onset progressive generalized weakness, muscle cramps, and tetany. The neurological examination revealed mild lower limb muscle weakness. Laboratory findings at presentation revealed severe hypokalemia and hypomagnesemia (Table 1). The fractional excretion of magnesium was 8% ($N = 0.5$–4%) and of potassium was 18% ($N \leq 10\%$). The urinary calcium/creatinine ratio was 0.011 mg/mg ($N \leq 0.2$). Urinary toxicological analysis for diuretics was negative. The patients were managed with intravenous magnesium and potassium supplements, with an impressive improvement.

Two years after the initial presentation of patient 1, his previously healthy younger brother, patient 2, presented at the age of 42 years to another hospital with muscle weakness. Biochemical analysis revealed hypokalemic–hypomagnesemic metabolic alkalosis.

Sequence analysis of the entire coding region and intron–exon boundaries of *SLC12A3* was performed on DNA extracted from peripheral blood lymphocytes of both patients. Mutation analysis revealed two apparent disease-causing mutations within the coding region of the gene in both siblings (Fig. 1). Sequence analysis also revealed a single nucleotide polymorphism (p.264G > A), carried in a heterozygous state in both siblings (Table 1).

Definition and epidemiology

GS is a rare autosomal recessive disorder, referred to as familial hypomagnesemia, hypocalciuria with hypokalemic metabolic alkalosis [1–3]. Loss of function mutations in *SLC12A3* gene encoding for the thiazide-sensitive NCCT is responsible for most of the cases. GS is inherited as an autosomal recessive trait, and homozygous and combined heterozygous mutations are expected. However, between 18 and 40% of patients with clinical GS are usually found to carry only one mutant allele after *SLC12A3* screening [10].

Table 1 Laboratory characteristics of the two affected brothers

Variable	Patient 1	Patient 2	Reference values
Blood tests			
BUN (Mg/dl)	20	22	7–20
Creatinine (mg/dl)	0.85	0.9	0.4–1.3
Glucose (mg/dl)	87	80	70–100
Sodium (mmol/l)	142	7.43	133–145
Potassium (mmol/l)*	2.4	3.2	3.5–5.3
Chloride (mmol/l)	96	98	95–110
Calcium (mg/dl)	9.8	10	8.5–10.8
Phosphate (mg/dl)	3.8	2.5	2.5–4.5
Magnesium (mg/dl)*	0.54	1.0	1.8–3.0
Aldosterone (pg/ml)	151	170	40–310
Renin (ng/ml/h)	8		1.7–3.9
pH	7.45	7.43	7.35–7.45
Bicarbonate (mmol/l)	33.5	38	22–26
Urine tests			
FEMg (%)*	8%	7.6%	0.5–4%
FEK (%)*	18%	24%	<10%
Calcium (mg/24 h)*	61	54	50–300
Calcium/creatinine (mg/mg)*	0.011	0.015	<0.2

* Abnormal values

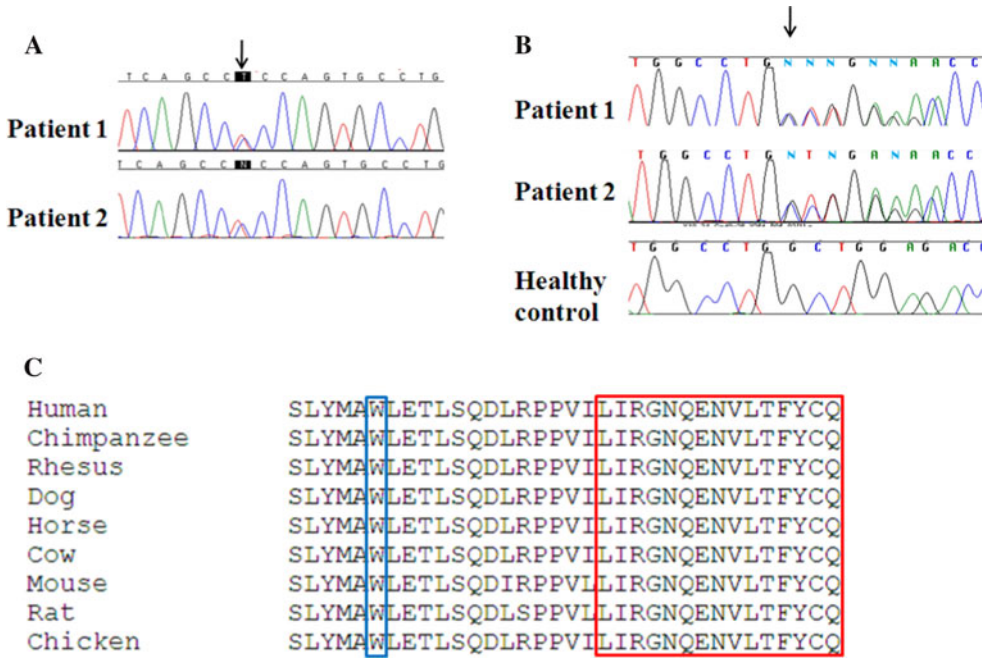

Fig. 1 Mutation analysis in two siblings affected with late-onset GS. Genomic DNA sequence analysis of *SLC12A3* in both affected brothers reveals **a** A heterozygous C → T transition in exon 16 at position g.21,137 (indicated by the *arrow*), causing proline-to-leucine exchange at amino acid position 643 of the protein sequence (P643L); **b** a heterozygous deletion of G nucleotide in exon 26 at position g.48,112 (*arrow*), resulting in a frame shift of the cDNA open reading frame, which causes a tryptophan to cysteine exchange at amino acid 1002 (W1002C) of the gene product, with an expected premature stop codon at amino acid 1016. Sequence analysis of a healthy control is shown below the chromatograms of affected individuals. **c** Multiple protein alignment of the C-terminal of NCCT: Analysis of conservation of the W1002C residue in NCCT was performed by sequence alignment of eight NCCT homolog proteins from various species

Clinical manifestation

GS patients usually present in the sixth year of life and in many cases the diagnosis is made only at adulthood. Most patients suffer from a range of tetany to paralysis during fever or when extra magnesium and potassium is lost because of vomiting or diarrhea [1, 13]. Paresthesia, and severe fatigue can interfere with daily activities. Severity of fatigue in GS is not completely related to the degree of the metabolic abnormalities. Polyuria and nocturia is usually absent or mild [14]. Some adult patients suffer from chondrocalcinosis, which is assumed to result from chronic hypomagnesemia. Hypokalemia and hypomagnesemia prolong the duration of the action potential of cardiomyocytes and consequently increase the risk of ventricular arrhythmia. Electrocardiograms of patients with GS have shown that, in about 50% of cases, the QT interval is indeed slightly to moderately prolonged [1, 5, 15].

Pathogenesis

GS is caused by mutations in the solute carrier family 12, member 3, *SLC12A3* gene, which encodes the renal thiazide-sensitive NCCT that is specifically expressed in the apical membrane of cells in the first part of the distal convolute tubule with consequent severe electrolyte wasting [14, 15]. This mutation in NCC activity causes less NaCl reabsorption in the DCT, such that more NaCl is present in the more distal nephron. The consequent volume depletion results in increased renin-aldosterone, ENaC activity, potassium wasting [15, 16]. Hypocalciuria, a marker of GS is the result of increased proximal tubule passive calcium reabsorption secondary to extracellular volume contraction (ECV). Thiazide administration (as in GS) induces hypocalciuria in transient receptor potential channel subfamily V, in which active distal Ca^{++} reabsorption is abolished because of inactivation of the epithelial Ca^{++} channel Trpv5 [17]. In general, there is extreme inter- and interfamilial phenotype variability in GS, emphasizing the lack of a correlation between the severity of symptoms in GS and the type of mutation in the *SLC12A3* gene. The mutation of nature and/or position of the *SLC12A3* gene combined with male gender seem to be associated with the severity of the syndrome. This observation is supported by earlier studies showing that the density of NCCT in DCT can be influenced by estrogen in rats [17–19].

A minority of patients with the GS phenotype have been shown to have mutations in the CLCNKB gene, encoding

the renal chloride channel CIC-Kb, located in the basolateral membrane of cells in the thick ascending limb of Henle, and in the distal tubule [1].

Genetic background

To date, more than 180 mutations of the thiazide-sensitive NCCT gene (SLC12A3) have been identified in patients with GS. Recent data [10] describe the results of the first screen by Direct Sequencing analysis in 448 index cases. Of these 448 cases, two affected alleles were identified in 315 patients (70%), 79 of them were homozygous (25%), and 236 were compound heterozygous (75%). Only one mutant allele was detected in 52 patients (18%), and the wild-type genotype was detected in 52 patients (11.6%). 172 different mutations were detected, spread throughout the gene. These included 64% missense, 14% frameshift, and 2% in-frame small deletions or insertions and 14% splice and 6% nonsense mutations. In spite of the growing number of causative mutations identified in patients with GS, up to 40% of patients are still found to carry only a single mutation in SLC12A3, instead of being compound heterozygous or homozygous [10].

Other authors described that the majority of GS patients are compound heterozygotes, carrying two different mutations on the parental alleles of *SLC12A3* [3, 15–17]. A minority of GS patients with no evidence for disease-causing mutations in *SLC12A3* have been shown to carry mutations in *CLCNKB* encoding the chloride channel ClC-Kb, located in the basolateral membrane of cells of the thick ascending loop of Henle [1, 21]. Deep intronic mutations in *SLC12A3* causing defective NCC expression can be identified with the RNA-Based approach in patients with GS [20, 21].

Treatment

Most asymptomatic patients with GS remain untreated and undergo ambulatory monitoring. Hypokalemia and hypomagnesemia are treated by oral potassium and magnesium salts [20, 22]. Oral therapy may be difficult, since large quantity of potassium chloride (>500 mmol of potassium/day in adults) may be required, and oral magnesium salts (whether in the form of sulfate, oxide, or chloride) may cause diarrhea. Magnesium chloride at a daily dose of 4–5 mg/kg in 3–4 divided doses may be better tolerated [1, 15]. In severely symptomatic cases, short-term intravenous electrolyte replacement can be highly effective. In cases of acute tetany, 20% $MgCl_2$ should be administered intravenously (0.1 mmol Magnesium/kg) and can be repeated every 6 h [22].

If symptomatic hypokalemia in not corrected by $MgCl_2$ administration, then it can be treated by aldosterone-antagonist drugs, or blocking the sodium channel ENaC [1, 21].

Discussion

GS is a rare autosomal recessive inherited disorder, characterized by hypokalemia–hypomagnesemia, hypocalciuria, with secondary hyperaldosteronism and metabolic alkalosis. Epidemiologic studies have demonstrated that there is no ethnic predilection for GS, and both sexes are equally affected. The marked similarity between the clinical features of patients with GS and patients with thiazide diuretics chronic use, has led to the conclusion that GS might have mutations that cause loss of function in the thiazide-sensitive contrasporter (TSC) NCCT which is located on the apical membrane in the distal convolute tubule. Based on a complete molecular investigation of the *SLC12A3* gene on the large cohort reported so far, and by combining several techniques, we can achieve a 91% mutation detection in GS.

GS patients generally present as clinically milder phenotype compared to BS [14], which is characterized by an older age at presentation and milder clinical symptoms. Prominent hypomagnesemia causes typical neuromuscular symptoms, like life-threatening cardiac arrhythmias [1, 14]. Although the diagnosis of GS is usually made between late childhoods to young adulthood, the range of age at diagnosis may vary widely from the neonatal period [1] to the seventh decade of life.

As shown in our patients, the most accurate means of diagnosing GS is by genetic analysis. Because 18–40% of suspected GS patients carry only one SLC12A3 mutant allele, large genomic rearrangements may account for unidentified mutations.

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