

Gitelman's Syndrome Associated with Chondrocalcinosis: A Case Study from the Azores Islands (Portugal)

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Abstract

Gitelman syndrome (GS) is a rare autosomal recessive inherited tubulopathy, characterized by defective tubular reabsorption of magnesium and potassium, mostly caused by mutations in the *SLC12A3* gene. The association of GS with chondrocalcinosis (CC) has been described in the literature as a typical example of hypomagnesemia-induced crystal deposition disease.

We are presenting one case where the genetic cause for GS was identified in a proband with secondary early onset CC. A 60 years-old male patient with CC, hypomagnesemia and hypokalemia was identified in our hospital as a result of clinical and laboratory assessments. The clinical diagnosis of GS was performed and *SLC12A3* gene was screened in the proband; variants detected were further searched in family members.

The proband was homozygous for the S615L mutation; additionally, only one from the seven family members which were heterozygous presents CC. The presence of CC in two other individuals is most likely sporadic, in agreement with their advanced age.

Keywords: Chondrocalcinosis; Calcium pyrophosphate dehydrate; Genetic studies

Introduction

Gitelman Syndrome (GS, OMIM 263800; ORPHA358) is a rare autosomal recessive tubulopathy, with a prevalence of approximately 1:40 000 in the Caucasian population. Onset is usually in adult life, but cases with onset in childhood are also known [1]. GS is characterized by hypomagnesemia, hypokalemia, metabolic alkalosis, hypocalciuria and hyperreninemic hyperaldosteronism. The clinical spectrum is wide and includes: cramps, myalgias, muscle weakness, tetania, and paralysis [2]. GS is mostly caused by loss of function mutations in the solute carrier family 12, member 3 (*SLC12A3* gene) which consists of 26 exons and is located on the long arm of 16th chromosome [1]; this gene encodes the thiazide-sensitive sodium-chloride cotransporter (NCCT), expressed in the distal convoluted tubule of the kidney [3].

NCCT is a polypeptide which consists of 1021 amino acids. Its 2-D structure is predicted to contain 12 transmembrane domains and intracellular amino and carboxyterminal regions [3]. At present, according to the Human Gene Mutation Database (<http://www.hgmd.cf.ac.uk/>), more than 100 different variants have been identified in patients with GS. They are missense, non-sense, frameshift, and splice-site variations and are scattered throughout the transporter protein with a possible clustering of variations in the carboxy-terminal tail [4].

The association of GS with chondrocalcinosis (CC) has been described in the literature as a typical example of hypomagnesemia-induced crystal deposition disease [2]. CC is characterized by deposition of crystals of calcium pyrophosphate dihydrate (CPPD) in articular hyaline and fibro-cartilage [2]. Seventeen cases of GS associated with CC due to CCPD have been published in the literature, including 10 females and 7 males with a mean age of 51.4 ± 15.9 years [2,5-15]. The role of hypomagnesemia in the development of CCPD is, however, not fully understood [16]. Magnesium is an important cofactor for alkaline pyrophosphatase, an enzyme that plays a key role by converting inorganic pyrophosphate (PPi) to orthophosphate (Pi). A reduction in the activity of this enzyme due to hypomagnesemia could induce CCPD by raising extracellular levels of PPi. Both PPi and calcium are crucial precursors for crystal nucleation. CCPD may be found in other conditions associated with hypomagnesemia, such as short bowel syndrome or in liver transplantation patients [16].

Case with Genetic Analysis

The proband, a 60 year-old caucasian male was first observed in the Rheumatic Diseases Clinic - HSEIT when he was 48 years old; he was born in Terceira island as well as both his parents. Symptoms started when he was 33 years old, mainly affecting knees, ankles, wrists, elbows and achilles tendons. In the proband, PPi crystals were identified in the synovial fluid aspirated from a right knee effusion. Since then he was under treatment with colchicine, NSAIDS (Nonsteroidal anti-inflammatory drugs), and oral potassium and

magnesium. Laboratory tests revealed normal leukocyte, erythrocyte and platelet count.

Blood urea was 33 mg/dl, creatinine 0.9 mg/dl and glucose 177 mg/dl. Serum electrolyte concentrations were as follows: sodium 139 mEq/L, potassium 3.2 mEq/L, calcium 9.8 mg/dl, and magnesium 1.1 mg/dl (Table 1). In spite of the treatment with colchicine, patient still maintain hypokalemia and hypomagnesemia, however he showed some improvements. Using the diagnostic criteria of Bettinelli et al. [17] a clinical diagnosis of GS was suspected, and a diagnosis of knee

CC was made after the identification of bilateral knee cartilage calcification (Figure 1). *SLC12A3* sequence analysis in the proband revealed that he was homozygous for a missense substitution in exon 15, previously described as associated to GS [18]. This mutation (CM014403), a C to T transition at position c.1869, changes the small size and polar amino acid serine to a medium size and hydrophobic leucine at position 615 (S615L), has a SIFT score of 0 and a Polyphen value of 0.996, both values suggestive of a deleterious variation.

Individuals	Sex	Age	Magnesium levels ^a	Potassium levels ^b	CC	Pathogenic mutation S615L
III.2	M	60	1.1	3.2	+	S615L/S615L
III.12	M	75	2.1	4.7	-	S615L/WT
III.13	F	67	2.2	4.1	-	WT/WT
III.16	M	75	2.0	4.6	+	WT/WT
III.19	F	79	1.9	4.7	+	S615L/WT
IV.1	M	35	2.3	3.7	-	S615L/WT
IV.2	F	54	2.2	4.2	-	S615L/WT
IV.4	M	46	1.9	4.0	-	S615L/WT
IV.14	F	51	1.9	4.0	-	WT/WT
IV.16	F	49	2.2	4.1	-	WT/WT
IV.17	F	45	1.9	4.3	-	WT/WT
IV.21	M	37	2.1	4.1	-	S615L/WT
IV.22	F	36	2.0	3.7	-	S615L/WT
V.1	F	25	2.0	4.7	-	WT/WT

^aNormal serum magnesium 1.5-2.5 mg/dL; ^bNormal serum potassium; 3.3-5.1 mmol/L; M:Male; F:Female; CC:Chondrocalcinosis; WT:Wild Type

Table 1: Characteristics and *SLC12A3* gene variants identified in the proband and in thirteen individuals of his family pedigree. The proband is indicated by bold.

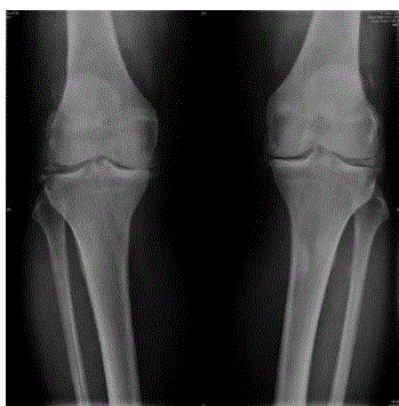


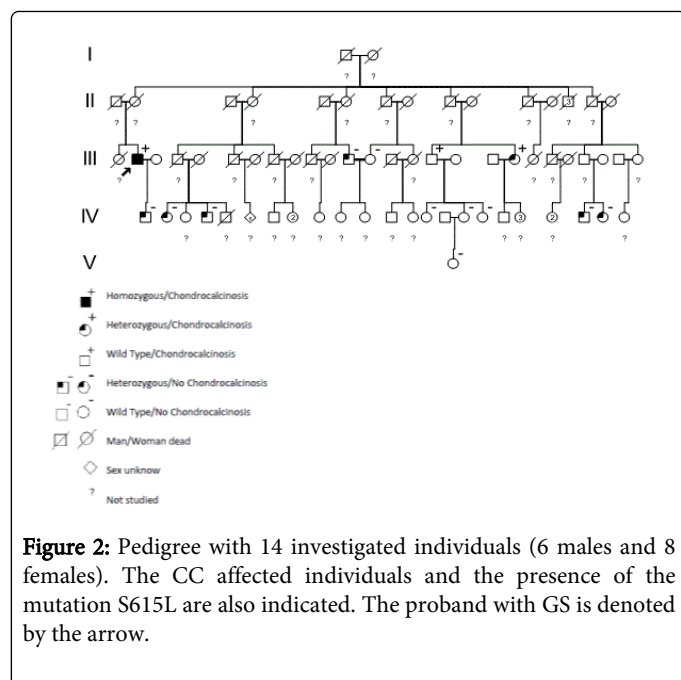
Figure 1: X-rays of proband showing classical chondrocalcinosis in knees.

Thirteen additional family members were investigated (5M: 8W; [25-79]; mean age 51); blood tests and x-rays were obtained for all of them (data not shown). The pedigree with investigated individuals is shown in Figure 2. The biochemical data in these patients show normal levels of serum magnesium ranging from 1.9 to 2.3 mg/dL and normal levels of potassium ranging from 3.7-4.7 mmol/L (Table 1).

When the S615L mutation was searched in the thirteen relatives of the proband, seven family members were found to be heterozygous, of which only one presented CC. Furthermore six individuals were wild-type homozygous; noteworthy, one of them (III.16) presented CC (Table 1 and Figure 2).

Discussion

The GS patient described in this study has the S615L variation in homozygosity, while the other cases of GS with this variation were reported in compound heterozygotes [2]. In our study, seven individuals heterozygous for the S615L did not have either hypokalemia or hypomagnesemia, confirming that they were asymptomatic carriers of this variation.



Hypomagnesemia and hypocalciuria are found in most cases of GS, however, some cases with mutations in the NCCT do not show these conditions [19]. It is believed that hypomagnesemia causes CC by increasing formation and reducing solubility of CCP crystals [16]. Excess of PPI is the main precursor for CPPD crystal nucleation. Because the magnesium is a necessary cofactor for numerous enzymes, such as pyrophosphatases, and considering the fact that it increases the solubility of CPPD crystals, low levels of magnesium could induce CPPD deposition disease by raising PPI and/or reducing the saturation product of CPPD crystals [14]. The prevalence of CC increases with age (10-15% for people between 65 and 75 years) and is hence called sporadic in patients older than 60 years, whereas in younger individuals there are several putative underlying disorders causing CPPD deposition disease, such as hemochromatosis, hyperparathyroidism, hypomagnesemia or hypophosphatemia [20].

The assumption that GS is caused by a defect in the NCCT cotransporter in the renal distal tubule has been proven by the identification of numerous variations in the *SLC12A3* gene in patients with GS [1,4,19,21]. In the present study the specific involvement of this cotransporter in the etiology of this disorder is further substantiated by the finding that the proband is homozygous for the S615L variation. The S615L identified in this study was previously described by Cruz and co-workers [18] in a study involved 36 kindreds from the United States, Canada and England and later reported in a study by Syrén et al. [22]. Although the *SLC12A3* variations reported in previous studies are distributed throughout the whole protein [4,23], the study of Lemmink (1998) indicates that the carboxy-terminal end represents a hot spot for variations [4]. S615L is located at the intracellular C-terminal end of the *SLC12A3* protein. It is conceivable that structural alterations due to *SLC12A3* variations in the C-terminal domain interfere with phosphorylation of the NCCT protein and as such with its regulation [4].

Evidence for an association between CC and GS comes from uncontrolled case reports, case series and only one cross-sectional study. As a result, its epidemiology remains unknown [16]. There have

been few cases described with a definite diagnosis of CC due to GS. In some patients with CPPD deposition disease secondary to hypomagnesemia, the stabilization of magnesium and potassium levels can reduce the deposition of CPPD crystals in the synovium and synovial fluid, reducing the frequency of attacks of articular pain [14].

Conclusion

GS is a hereditary disease characterized by defective tubular reabsorption of magnesium and potassium, mostly caused by mutations in the *SLC12A3* gene. Sometimes GS patients, as in our case, might come with a CC diagnosis. We identified the genetic cause for GS in a proband with secondary early onset CC. Further studies are needed in order to enlighten the pathophysiology and prevalence of CC in patients with GS.

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