BJMG 26 (1), 2023 • 69-74
DOI: 10.2478/bjmg-2023-0009
sciendo

CASE REPORT

TWO BROTHERS FROM MACEDONIA WITH GITELMAN SYNDROME

Janchevska A[1], Tasic V[1], Jordanova O[1], Gucev Z[1], Jenkins L[2], Jovanovska N[3], Plaseska – Karanfilska D[3], Ashton E[2], Bockenhauer D[2]

[*]**Corresponding Author:** Ass. Prof. Dr. Aleksandra Janchevska, University Children's hospital, Skopje, Rep. of N. Macedonia; Email: dr.sasha1969@yahoo.com

ABSTRACT

Gitelman syndrome (GS) is a rare renal tubulopathy with an autosomal recessive mode of inheritance, caused by biallelic pathogenic variants in the *SLC12A3* gene. The clinical features may overlap with other disorders, such as Bartter syndrome type 3, HNF1B nephropathy or even mitochondrial disease, but can be distinguished by molecular genetic analysis.

Here we report on two preschool brothers, who presented with a several months' history of episodes of carpopedal spasms and muscle aches.

The biochemical analyses revealed hypokalemia and hypomagnesemia without metabolic alkalosis. A 24-h urine sample demonstrated hypocalciuria.

The molecular analyses showed that both patients were heterozygous for 3 (likely) pathogenic variants in *SLC12A3*: c.1805_1806del; p. (Tyr602Cysfs*31), c.2660+1G>A and c.2944 A>T; p. (Ile982Phe). Analysis of the parents showed that the mother was heterozygous for the c.2944 A>T p.(Ile982Phe) variant, and the father carried the other 2 variants (c.1805_1806del and c.2660+1G>A).

Herein we present two children in a family from N. Macedonia with clinical manifestations and electrolyte imbalances suggestive of GS. The results of the tubulopathy next generation sequencing (NGS) panel confirmed the diagnosis. The boys are treated with a high salt diet and oral potassium and magnesium supplements.

Key words: Gitelman syndrome, hypokalemia, hypomagnesemia, hypocalciuria, SLC12A3

[1] University Children's hospital, Skopje, Rep. of N. Macedonia

[2] North East Thames Regional Genetic Laboratory, Great Ormond Street Hospital for children, London, UK

[3] Research Center for Genetic Engineering and Biotechnology "Georgi D Efremov", Macedonian Academy of Sciences and Arts, Skopje, Rep. of N. Macedonia

INTRODUCTION

Gitelman syndrome (#MIM 263800, GS) is a rare and autosomal recessive renal disorder, characterized by hypokalemic metabolic alkalosis, hypomagnesemia and hypocalciuria [1]. It was first described in 1966 by Gitelman, Graham and Welt [2], and the underlying gene *SLC12A3* was identified in 1996 [3]. The prevalence has been variably estimated, but it is typically around 1 in 40,000 among Caucasians [4]. Yet, based on the frequency of pathogenic variants, the actual prevalence maybe as high as 1:1250, suggesting that many patients may go undiagnosed [5].

Although presentation in the neonatal period has been reported, only a few patients are actually diagnosed because of symptoms in childhood [6]. Most patients present with symptoms in adolescence or adulthood. The spectrum of clinical manifestations is wide, from asymptomatic to severe forms. Salt craving, episodes of muscle weakness or symptoms of neuromuscular irritability, fatigue, excessive thirst, growth delay or delayed puberty and abdominal pain have all been reported [7]. There is also variability in the biochemical manifestations [8]. Several other disorders, especially Bartter syndrome type 3, have similar symptoms [8].

Biallelic disease-causing variants in *SLC12A3* have been found in the majority of patients with GS. This gene is located on the long arm of chromosome 16 (16q13) and contains 26 exons [3, 9]. In some patients, only a single heterozygous coding variant is identified, but with the advent of new sequencing technology, intronic variants on the other allele, copy number variants or complex genomic rearrangements may be found [10].

SLC12A3 encodes the 1030 amino-acid thiazide-sensitive sodium chloride cotransporter (NCCT) protein (NM_000339.2; OMIM 600968) that mediates apical sodium-chloride uptake in the distal convoluted tubule (DCT) [11].

Here, we report on two preschool boys with clinical presentation of Gitelman syndrome, confirmed by mo-

lecular analysis. They are, to the best of our knowledge, the first described and confirmed cases of GS in childhood in our country. Yet, given the frequency of the disorder around the world, the question arises, whether other cases have remained undiagnosed. We therefore present these patients to raise awareness of this disorder in the Republic of N. Macedonia.

CASE REPORT

A 7-year-old boy was admitted with episodes of carpopedal spasms and muscle aches, after vomiting in the preceding months. He was born at term, in the 39th gestation week of a normal pregnancy, with a birth weight (BW) of 3150gr (-0.58 SDS) and length (BL) 50cm (-0.04 SDS). His past medical history and family history were unremarkable.

The carpopedal spasms and muscle aches later also manifested in his younger brother at the age of 7.5 years. His birth parameters, BW of 3670gr (0.23 SDS) and BL (0.16 SDS) were also within reference range with an otherwise unremarkable past medical history.

The boys' height (-0.5 SDS in older versus -0.91 SDS in younger brother) and weight (1.24 SDS versus -1.96 SDS) and pubertal stage, A1B1P1, at the onset of symptoms, and during the follow-up period, have been appropriate for their sex and age.

Our patients never had clinical signs of dehydration.

The initial and follow-up measured biochemical parameters revealed electrolyte imbalances shown in Tables 1a and 1b.

The serum parameters of hepatic and renal function were within reference range, as were those for parathyroid and thyroid function. Although hypokalemia and hypomagnesemia may contribute to prolongation of the Q-T interval [8], the performed electrocardiogram and the measured Q-T interval (0.36 sec) were normal, as were kidney ultrasound, audiogram and electroencephalogram.

The molecular analysis was performed after written informed consent had been obtained from the parents. A next generation sequencing (NGS) panel, as described previously [12] was used and revealed in the older brother three heterozygous variants in *SLC12A3* (NM_000339.3): c.1805_1806del, c.2660+1G>A and c.2944 A>T, all confirmed by Sanger sequence analysis. The same constellation was found in the younger brother.

Their mother was found to be heterozygous for the c.2944 A>T; p. (Ile982Phe) pathogenic variant and their asymptomatic father was heterozygous for the other two pathogenic variants *SLC12A3* c.1805_1806del; p. (Tyr602Cysfs*31) and c.2660+1G>A, thus confirming that these two variants are on the same allele.

Table 1: The pertinent biochemical values in blood (1a) and urine (1b) are shown bellow. Values are in mmol/l, unless otherwise indicated. Age-appropriate reference ranges from our hospital are provided in brackets were applicable.

1a. The initial and current follow-up measured biochemical parameters in serum

Patients	At the onset of the disease		Follow up	
	Older boy age 7 years	Younger boy age 7 years	Older boy age 12 years	Younger boy age 8 years
Urea (2.6-6.4)	2.7	3.5	3.1	3.9
Creatinine (0-104 mcmol/l)	43	42	42.4	39.1
Total proteins 64-83 g/l	78	80	72	77
Sodium (136-145)	140	138	136	139
Potassium (3.5-5.1)	2.9	3.2	3.4	3.4
Chloride (98-107)	100	99	95	98
Calcium (2.1-2.55)	2.57	2.7	2.74	2.53
Phosphorus (0.85-2.15)	1.62	1.61	1.62	1.29
Magnesium (0.7-1.0)	0.66	0.67	0.68	0.68
pH (7.35-7.45)	7.38	7.43	7.41	7.43
Bicarbonate (23-29)	23.8	24.2	23.3	23.4
Parathormone (PTH) 9.2-44.6 pg/ml	43.6	28.1	27.5	19.9

1b. The initial and current follow-up measured biochemical parameters in urine

Patients	At the onset of the disease		Follow up	
	Older boy	Younger boy	Older boy	Younger boy
Sodium	171	143	55	191
Potassium	33.5	143.5	62.1	97.7
Chloride	164	187	57	240
Calcium	<0.5	<0.5	<0.5	<0.5
Phosphorus	15.34	36.38	6.75	7.61
Magnesium	3.58	4.85	1.89	5.4
Creatinine (mcmol/l)	3802	4288	7412.5	10715.9
Urine Calcium/ Creatinine ratio [mg/mg] <0.14	<0.0465	<0.0412	<0.0238	<0.0165

The paternal variants are considered pathogenic. The c.2660+1G>A variant affects the canonical donor splice site of exon 22 and has been recurrently identified

in Gitelman syndrome [12, 13]. The other variant results in a frameshift. Thus, both are null variants (PVS1), that are absent in controls (PM2) and in a gene that is highly specific for the phenotype (PP3) and are thus annotated as pathogenic.

The maternal variant c.2944 A>T; p. (Ile982Phe) is absent in controls (PM2) and was found *in trans* with a pathogenic variant (PM3). *In silico* tools predict that this variant is likely damaging (PP3) and as the phenotype is highly specific for this gene (PP4), this variant was classified as likely pathogenic.

The boys were advised to maintain an increased salt intake and were prescribed oral potassium and magnesium supplements. The follow up period has been uneventful with occasional episodes of paresthesia.

DISCUSSION

Gitelman syndrome is an inherited renal tubulopathy [11, 14] typically diagnosed in adolescence or adulthood. There are only a few reports of it being diagnosed before the 6th year of age [4, 6, 15, 16, 17].

The non-specificity of the symptoms, and their mild presentation are perhaps the reason for the late recognition of the syndrome. Indeed, many patients may have no apparent symptoms and are identified when a blood test is obtained for unrelated reasons. Thus, hypokalemic metabolic alkalosis with hypomagnesaemia should prompt the suspicion of this diagnosis [18, 19, 20, 21, 22] which should be then confirmed by genetic testing. A Gitelman-like phenotype can also be acquired: for instance, chronic diarrhea [23], vomiting, bulimia, anorexia, long-term abuse of laxatives or diuretics may mimic the symptoms of mild forms of GS.

In 2011 Vargas et al. [24] reported 172 mutations in a large cohort of 448 GS patients, of which 59% were missense mutations detected by direct sequencing and in approximately 6%, large rearrangements were found by MLPA analysis.

Glaudemans et al., 2012 [25], in a cohort of 163 patients with clinical signs of GS detected 114 mutations in the *SLC12A3* gene by direct sequencing of which 31 were novel.

No specific genotype-phenotype correlations have been reported.

In 2018, Ashton et al. [12], identified 269 variants in 27 genes in 410 patients by using a specially designed kit for targeted amplification of 37 known tubulopathy disease genes. The emergence of simultaneous sequencing of a panel of multiple genes has become an increasingly used method. They genetically confirmed 63 GS patients with childhood-onset symptoms by the panel sequencing. This comprehensive method distinguished the overlapping phenotypes and enabled an accurate diagnosis. A few isolated cases of GS patients have been associated with other rare conditions such as nephrotic syndrome, parathyroid adenoma or growth hormone deficiency, which presumably just reflects the frequency of the syndrome, as these symptoms are likely unrelated [26, 27, 28].

The two brothers with GS fulfilled already established biochemical diagnostic criteria by the KDIGO consensus report [17] and the tubulopathy panel sequencing followed by the Sanger sequencing confirmed the diagnosis. The treatment with a high salt diet and oral potassium and magnesium supplements followed the recommendations from the consensus guidelines. It has helped to maintain the biochemical parameters almost within reference range and reduced the frequency of the symptoms to a minimum.

Some GS patients with severe symptoms have been treated with intravenous potassium and/or magnesium supplements and/or potassium-sparing diuretics, pain medications, renin angiotensin system blockers and nonsteroidal anti-inflammatory drugs [17, 29, 30, 31]. The efficacy of these treatments remains to be established.

The frequency and severity of symptoms in patients with GS is variable, but the majority of them have a good prognosis although, some cases may develop chronic renal insufficiency [31] or cardiac arrhythmias [1, 32].

CONCLUSIONS

We present the first two siblings, aged 7 years and 7.5 years at the time of the diagnosis, from N. Macedonia with typical clinical features and electrolyte disturbances of Gitelman syndrome. The findings of the next generation sequencing methods were consistent with the clinical diagnosis of GS. A treatment with a high salt diet and oral potassium and magnesium supplements allows our patients a good quality of life and maintains their biochemical parameters within the normal range.

REFERENCES

1. Graziani G, Fedeli C, Moroni L, Cosmai L, Badalamenti S, Ponticelli C. Gitelman syndrome: pathophysiological and clinical aspects. QJM. 2010; 103(10):741-748.
2. Gitelman HJ, Graham JB, Welt LG. A new familial disorder characterized by hypokalemia and hypomagnesemia. Trans Assoc Am Phys. 1966; 79: 221-235.
3. Simon DB, Nelson-Williams C, Bia MJ, Ellison D, Karet FE, Molina AM et al. Gitelman's variant of

Bartter's syndrome, inherited hypokalaemic alkalosis, is caused by mutations in the thiazide-sensitive Na-Cl cotransporter. Nature Genet. 1996; 12: 24-30.

4. Knoers NVA, Levtchenko EN. Gitelman syndrome. Orphanet J Rare Dis. 2008 30;3: 22.
5. Blanchard A, Vallet M, Dubourg L, Hureaux M, Allard J, Haymann JP et al. Resistance to Insulin in Patients with Gitelman Syndrome and a Subtle Intermediate Phenotype in Heterozygous Carriers: A Cross-Sectional Study. J Am Soc Nephrol. 2019 Aug;30(8):1534-1545.
6. Tammaro F, Bettinelli A, Cattarelli D, Cavazza A, Colombo C, Syrén ML et al. Early appearance of hypokalemia in Gitelman syndrome. Pediatr Nephrol. 2010; 25:2179–2182.
7. Zelikovic I: Molecular pathophysiology of tubular transport disorders. Pediatr Nephrol.2001; 16:919–935.
8. Al Shibli A, Narchi H. Bartter and Gitelman syndromes: Spectrum of clinical manifestations caused by different mutations. World J Methodol. 2015; 26;5(2):55-61.
9. Mastroianni N, Bettinelli A, Bianchetti M, Colussi G, De Fusco M, Sereni F. et al. Novel molecular variants of the Na-Cl cotransporter gene are responsible for Gitelman syndrome. Am J Hum Genet.1996; 59: 1019-1026.
10. Viering DHHM, Hureaux M, Neveling K, Latta F, Kwint M , Blanchard A et al. Long-Read Sequencing Identifies Novel Pathogenic Intronic Variants in Gitelman Syndrome. J Am Soc Nephrol. 2023 1;34(2):333-345.
11. Kleta R, Bockenhauer D. Salt-Losing Tubulopathies in Children: What's New, What's Controversial? J Am Soc Nephrol. 2018;29(3):727-739.
12. Ashton E, Legrand A, Benoit V, Roncelin I, Venisse A, Zennaro MC et al. Simultaneous sequencing of 37 genes identified causative mutations in the majority of children with renal tubulopathies. Clin Invest. 2018; 93: 961-967.
13. Hureaux M, Ashton E, Dahan K, Houillier P, Blanchard A, Cormier C et al. High-thro-ughput sequencing contributes to the diagnosis of tubulopathies and familial hypercal-cmia hypocalciuria in adults. Kidney Int 2019;96(6):1408-1416.
14. Leung JC. Inherited renal diseases. Curr Pediatr Rev. 2014;10(2):95-100.
15. Gitelman syndrome. Genetics Home Reference. February 2011.
16. Walsh PR, Tse Y, Ashton E, Iancu D, Jenkins L, Bienias M, et al. Clinical and diagnostic features of Bartter and Gitelman syndromes. Clin Kidney J. 2018;11(3):302-309.
17. Blanchard A, Bockenhauer D, Bolignano D, Calò LA, Cosyns E, Devuyst O et al. Gitelman syndrome: consensus and guidance from a Kidney Disease: Improving Global Outcomes (KDIGO) Controversies Conference. Kidney Int. 2017; 91(1):24-33.
18. Cruz DN, Shaer AJ, Bia MJ, Lifton RP, Simon DB, for Yale Gitelman's and Bartter's Syndrome Collaborative Study Group. Gitelman's syndrome revisited: an evaluation of symptoms and health-related quality of life. Kidney Int. 2001; 59:710–717.
19. Pachulski RT, Lopez F, Sharaf R. Gitelman's not-so-benign syndrome. N Engl J Med. 2005; 353:850–851.
20. Peters M, Jeck N, Reinalter S, Leonhardt A, Tönshoff B, Klaus G, et al. Clinical presentation of genetically defined patients with hypokalemic salt-losing tubulopathies. Am J Med. 2002; 112:183–190.
21. Emmett M, Ellison D. Bartter and Gitelman syndromes. In: Sterns RH, Forman JP. UpToDate. Waltham, MA: UpToDate; Last updated February 7, 2018; Accessed 2/22/2018.
22. Ungaro CM, Odstrcil-Bobillo MS, Russo PM. Gitelman syndrome. Medicina (B Aires). 2020;80(1):87-90.
23. Matsunoshita N, Nozu K, Yoshikane M, Kawaguchi A, Fujita N, Morisada N et al. Congenital chloride diarrhea needs to be distinguished from Bartter and Gitelman syndrome. J Hum Genet. 2018 Jul;63(8):887-892.
24. Vargas-Poussou R, Dahan K, Kahila D, Venisse A, Riveira-Munoz E, Debaix H et al. Spectrum of mutations in Gitelman syndrome. J Am Soc Nephrol. 2011;22(4):693-703.
25. Glaudemans B, Yntema HG, San-Cristobal P, Schoots J, Pfundt R, Kamsteeg, EJ et al. Novel NCC mutants and functional analysis in a new cohort of patients with Gitelman syndrome**.** Europ. J. Hum. Genet. 2012; 20: 263-270.
26. Rosado Rubio C, Fraile Gómez P, Gómez Muñoz MA, Garcia-Cosmes P, Lerma Márquez JL. C1q nephropathy in a patient with Gitelman syndrome. NDT Plus. 2011;4(6):392-393.
27. Rego T, Fonseca F, Cerqueira R, Agapito A. Gitelman syndrome and primary hyperparathyroidism: a rare association. BMJ Case Rep. 2018; 5; pii: bcr-2017-223663.

28. Huang K, Dai YL, Zhang JW, Zhang L, Wu W, Dong GP, et al. Gitelman syndrome combined with growth hormone deficiency: Three cases report. Medicine (Baltimore). 2019;98(40): e17244.
29. Sinha A, Lněnička P, Basu B, Gulati A, Hari P, Bagga A. Gitelman syndrome: novel mutation and long-term follow-up. Clin Exp Nephrol. 2012;16(2):306-309.
30. Filippatos TD, Rizos CV, Tzavella E, Elisaf MS. Gitelman syndrome: an analysis of the underlying pathophysiologic mechanisms of acid-base and electrolyte abnormalities. Int Urol Nephrol. 2018;50(1):91-96.
31. Klemmer PJ. Gitelman Syndrome. National Organization for Rare Disorders (NORD). 2015.
32. Lee JH, Lee J, Han JS. Gitelman's syndrome with vomiting manifested by severe metabolic alkalosis and progressive renal insufficiency. Tohoku J Exp Med. 2013; 231(3):165-169.