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CLINICAL INVESTIGATION

Genetic heterogeneity in tubular hypomagnesemia-hypokalemia with hypocalcuria (Gitelman's syndrome)

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Genetic heterogeneity in tubular hypomagnesemia-hypokalemia with hypocalciuria (Gitelman's syndrome). To better clarify the genetic inheritance of primary tubular hypomagnesemia-hypokalemia with hypocalciuria, or Gitelman's syndrome (GS), we studied eight families (10 patients aged 11 to 22 years; 16 parents; 9 siblings) in which at least one offspring had GS (plasma magnesium < 0.65 mmol/liter; plasma potassium < 3.6 mmol/liter; high magnesium and potassium fractional excretions; molar urinary calcium/creatinine < 0.10). Two families each had two offspring of different sex with GS, who all had tetanic episodes and/or marked weakness during childhood or adolescence, whereas in three other families two mothers and three offspring presented GS and one father and two other offspring had hypomagnesemia and hypocalciuria but normal plasma potassium. The mean plasma magnesium and potassium levels of the patients of the first two families were significantly lower ($P < 0.05$) than those of the other three families. Intralymphocytic but not intraerythrocytic magnesium and potassium were significantly lower ($P < 0.05$) in patients compared to controls. We hypothesize that there are two different types of genetic transmission of GS, one autosomal recessive and one autosomal dominant with high phenotypic variability. It seems that this genetic heterogeneity is associated with a different clinical expression with frequent tetanic episodes and lower plasma potassium and magnesium levels in the autosomal recessive form.

Magnesium deficiency and hypocalciuria are, in addition to hypokalemia, the biochemical hallmarks of Gitelman's syndrome (GS), a recognized variant of Bartter's syndrome, sometimes referred to as primary tubular hypomagnesemia-hypokalemia with hypocalciuria [1–6]. It has been postulated that in GS the renal tubular defect is in the distal convoluted tubule [2, 3, 5, 6], but the exact site of the abnormality is unknown.

GS is considered to be transmitted in an autosomal recessive fashion as suggested by the familial cases described to date [5–9]. Thus far only a few families have been studied [4–9], and therefore to help clarify the mode of inheritance we investigated eight families with at least one affected member.

Methods

Subjects

We studied eight families (I to VIII) in which at least one offspring had GS. The families included 10 patients (4 males and 6 females, aged 11 to 22 years; their 16 parents, aged 34 to 53 years; 9 siblings, 5 males and 4 females, aged 13 to 24 years). No consanguinity was reported in the eight families. The patients' main clinical characteristics are presented in Table 1 (the first 4 patients are 2 pairs of siblings). Four subjects had documented tetanic episodes at first admission or subsequently. Severe weakness, considered as generalized fatigue causing substantial disruption of usual daily activities, was reported in three patients (no. 1 to 3). The remaining seven patients (no. 4 to 10) had no severe symptoms of magnesium or potassium deficiency, and GS was only diagnosed when electrolyte measurements were performed during investigations for mild weakness (considered as subjective generalized fatigue without any substantial effect on usual daily activities) associated or not with other diseases (enteritis, appendicopathy, urinary tract infection). Apart from normal blood pressure and negative screen for diuretics, the diagnosis of GS was based on the following criteria: (1) hypomagnesemia of renal origin, defined as plasma magnesium < 0.65 mmol/liter (by flame atomic absorption spectrophotometry) in the presence of inappropriately high magnesium excretion (fractional excretion of magnesium $> 4.0\%$); (2) hypokalemia of renal origin, defined as plasma potassium < 3.6 mmol/liter in the presence of inappropriately high potassium excretion (fractional excretion of potassium $> 16.0\%$); (3) urinary calcium/creatinine molar ratio < 0.10. At the time of the study the patients had not taken any medication for at least one month. We also studied 13 healthy subjects aged 16 to 22 years who served as controls. No biochemical data were available on the patients' parents or siblings at the beginning of the study, and none of them had symptoms of any disease. Informed consent was obtained from all the subjects studied.

Methods

Dietary records were kept by all the subjects for three days before the study, and 24-hour urine was collected the day before. A blood sample was taken at the end of the 24-hour urine collection. Blood hydrogen ion concentration, blood carbon dioxide tension, plasma and urinary creatinine, sodium, chloride, inorganic phosphate, total calcium and magnesium were measured in all subjects. Intralymphocytic and intraerythrocytic magnesium and potassium were assayed in samples from the 10 patients, 13 parents, 8 siblings and all the controls. Blood hydrogen ion concentration and blood carbon dioxide tension (by

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Table 1. Main clinical data in patients with Gitelman's syndrome

Patient	Sex	Family	Age at diagnosis *years*	Age at time of the study	Clinical signs	
					Tetany	Weakness
1	F	I	20	22	+	++
2	M	I	7	18	+	++
3	F	II	12	19	−	++
4	M	II	13	20	+	+
5	F	III	13	15	−	+
6	F	IV	11	16	−	+
7	M	V	13	14	−	+
8	F	VI	6	20	+	+
9	F	VII	6	11	−	+
10	M	VIII	10	16	−	+

Symbols are: ++, severe weakness; +, mild weakness.

selective electrodes) were measured immediately after blood collection. Blood was prepared for intralymphocytic and intraerythrocytic magnesium and potassium determinations as previously described [10]. Plasma, urinary and intracellular magnesium and potassium were measured by flame atomic absorption spectrophotometry, sodium and chloride by direct flame photometry, and total calcium and phosphate by colorimetry (Kodak Ektachem). Creatinine was measured by an enzymatic method (Hitachi 747). Plasma bicarbonate was calculated from blood hydrogen and blood carbon dioxide tension according to the Henderson-Hasselbach equation with an acidity exponent of 6.10 and a solubility coefficient of 0.0301. Fractional electrolyte excretions were calculated using standard formulae. Creatinine clearance was measured in 24-hour urines.

Statistical analysis

Data are reported as mean ± standard deviation (SD). Analysis of variance was used to test differences among groups. When a statistically significant result was found, differences between individual means were tested using multiple comparison techniques. A two-side P value of less than 0.05 was considered statistically significant.

Results

The biochemical findings and the dietary habits in patients with GS, their parents, their siblings and the control group appear in Table 2. The four study groups did not differ with respect to creatinine clearance, total plasma calcium, plasma and urinary phosphate, urinary sodium excretion, and dietary intake of calcium, magnesium, potassium and sodium. Apart from hypokalemia with inappropriate urinary potassium excretion, hypomagnesemia with inappropriate urinary magnesium excretion and hypocalciuria (inclusion criteria for GS), patients with GS showed a significant tendency to reduced plasma sodium, hypochloremia with inappropriate urinary chloride excretion, and increased plasma pH and bicarbonates. Mean plasma and urinary potassium, sodium, chloride and magnesium, blood pH and plasma bicarbonate were comparable in the parents, siblings and controls. Intralymphocytic potassium was lower in the patients with respect to the other groups and intralymphocytic magnesium was lower only with respect to the controls (Table 3), whereas no significant difference was found in intraerythrocytic values.

The pedigrees of the eight families and the corresponding individual values of plasma magnesium and potassium and molar urinary calcium creatinine are given in Figure 1. These families were divided into three groups. Families I and II (group A) each included 2 siblings of different sexes who had GS with a history of tetanic episodes (patients 1, 2 and 4) and/or severe weakness (patients 1, 2 and 3). Plasma magnesium and potassium were normal in the four parents and the three siblings. Three families (families III, IV and V) (group B) had one parent with all or some of the biochemical features of GS, and the patients (no. 5, 6 and 7) presented a history of mild weakness but no tetanic episodes. Two mothers (families IV and V) had all the biochemical features of the syndrome, namely, hypomagnesemia, hypokalemia and hypocalciuria. In family III the father and the two siblings had hypomagnesemia and hypocalciuria but not hypokalemia; the abnormalities in plasma magnesium and calciuria of both the father and siblings were borderline compared to reference values. None of the eight subjects with electrolyte abnormalities in group B had major signs or symptoms attributable to hypomagnesemia or hypokalemia. Each of the remaining three families (VI, VII and VIII) (group C) had only one offspring with GS. Patient 8 had a history of tetanic episodes during childhood. The remaining members of these families had normal plasma levels and fractional excretion of potassium and magnesium and a normal urinary calcium/creatinine molar ratio.

The mean plasma levels of magnesium and potassium were significantly lower in hypomagnesemic subjects of group A than in those of group B (plasma magnesium, 0.40 ± 0.9 vs. 0.57 ± 0.07 mmol/liter; plasma potassium, 2.90 ± 0.28 vs. 3.60 ± 0.56 mmol/liter; $P < 0.05$). The mean intralymphocytic magnesium and potassium values of the hypomagnesemic subjects of subgroup A were slightly but not significantly lower than those of group B (intralymphocytic magnesium 50.9 ± 6.6 vs. 59.2 ± 21 nmol/mg protein; intralymphocytic potassium 441.0 ± 66.7 vs. 517.9 ± 123.1 nmol/mg protein). None of the other plasma, urinary and intracellular variables differed significantly in the groups.

Discussion

This study includes the largest reported number of families with members affected by GS. Previous reports suggested that this disorder could have an autosomal recessive mode of inheritance as affected siblings of different sexes have been observed with clinically asymptomatic parents [5–9].

No information is available so far on the molecular defect of this hereditary condition. For this reason our evaluation of the possible mode of inheritance was based only on clinical and biochemical data of affected patients and their relatives. Analysis of the eight families confirmed the existence of an autosomal recessive form of GS. In the group A families (I and II) siblings of different sex were similarly affected. The parents of these children were completely healthy and did not show any abnormality of magnesium, potassium and calcium metabolism. Also in families III, IV and V none of the parents had a clinically overt GS, but in two mothers we found all the biochemical features of the syndrome. In family III the father and two siblings without any clinical disturbance presented tubular hypomagnesemia and hypocalciuria but without any hypokalemia; an X-linked recessive transmission can be excluded in this family because of the father-to-son transmission. The biochemical features observed in the latter three subjects resemble those reported by Geven et al in a renal magnesium wasting disorder with autosomal dominant mode of inheritance in two families [11]. In family V both an

Table 2. Biochemical findings and dietary habits in 10 patients with GS, 16 parents of patients with GS, 9 siblings of patients with GS, and a control group of 13 healthy subjects

	Patients	Siblings	Parents	Controls
Creatinine clearance *ml/min/1.73 m²*	119 ± 31	111 ± 39	109 ± 28	119 ± 24
Plasma K *mmol/liter*	3.0 ± 0.3[b]	4.3 ± 0.2	4.2 ± 0.3	4.2 ± 0.3
FE_K, %	20.0 ± 3.6[b]	9.1 ± 3.2	9.9 ± 5.1	9.6 ± 3.2
Plasma Na *mmol/liter*	137.0 ± 1.4[a]	139.2 ± 1.8	140.1 ± 1.7	139.5 ± 1.9
FE_{Na}, %	1.1 ± 0.3	0.8 ± 0.5	0.9 ± 0.3	0.9 ± 0.4
Plasma Cl *mmol/liter*	94.9 ± 2.4[a]	100.8 ± 1.9	101.9 ± 1.6	101.8 ± 2.0
FE_{Cl}, %	1.7 ± 0.5[a]	1.2 ± 0.6	1.2 ± 0.3	1.2 ± 0.5
Venous pH	7.422 ± 0.035[a]	7.340 ± 0.039	7.383 ± 0.026	7.346 ± 0.034
Plasma HCO_3^- *mmol/liter*	32.0 ± 2.3[a]	28.0 ± 1.8	27.6 ± 2.2	28.0 ± 1.8
Tot plasma Mg *mmol/liter*	0.48 ± 0.11[b]	0.75 ± 0.07	0.75 ± 0.12	0.74 ± 0.04
FEM_g %	6.7 ± 2.7[b]	3.6 ± 1.1	2.8 ± 0.9	3.0 ± 0.6
Tot plasma Ca, *mmol/liter*	2.46 ± 0.06	2.46 ± 0.05	2.37 ± 0.04	2.41 ± 0.11
Molar $U_{Ca/Cr}$	0.030 ± 0.017[b]	0.224 ± 0.129	0.286 ± 0.171	0.384 ± 0.220
Plasma PO_4, *mmol/liter*	1.26 ± 0.16	1.39 ± 0.18	1.10 ± 0.13	1.20 ± 0.13
TRP, %	87.3 ± 4.9	88.1 ± 2.9	84.8 ± 5.3	85.9 ± 2.2
Dietary Ca, *intake mg/day*	616 ± 233	622 ± 337	772 ± 473	607 ± 477
Dietary Mg, *intake mg/day*	236 ± 114	219 ± 49	256 ± 105	212 ± 58
Dietary K, *intake mg/day*	2779 ± 644	2227 ± 644	2422 ± 916	2073 ± 592
Dietary Na, *intake mg/day*	1756 ± 718	1473 ± 346	2063 ± 997	1446 ± 729

Data are mean ± SD. Abbreviations are: FE, fractional excretion; TRP, tubular reabsorption of phosphate.
[a] $P < 0.05$, patients vs. controls, parents, and siblings, respectively
[b] Not compared in the patients and controls; a significant difference was predictable as the value was by definition abnormal in a patient with GS

Table 3. Magnesium and potassium levels in lymphocytes and erythrocytes of the four study groups

	Patients ($N = 10$)	Siblings ($N = 8$)	Parents ($N = 13$)	Controls ($N = 8$)
Lymphocyte magnesium *nmol/mg protein*	55 ± 9[a]	64 ± 15	63 ± 15	66 ± 9
Lymphocyte potassium *nmol/mg protein*	459 ± 77[b]	568 ± 76	542 ± 68	534 ± 95
Erythrocyte magnesium *mmol/liter*	1.9 ± 0.2	2.1 ± 0.3	2.1 ± 0.2	2.0 ± 0.2
Erythrocyte potassium *mmol/liter*	76 ± 4	74 ± 8	76 ± 5	78 ± 7

Data are mean ± SD.
[a] $P < 0.05$ patients vs. controls, parents and siblings, respectively
[b] $P < 0.05$ patients vs. all other groups

autosomal dominant and an X-linked recessive mode of transmission with clinical expression in the carrier may be postulated. In family IV the mode of inheritance seems autosomal dominant although X-linked recessive transmission could not be formally excluded. In our opinion, taking all these data together, the most likely hypothesis appears to be that GS was transmitted in autosomal dominant fashion with highly variable clinical expression in the group B families.

In our GS patients with recessive mode of inheritance the clinical features were severe and included both muscular weakness and tetany. In those with a hypothesized dominant transmission the clinical features of GS were mild, including muscular weakness but without tetany, and mean plasma and magnesium levels were significantly higher than in the patients with autosomal recessive mode of inheritance (group A). Furthermore, as in most disorders with this pattern of inheritance, the clinical and biochemical expression of GS was highly variable in our patients with hypothesized dominant inheritance. In families VI, VII and VIII (group C) only one offspring was affected and the biochemical evaluation of the parents did not show any specific abnormality related to GS. These families do not afford useful information on the mode of transmission of the disease because this situation could be explained both as a new mutation of an autosomal dominant gene and as homozygosity for an autosomal recessive form.

Our study also provides some new data on the biochemical and intracelluar alterations in GS. Experimental hypomagnesemia promotes both tubular calcium reabsorption and potassium depletion [12–14], whereas elevated extracellular magnesium results in an inhibition of tubular calcium reabsorption [14]. It has therefore been suggested that in GS hypokalemia and hypocalciuria are secondary to hypomagnesemia [5]. We observed asymptomatic hypomagnesemia and hypocalciuria without any associated hypokalemia in three healthy members of a family with dominantly transmitted GS. We speculate that in GS both hypomagnesemia and hypocalciuria may precede potassium deficiency. Not surprisingly, therefore, correction of hypomagnesemia in GS corrects only the tendency to potassium deficiency [5] without any consistent influence on urinary calcium excretion [3], indicating that hypomagnesemia is not the cause of hypocalciuria [3]. However, the pathogenesis of GS is still unclear and this suggestion needs further evaluation.

Muscular weakness and tetanic episodes, the main clinical features of GS, have been imputed not only to hypokalemia and hypomagnesemia but also to a reduced skeletal muscle content of the mentioned ions [15, 16]. Intracellular values of magnesium and potassium, which have not previously been investigated in GS, were depleted in our patients, at least as far as lymphocytic content is concerned. Intralymphocytic magnesium has been demonstrated to correlate well with the cation content in muscle and bone [17, 18], two compartments containing 80% of whole body magnesium [19, 20]. Other studies have shown that intraerythrocytic magnesium and potassium do not reflect intracellular content as intralymphocytic content [10, 18], and this may

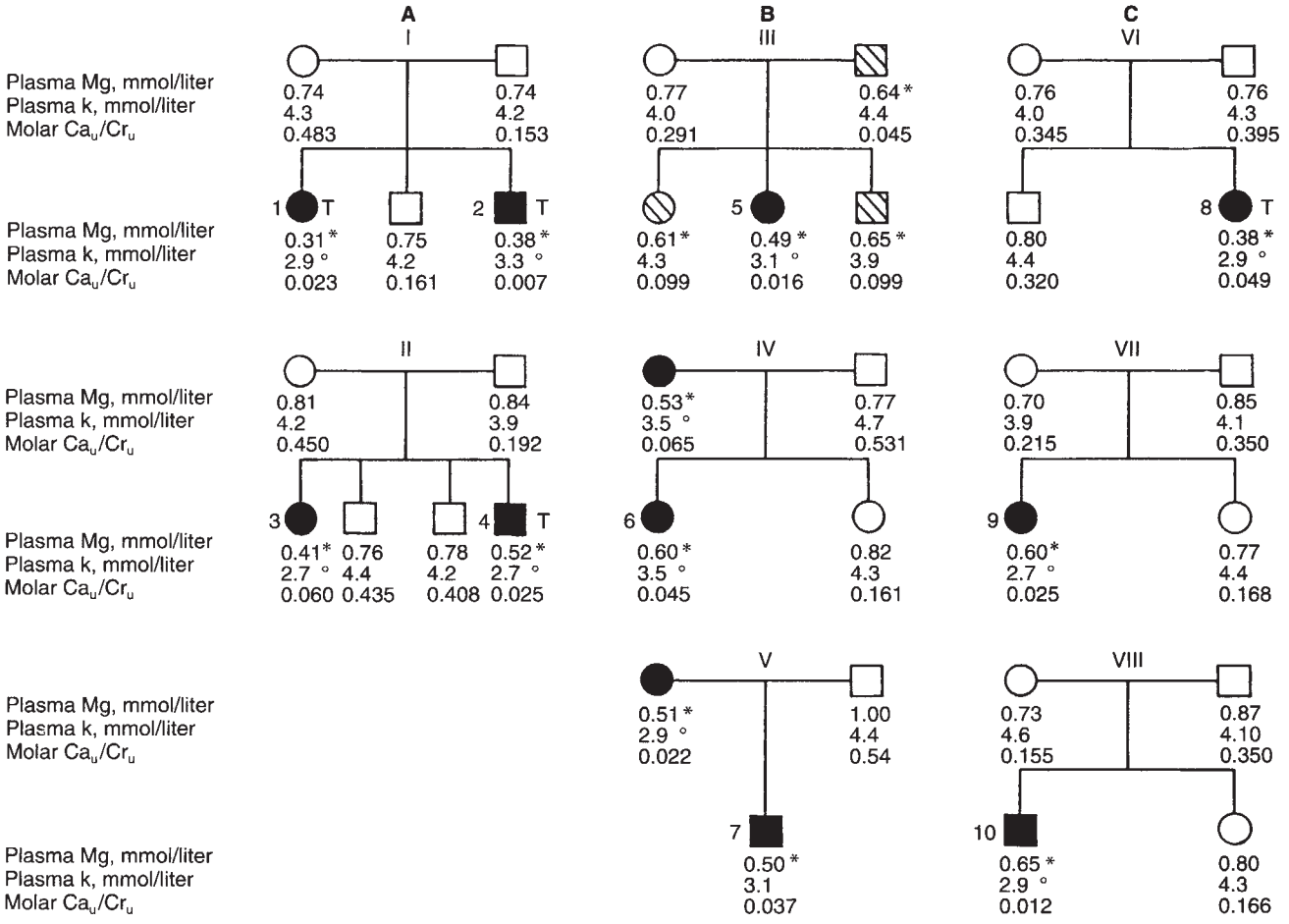

Fig. 1. *Subdivision of 8 families with GS according to the hypothesis of genetic transmission.* Symbols are: (■ and ●) subjects with hypomagnesemia (plasma magnesium < 0.65 mmol/liter), hypokalemia (plasma potassium < 3.6 mmol/liter) and hypocalciuria (molar urinary calcium over creatinine < 0.10); (▧ and ▨) subjects with only hypomagnesemia and hypocalciuria. F = frank tetany. Plasma magnesium, potassium and molar urinary calcium/creatinine ratio are reported for all studied subjects. *Fractional excretion of magnesium > 4.0%; ° fractional excretion of potassium > 16%.

explain the absence of intraerythrocytic differences between our patients and the other groups studied.

In conclusion, our data in eight families provide evidence for a genetic heterogeneity of GS with the existence of at least two different forms: one with autosomal recessive inheritance presenting with frequent tetanic episodes and lower plasma potassium and magnesium levels, and a new one with autosomal dominant inheritance presenting only with mild muscular weakness or even without any symptoms. It is relatively rare but not unknown for the same disease to be transmitted in both dominant and recessive fashions, and molecular biology studies are required to resolve this question.

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