The mutation c.1196_1202dup7bp (p.Ser402X) in the SLC12A3 gene clusters in Italian Gitelman syndrome patients and reflects the presence of a common ancestor

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Abstract

Background. Inactivating mutations in the SLC12A3 gene are the main cause of Gitelman’s syndrome (GS), a renal tubular disorder inherited as an autosomal recessive trait. In our cohort of patients, we identified 11 probands from 11 apparently unrelated Italian families that carry the c.1196_1202dup7bp mutation, which appears to be more frequent than other mutations in Italian GS patients. Therefore, we characterized in greater detail the SLC12A3 locus and its vicinity in those patients that carry this mutation in order to detect a possible shared haplotype. Three further probands characterized in France, carrying the same mutation, were also included in this study.

Methods. Sequence or fragment analyses were carried out to investigate seven intragenic polymorphisms (rs3217425, rs381619, rs2304483, rs2278489, rs2289116 and rs2289115) that flank the mutation, as well as two extragenic markers, D16S3071 and D16S3057, flanking the SLC12A3 locus in the 5’ and 3’ termini, respectively.

Results. A shared haplotype co-segregates with the mutation both in Italian and French probands. Moreover, all the Italian families originate from a restricted area of Italy. Likewise, the French probands come from an area of France close to the north of Italy.

Conclusion. It is likely that the c.1196_1202dup7bp mutation in the SLC12A3 gene reflects the presence of a common ancestor in an area covering the northern-central part of Italy and eastern France. A modified genotyping strategy for GS patients originating from this area has to be considered.

Keywords: common ancestor; Gitelman’s syndrome; population-specific mutation

Introduction

Gitelman’s syndrome (GS) (OMIM#263800), the most common hereditary renal tubulopathy, is characterized by salt losing, hypokalaemic metabolic alkalosis, secondary hyperreninaemia and hyperaldosteronism, normal–low blood pressure and an ~1% prevalence of heterozygotes in the European population [1].

The gene implicated in most cases of GS, SLC12A3 (OMIM*600968), is located on chromosome 16 and encodes the thiazide-sensitive Na/Cl co-transporter (NCC) [2], which is expressed at the apical membrane of the distal convoluted tubule in the kidney [3]. GS is an autosomal disease recessively inherited and caused by inactivating mutations, of which more than 190 have been documented.

In our cohort of Italian patients with a clinical and molecular diagnosis of GS (103 subjects), 75 different mutations including missense, nonsense, duplication, deletion and splice-site mutations have been detected which were spread throughout the gene without concentrating in any hot spot. The most frequent mutation identified is a duplication of 7 bp in exon 10, c.1196_1202dupGTGATGC, which leads to a premature termination codon: p.Ser402X.

We identified 14 alleles carrying this duplication (GSdup alleles) in 11 patients. The higher frequency of
the c.1196_1202dup7bp mutation, 6.8% of all mutated alleles identified in Italian GS patients, compared with other populations, raised the question of the presence of a common ancestor. Thus, the aim of the study was to perform an extensive genotyping using seven intragenic polymorphic markers flanking exon 10 and two extragenic markers in order to detect a possible shared haplotype among the patients and evaluate if the hypothesis of a founder effect is well-grounded.

We included in this study two French patients who were previously reported to carry this duplication, the mutation being described as c1228insGTGATGC according to a previous nomenclature [4]. A third French patient was included, who has never been described. We confirmed the presence of the duplication in one allele in each subject. Unfortunately, it was not possible to trace an additional proband carrying the same mutation described in a North American study [5].

Materials and methods

Ten Italian (one was not available for analysis) and three French GS patients, collected between 1997 and 2007, carrying the c.1196_1202dupGTGATGC mutation on 16 alleles totally were investigated. All the patients had chronic hypokalaemia of renal origin, normal blood pressure and a clinical history consistent with GS. The mutations of the Italian and French patients are summarized in Table 1. The 10 pairs of parents and one sibling of each of three probands (A, H, J) were also analysed in order to define the haplotype inheritance. The segregation analysis was performed in two of the French probands, as for the third proband, the parents were not available for analysis.

The extensive genotyping included seven intragenic polymorphic markers flanking exon 10 and two extragenic markers. The selected intragenic single-nucleotide polymorphisms (SNPs), rs3816119, rs2304483, rs2278490, rs2278489, rs2289116 and rs2289115, and the multiallelic tetranucleotide repeat, rs3127425, are located in intronic regions. D16S3071 and D16S3057 are ~200 kb proximally and 600 kb distally located to the SLC12A3 locus, respectively (Figure 1). On the Généthon map, they are localized to 73.5 and 75.5 cM, respectively.

The markers and SNPs were also studied in 55 healthy controls and 60 GS patients with mutations other than the 7-bp duplication (GSmut). Genomic DNA was extracted from peripheral white blood cells by standard methods, and specific primer pairs were used (Supplementary Table 1) to amplify the regions of interest by polymerase chain reaction. Sequencing and fragment analysis were evaluated on an Applied Biosystems 3130xl (Applied Biosystems, Foster City, CA, USA) and analysed with the Sequencing Analysis 3.7 and GeneMapper softwares, respectively.

The nomenclature is based on the recommendations of the Human Genomic Variant Society, using the nomenclature of Reference Sequence NM_000339. Statistical significance was estimated using the chi-square test.

Results

We first investigated the place of origin of the families and discovered a common and restricted geographical origin including Lombardy, Emilia Romagna and Tuscany for the Italian patients (seven, one and two families, respectively) and Lyon and Grenoble for the French patients (Figure 2). Comparing the number of the GSdup alleles with the GSmut alleles originating from northern Italy, the frequency increased to 19.54%. The healthy controls showed similar allelic distribution frequencies for the multiallelic markers and SNPs regardless of geographic origin in Italy (data not shown). Neither did the geographic origin influence the pattern of allelic distribution among the GSmut patients, except for the two SNPs that flank exon 25, rs2289116 and rs2289115. The GSmut patients from the northern or the rest of Italy carried mainly the reference or minor allele in these SNPs, respectively (data not shown).

Because of the bias in allelic distribution, the GSdup results presented here are compared with the results obtained from northern Italy only. Thus, the control groups were restricted to 25 healthy subjects (50 alleles) and 30 GSmut patients (60 alleles) plus 10 second GSmut alleles of the Italian and French compound heterozygous GSdup patients, for a total of 70 alleles.

Seven intragenic markers flanking the duplication and two extragenic markers flanking the SLC12A3 locus were studied.

There was a significantly different distribution among the groups for the three multiallelic markers rs3217425, D16S3071 and D16S3057, P < 0.001, P < 0.001 and P < 0.05, respectively. The intragenic marker rs3217425 in intron 8 (heterozygosity of 70.8%) showed only the 258-bp fragment in the GSdup alleles, whereas the extragenic markers D16S3071 (heterozygosity of 77.2%) and D16S3057 (heterozygosity 68.2%) revealed the 86-bp allele

| Table 1. Mutations identified in the 13 GSdup patients studied (A–M) |

<table>
<thead>
<tr>
<th>Patient</th>
<th>Mutation</th>
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<tbody>
<tr>
<td>A</td>
<td>c.[1196_1202dup7bp]+c.[1196_1202dup7bp]</td>
</tr>
<tr>
<td>B*</td>
<td>c.[1196_1202dup7bp]+c.[1196_1202dup7bp]</td>
</tr>
<tr>
<td>C</td>
<td>c.[1196_1202dup7bp]+c.[1196_1202dup7bp]</td>
</tr>
<tr>
<td>D</td>
<td>c.[1196_1202dup7bp]+c.[1924C&gt;G]</td>
</tr>
<tr>
<td>E</td>
<td>c.[1196_1202dup7bp]+c.[20_21delCA]</td>
</tr>
<tr>
<td>F</td>
<td>c.[1196_1202dup7bp]+c.[20_21delCA]</td>
</tr>
<tr>
<td>G</td>
<td>c.[1196_1202dup7bp]+c.[20_21delCA]</td>
</tr>
<tr>
<td>H</td>
<td>c.[1196_1202dup7bp]+c.[20_21delCA]</td>
</tr>
<tr>
<td>I</td>
<td>c.[1196_1202dup7bp]+c.[20_21delCA]</td>
</tr>
<tr>
<td>J</td>
<td>c.[1196_1202dup7bp]+c.[20_21delCA]</td>
</tr>
<tr>
<td>K</td>
<td>c.[1196_1202dup7bp]+c.[20_21delCA]</td>
</tr>
<tr>
<td>L</td>
<td>c.[1196_1202dup7bp]+c.[20_21delCA]</td>
</tr>
<tr>
<td>M</td>
<td>c.[1196_1202dup7bp]+c.[20_21delCA]</td>
</tr>
</tbody>
</table>

*Patient B has an affected symptomatic mother with GS genotype c.[1196_1202dup7bp]+c.[2981G>A].
and the two fragments 195 bp (80%) and 201 bp (20%), respectively (Supplementary Figure 1a–c).

The six remaining markers are bi/triallelic and localized in introns 10, 13, 20, 24 and 25 downstream of the duplication mutation. All GSdup alleles shared the same allelic variant; the distribution of the alleles in healthy controls, GSmut and GSdup reached a statistically significant difference for three of the SNPs: rs2304483 (P < 0.001), rs2278489 (P < 0.001) and rs2289115 (P < 0.01) in introns 13, 20 and 25, respectively (Supplementary Table 2).

The results obtained from the extensive genotyping with polymorphic markers allowed us to identify a conserved haplotype, 86bp-258bp-c.1196_1202dup7bp-C-T -C-T -G-C (Figure 1), spanning from the D16S3071 5′ extragenic locus to the 3′ intragenic rs2289115 marker and in linkage disequilibrium with the mutation in the Italian families and one F-

![Image](https://example.com/image.png)

**Fig. 1.** Genetic markers used for segregation analysis of the SLC12A3 locus and frequencies of polymorphic markers in the GSdup, GSmut and healthy control allele groups. Schematic representation of the positions of extragenic and intragenic (1–7) markers (box) in relation to the SLC12A3 locus and the 26 exons (ticks), respectively (short arrows), and the c.1196_1202dup7bp mutation in exon 10 (long arrow). Superscripted letter a denotes shared haplotype (bold) constituted by the polymorphism identified for each marker and superscripted letter b denotes relative frequencies of each polymorphism in the three groups. Statistical significance: ***P < 0.01, ****P < 0.001 (chi-square test).

![Image](https://example.com/image.png)

**Fig. 2.** Geographic origin of the 10 Italian and 3 French GS patients studied (black circles) that carry the c.1196_1202dup7bp mutation in the SLC12A3 gene.
French family. It could be traced in the available heterozygous parents and in the siblings who had inherited the mutation. In contrast to the GS<sup>mut</sup> alleles, the GS<sup>sup</sup> and wild-type alleles showed random haplotypes as shown in the examples of two affected families and one healthy family in Figure 3. The two remaining French GS patients for whom the phases could not be established by means of uninformative/unavailable parents' analysis did not give any results in contrast to our findings.

One healthy control was shown to carry the shared haplotype without the duplication in one allele; it was not possible to exclude its presence in three other controls or in one GS<sup>mut</sup> patient since we could not establish the phases as one or both parents were not available for analysis.

**Discussion**

We here describe the frameshift mutation c.1196_1202dup7bp (p.Ser402X) in exon 10 of the SLC12A3 gene, which is one of the most frequent among Italian GS patients, representing 6.8% of all of the variants we have identified. It does not seem to be particularly frequent in other populations because, as far as we know, it has been found in four alleles of four GS patients.

This mutation must be regarded as pathogenetic as the duplication creates a stop codon in amino acid residue 402, i.e. before the two crucial N-glycosylation sites Asn406 and Asn426; the importance of the glycosylation for the protein function has been demonstrated in vitro for mouse [6], rat [7] and human [8] NCC. Functional expression studies on *X. laevis* oocytes of N-glycosylation-deprived proteins show a decreased <sup>22</sup>Na<sup>+</sup> uptake, demonstrating that N-glycosylation is essential for biologic activity, protein folding and cell surface expression. The introduction of a stop codon could also lead to degradation of the corresponding transcript by the process of nonsense-mediated mRNA decay [9].

The duplication segregates with a unique haplotype, 86bp-258bp-c.1196_1202-C-T -C-T -G-C, identified by eight out of nine markers analysed. The extragenic marker D16S3057, ~600 kb downstream the 3' end of the gene, is no longer conserved in the probands. Of the two alleles present in the GS<sup>sup</sup> subjects in this marker, 195 and 201 bp, most probably, the more frequent (195 bp) is the ancestral one. The appearance of the 201-bp allele in the cohort ought to be a subsequent evolutionary divergence and its introduction sets the limit in the 3' end of the haplotype shared. Thus, the unit of conserved transmitted DNA that harbours the duplication extends at least 255 kb (55 kb of the SLC12A3 gene plus 200 kb upstream the locus).

Although our laboratory receives samples from all over Italy, the subjects carrying c.1196_1202dup7bp all come from the north and central part of the country. The haplotype could not be excluded in two French probands; it was confirmed in the only case for which the parents of the proband were available and informative. Actually, the results obtained never contradicted our hypothesis on a common conserved haplotype. The French patients come from a region relatively nearby Italy (Lyon and Grenoble), thus extending the area where the mutation is concentrated (Figure 2). In fact, the allelic frequency increases to 19.54% among GS patients that origin from this part of Italy. As a consequence, since one out of five of northern Italy GS patients is expected to carry the GS<sup>dup</sup> mutation, the first mutational analysis in these subjects has to focus on exon 10. The method of choice remains sequencing analysis and not a more rapid screening method based on restriction enzymes or allele-specific oligonucleotide, as in this geographic area, exon 10 also presents other mutations as well (data not shown).
The mutation c.1196_1202dup7bp (p.Ser402X) in the SLC12A3 gene

Even though the area in which the mutation appears is extended beyond the Alps, the low number of French probands studied does not permit to draw any similar conclusions regarding a possible local genotyping strategy.

The haplotype has been identified in one healthy subject, suggesting that it is not confined to the GS community; we have no sufficient data to assert that the founder originates from this area, but it is conceivable that the major concentration of mutants in northern Italy is due to the offspring of a common ancestor (identity by descent). If the haplotype discovered in this control subject is not related to the ancestral chromosome on which the mutation occurred, its presence should result from evolutionary events of different origins (identity by state).

The shared mutated haplotype of the 13 GSdup subjects reflects the presence of a founder mutation that excludes the alternative hypothesis of a DNA region especially inclined to mutations and thus supports previous reports regarding the absence of mutational hot spots in the SLC12A3 gene [10]. Not only the observation made here is consistent with earlier studies regarding population-specific mutations in GS: the splice-site mutation IVS9(+1)g>t among Roma derives from a common ancestor [11], as does the deletion mutation of exons 1–7 in the Amish [12]; the missense mutation Thr60Met seems to be common among Japanese and Chinese patients but has not been identified in other populations [13,14]. It would be interesting to clarify if also this latter mutation might be the result of a founder.

For some disease traits, a founder mutation preserved in evolution proved beneficial since an allele with a specific mutation conferred some survival advantage. Well-known examples are the sickle cell disease caused by mutations of the HbS gene or the case of thrombosis caused by a mutation in the Factor V Leiden gene where subjects carrying one particular mutated allele are protected against malaria or systemic bacterial infection, respectively [15,16].

Whether the mutation reported here is of any particular advantage is not known; GS patients are protected from developing hypertension [12], but it is worth noting that the carrier state may benefit from this protection [17,18].

The data considered here support our hypothesis that the c.1196_1202dup7bp mutation in exon 10 of the SLC12A3 gene found in Italian GS patients is the effect of a founding mutation that occurred in a common ancestor and propagated in an area ranging from northern Italy to eastern France near Italy. Besides the speculative interest of our observation, there are practical implications. Since one out of five GS patients from northern Italy is expected to carry the GSdup mutation, the genotyping of any patient originating from this area should focus at first on exon 10.

Supplementary data

Supplementary data is available online at http://ndt.oxfordjournals.org.

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Conflict of interest statement. None declared.

References


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