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Case Report

A pseudo-dominant form of Gitelman's syndrome

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Abstract
Gitelman's syndrome is an autosomal recessive salt losing nephropathy caused by inactivated mutations of the *SLC12A3* gene, encoding the NaCl cotransporter of the distal convoluted tubule. We report a French family with five affected members over two generations suggesting a dominant transmission. After *SLC12A3* sequencing of seven individuals, four mutations were detected. Pseudo-dominant transmission was explained by the union of a compound heterozygous woman (two mutations on one allele and one mutation on the other) with a heterozygous healthy man. This study shows the importance of complete genetic analysis of families with unusual presentation.

Keywords: Gitelman syndrome; mutation in *cis*; mutation in *trans*; pseudo-dominant inheritance

Background

Gitelman's syndrome (GS; OMIM 263800) is an autosomal recessive disease characterized by a defect in renal tubular sodium reabsorption with secondary hyperaldosteronism, renal hypokalaemia metabolic alkalosis, hypomagnesaemia and hypocalciuria [1]. GS results from inactivated mutations of the *SLC12A3* gene, encoding the NaCl cotransporter of the distal convoluted tubule [2]. Most subjects carry two different heterozygous mutations inherited from each parent. Estimated prevalence of GS and of heterozygous carriers is ~1/40 000 and 1/100 in the Caucasian population [3]. We report phenotypic and genotypic data of a French family with two unusual features: five members affected over two generations and the presence of four different mutations.

Case report

A 20-year-old man (Patient II.2, Figure 1) was referred for severe hypokalaemia (2 mmol/L) with hypomagnesaemia discovered 2 months before. He reported cramps, episodes of tetany and weakness. He had no past medical history, particularly, no prematurity or growth retardation. He was treated with potassium and magnesium supplementation. Normal blood pressure, orthostatic tachycardia, hyperreninaemic hyperaldosteronism, hypokalaemia, hypomagnesaemia, metabolic alkalosis and high urinary excretion of NaCl, K and Mg were confirmed. There was no polyuria or hypercalciuria.

Familial history of hypokalaemia was present in his mother, his maternal aunt and one of his maternal uncles. We first investigated his mother and then five other family members. Clinical and biochemical data are summarized in Table 1.

The phenotypes of Patients I.1, I.3, I.4, II.1 and II.2 were similar and strongly suggested GS. Genetic investigations were performed after patients' informed consent was obtained. DNA was extracted from blood leucocytes by standard procedures. Mutation analysis was performed by polymerase chain reaction amplification and direct sequencing of the *SLC12A3* gene, as previously described [4]. Molecular genetic analysis showed the following results (Figure 1).

The proband (Patient II.2) was compound heterozygous for one frameshift mutation in exon 10 (c.1196_1202dup, p.Ser402X, mutation *a*) and one splice-site mutation in intron 23 (c.2747 + 1G > A, mutation *b*). For his mother (Patient I.3), we first analysed Exons 10 and 23 but only mutation *b* was detected. Analysis of the remaining exons revealed two additional mutations: one frameshift mutation in Exon 14 (c.1805_1806del, p.Tyr602CysfsX31, mutation *c*) and another splice mutation in intron 22 (c.2660 + 1G > A, mutation *d*). Patients I.1 and I.4 carried the same three mutations as Patient I.3. Thus, Patient II.1 carried heterozygous mutations *a*, *c* and *d*. The healthy father (Patient I.2) was heterozygous for mutation *a*, and the healthy sister (Patient II.3) was heterozygous for mutations *c* and *d*. From these results, we deduced that mutations *c* and *d* were on the same allele (i.e. in *cis*) (Figure 1). Mutations *a* and *c* have been previously described [5, 6]. Mutation *b* has been

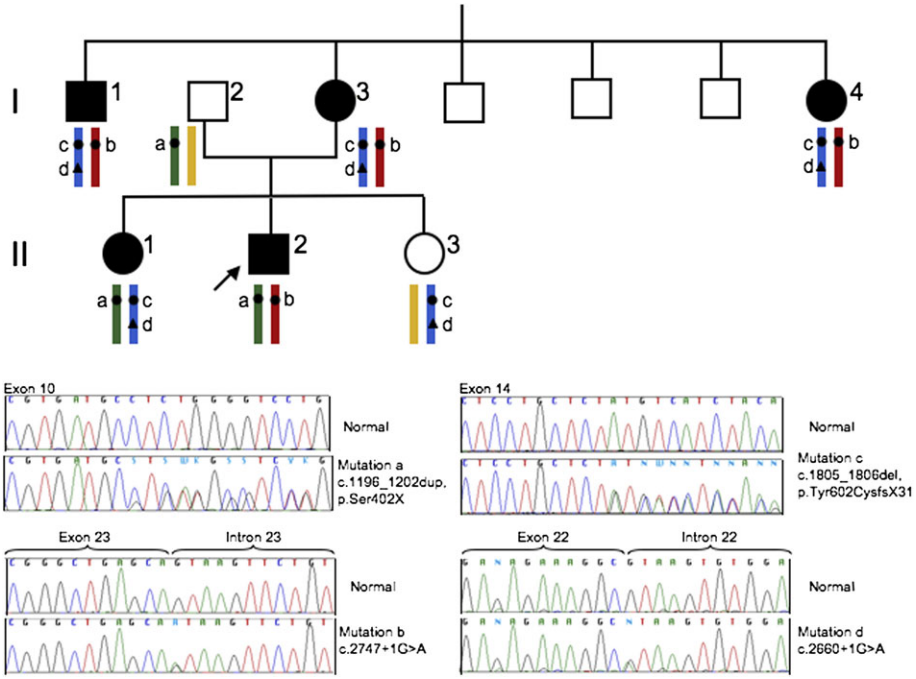

Fig. 1. Family tree illustrating the transmission of GS over two generations. Sequencing chromatograms and segregation of the four detected mutations in the *SLC12A3* gene are shown. Affected subjects are represented by dark symbols. The index patient is II.2. Mutations *c* and *d* are on the same allele.

detected only in the proband of this family in our cohort [4] and finally, this is the first description of mutation *d*. We did not perform RNA analysis, but mutation *d* was not detected in 200 normal chromosomes and four different bioinformatic methods integrated in the Alamut V.2 software predict a loss of donor splice-site (Interactive Biosoftware, Rouen, France; http://www.interactivebiosoftware.com/).

Discussion

We report a French non-consanguineous family with apparent autosomal-dominant transmission of GS with five cases over two generations. Direct *SLC12A3* sequencing has allowed us to show that the dominant-like transmission of the disease was explained by the union of a compound heterozygous woman, carrying two mutations on one allele and another mutation on the other, with a healthy man heterozygous for the mutation p.Ser402X. Consequently, the proband and his affected sister differed by the allele transmitted by their mother. An apparent dominant transmission of GS has been previously reported. Bettinelli *et al.* [7] in 1995 hypothesized that two different genetic transmissions of GS exist, autosomal recessive or autosomal dominant. Subsequently, the molecular analysis of the family described by Bettinelli *et al.* and another similar family showed that the affected parent was compound heterozygous with two distinct mutations and the other parent was heterozygous for a third mutation [5, 8]. It is well established that GS is transmitted as an autosomal recessive trait, and patients presenting typical GS phenotype are homozygous or compound heterozygous (i.e. bearing two pathogenic mutations in *trans*). The estimated prevalence of *SLC12A3* heterozygous carriers is ~1% [3]. Recently, this prevalence was estimated to 0.48% in unrelated subjects of the Framingham Heart study population [9]. Hence, in non-consanguineous families, the theoretical probability to have an affected child when one of the parents has GS is at least 1/200 and for a couple of one heterozygous carrier and one homozygous or compound heterozygous GS patient, as in the family described here, the probability to have an affected child is 50%.

The four mutations detected in this family are two frameshift and two splice-site mutations probably resulting in unstable/abnormal messenger RNAs or truncated proteins. Interestingly, two of them are located on the same allele. In GS and other recessive diseases such as cystic fibrosis, two mutations on the same allele have been described; they are often missense mutations, which could be either a frequent not pathogenic variant or be functional and contribute to the phenotype severity [10–12]. Here, the two mutations detected on the same allele are pathogenic and rare. Indeed, in our cohort of 396 GS patients harbouring *SLC12A3* mutations [4], mutation *c* was detected in only one other proband and for mutation *d*, it is the first ever description. This finding of triple pathogenic mutations appears to be a rare phenomenon (0.7% in our cohort) but illustrates the importance of sequencing all the exons for probands and parents to check the segregation and to determine if the mutations are located in *cis* or in *trans*. Otherwise, individuals with two mutations on the same allele, as sister II.3 in this family, could be wrongly considered as affected.

In summary, complete phenotypic and genotypic characterization are critical to define autosomal recessive diseases with vertical transmission. The probability of a union between a GS-affected subject and a healthy heterozygous subject must be considered to provide accurate genetic advice.

Table 1. Clinical and biological data of the five GS patients and the two heterozygous carriers

	Standards	II-2 (proband)	II-1 (affected sister)	II-3 (unaffected sister)	I-1 (affected uncle)	I-2 (father)	I-3 (mother)	I-4 (affected aunt)
Age, years		20	24	19	52	52	51	41
Age at hypokalaemia detection, years		20	24		42		47	21
Medical history and symptoms		Cramps, tetany and weakness	Tetany and weakness		Faintness and tetany at 42	Hypertension	Pre-term, generalized seizures at 31 and 41, transient right hemiparesis at 47. Normal pregnancies	Generalized seizures
Treatment (dose/day)								
Potassium		50 mmol	90 mmol		100 mmol		70 mmol	40 mmol
Magnesium		15 mmol			10 mmol			5.5 mmol
Spironolactone					100 mg			
Recumbent blood pressure, mmHg (cardiac frequency, bpm)		112/55 (46)	105/53 (52)		119/68 (55)	132/78 (47)	114/60 (59)	110/60 (72)
Standing blood pressure, mmHg (cardiac frequency, b.p.m.)		110/54 (115)	118/63 (93)		87/43 (66)	136/84 (52)	116/63 (79)	63/37 (106)
Plasma parameters								
Sodium, mmol/L	135–145	140	140	137	138	140	139	137
Potassium, mmol/L	3.5–4.5	2.6	2.4	3.4[a]	2.5	4.3	2.2	2.5
Chloride, mmol/L	95–105	94	99	101	92	103	95	94
Bicarbonate, mmol/L	22–27	33	29	26	37	29	35	36
Magnesium, mmol/L	0.64–0.90	0.52	0.62	0.90	0.49	0.87	0.58	0.64
Standing renin, mU/L	15–50	272	197		133		124	104
Standing aldosterone, pmol/L	208–1000	943	1961		1488		596	917
Urine parameters								
Urine volume, L/24 h		2.01	0.76		1.93		2.00	1.99
Sodium, mmol/24 h	<10 if NaCl depletion	258	123		245		168	247
Potassium, mmol/24 h	<20 if K depletion	119	87		179		132	127
Chloride, mmol/24 h	<10 if NaCl depletion	286	106		345		251	324
Magnesium, mmol/24 h	<1 if Mg depletion	2.9	0.7		6.2		3.8	2.1
Calcium, mmol/24 h	Man <7.5, woman <6.25	2.5	0.1		5.8		3.8	3.4
Creatinine mmol/kg/day	Men 0.17–0.23, women 0.12–0.19	0.28	0.17		0.17		0.12	0.11

[a]Plasma potassium concentration 1 year before was normal.

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Conflict of interest statement. None declared.

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