Molecular analysis of the SLC22A12 (URAT1) gene in patients with primary gout

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Objective. To analyse the SLC22A12 (URAT1) gene in primary gout patients, first-grade relatives and healthy controls and the possible association of them with demographic and clinical data. Subjects and Methods. We included 69 consecutive patients with diagnosis of primary gout, as well as 29 first-grade relatives and 120 healthy volunteers. Demographic and clinical data were obtained from the patients and relatives. DNA was purified from peripheral blood and all 10 exons of the SLC22A12 (URAT1) gene were sequenced. Results. We found six different mutations in the SLC22A12 gene in 16 out of 69 (23%) patients with primary gout. Five mutations were in exon 5 and one in exon 4; five out of six mutations were heterozygous (one compound heterozygous) and one homozygous. The C850G mutation (exon 5) was found in 11 gout patients, these patients have lower levels of triglycerides than the rest of the group: 160 ± 56 vs 292 ± 203 mg/dl (P = 0.038). In one family, we found SLC22A12 mutations in three relatives within exon 5. We did not find mutations in the other exons studied (1–3 and 6–10), nor in any of the 10 exons of the 120 healthy volunteers. Conclusions. We found several mutations in SLC22A12 gene associated with primary gout, the definite role of these mutations in URAT1 activity needs to be further studied.

Key words: Gout, URAT1, Uric acid, SLC22A12.

Introduction

Urate is the final product of purine metabolism in humans. Approximately 90% of such metabolites are reabsorbed as anions through mechanisms involving transporting molecules at the proximal renal tubule. The urinary excretion of urate depends of several mechanisms, particularly secretion and reabsorption. The latter predominates in humans, dogs and rats [1–6]. Urate reabsorption is mostly determined by urate transporter 1 (URAT1), a 555 amino acid protein that seems to be exclusively localized in the luminal epithelial of the proximal tubule in humans and other species [7, 8]. URAT1 transports and interchanges urate for organic anions, particularly when the intracellular concentration of the latter increases. Agents and drugs with affinity for URAT1 may have paradoxical uricosuric or anti-uricosuric properties when they accumulate in the lumen or within the cell.

Nowadays, URAT1 is considered the most important element in the mechanisms involved in the reabsorption and urinary excretion of uric acid. URAT1 action is directly modified by organic anions (i.e. lactate) and several uricosuric or anti-uricosuric drugs including probenecid, benzobromaron, sulfipyrazone, phenylbutazone, non-steroidal anti-inflammatory drugs, losartan and diuretics [1–7].

Interestingly, testosterone increases the promoter activity of human URAT1 gene [9], and its expression in male mice is 2.3 times higher than in females [8]. On the other hand, the interaction between PDZK1 protein and the PDZ (PSD-95, Dg1A and ZO-1) domain of URAT1 in the C-terminal region regulates its function [10].

URAT1 is encoded by the SLC22A12 gene (11q13), which is constituted by 2642 base pairs in 10 exons. While several SLC22A12 gene mutations have been found in Japanese patients with idiopathic renal hypouricaemia (IRH), the healthy Japanese also have various polymorphisms of the same gene [7, 11, 12]. Gout is a multifactorial metabolic disease characterized by hyperuricaemia and monosodium urate (MSU) crystals-induced synovitis. Since 25–40% of the patients have a positive familial history of the disease, a number of studies have searched for enzymatic deficiencies or disease associations with human leucocyte antigen markers [13], apolipoprotein phenotypes [14, 15] and the methylene tetrahydrofolate reductase gene [16], but results are inconsistent thus far. The National Heart, Lung and Blood Institute (NHLBI) Family Heart Study has estimated that the contribution of heredity to the level of uric acid in the serum is around 40%. In the same study, the correlation coefficient among siblings was 22% [17].

Approximately 90% of asymptomatic hyperuricaemics and patients with gout are uric acid under-excretors, which is probably due to an abnormal functioning of the proximal tubule that also reduces the excretion of xanthines, phosphates and other anions [1–6, 18]. In this context, we hypothesized that URAT1 may play a role in urate renal under-excretion in patients with primary gout and, therefore, looked for the presence of mutations in the SLC22A12 gene.

Subjects and methods

We included 69 non-related, consecutive out-patients with primary gout according to the American College of...
Rheumatology diagnostic criteria [19]. At the time of the study, most patients were not on a regular treatment of the disease, and none of them received losartan, thiazide drugs or fenofibrate. We also invited to participate in the study healthy first-degree relatives (parents, siblings or their offspring) of the patients and 120 healthy controls with no personal or familial history of hyperuricaemia or gout. All patients, relatives and healthy controls were of the same predominant genetic background (Mexican mestizos). The protocol of this research was approved by the Institutional Review Board of our institution and all participants received information about the study, agreed and signed an informed consent.

Demographic, medical history, therapeutic and clinical data were obtained from all individuals. Blood chemistry, lipid profile, creatinine clearance (CrCl) adjusted to 1.73 m² body surface and urinary uric acid excretion in 24 h urine sampling (adjusted/C6) were examined by standard methods. Hyperuricaemia was defined as the level of serum uric acid in males and females. Adjusted urate excretion (FEUA) was calculated by the Fisher’s exact test, chi-square test and the U Mann–Whitney test. The association between demographic and clinical variables and mutations was calculated by the Fisher’s exact test, chi-square test and the U Mann–Whitney test.

**Results**

Eighty-five percent of the patients were uric acid under-excretors (Mexican mestizos). The protocol of this research was approved by the Institutional Review Board of our institution and all participants received information about the study, agreed and signed an informed consent.

The molecular analysis of the SLC22A12 genes was performed in genomic DNA obtained from the peripheral blood by conventional methods. The polymerase chain reaction (PCR) primers are described in Table 1. In general, PCR consisted of 30 cycles of denaturation at 94 °C for 60, annealing as in Table 1 and extension at 72 °C for 60. Exon amplifying conditions were as follows: DNA 500 ng, primers 0.4 μM, dNTP 0.08 mM, MgCl2 1.5 mM, buffer 1x, Taq Pol 1.5 U, vol 50 μl. PCR products were purified with a PCR purification kit (Qiagen, Valencia, CA). DNA sequencing analysis was performed in ABI PRISM 310 genetic analyser (Applied Biosystems, Foster City, CA) according to the supplier’s conditions. All assays were always performed twice with a normal control included.

**Statistical analysis**

The association between demographic and clinical variables and mutations was calculated by the Fisher’s exact test, chi-square test and the U Mann–Whitney test.

The data discussed in this publication have been deposited in NCBI’s GenBank (http://www.ncbi.nlm.nih.gov/genbank/), and are accessible through accession numbers DQ514592–DQ514596.

**Results**

There were 69 patients with gout (65 males, four females) with uric acid under-excretion in 41% and uric acid urinary under-excretion in 37.6% of the patients’ offspring, 34% were siblings. Their means (S.D.) of ages at onset and at study visit of 37.6 ± 11.5 yrs and 50.5 ± 12 yrs included in the study. While 52% of the patients had tophaceous gout, 20% had typical MSU crystals in the synovial fluid, and 28% fulfilled at least six ACR clinical criteria for the diagnosis of gout. None of the patients had secondary gout.

At inclusion, their means (S.D.) of serum uric acid, CrCl, UCl and FEUA were 0.62 ± 0.17 mg/dl (417 ± 125 μM/l), 76.4 ± 29.7 ml/min, 3.6 ± 1.4 ml/min, and 4.7 ± 2.22%, respectively. Eighty-five percent of the patients were uric acid under-excretors and nine had chronic renal failure diagnosed at least 10 yrs after the onset of gout.

There were 14 males and 15 females [mean age (S.D.) 34.5 ± 13.9 yrs] among first-degree relatives; while 65% of them were the patient’s offspring, 34% were siblings. Their means (S.D.) of serum uric acid, CrCl, UCl and FEUA were 6.4 ± 1.7 mg/dl (381 ± 101 μM/l), 108.4 ± 42.9 ml/min, 6.01 ± 2.99 ml/min and FEUA 4.68 ± 1.51%, respectively. There was asymptomatic hyperuricaemia in 41% and uric acid urinary under-excretion in 40%. The group of healthy controls included 45% of males and 55% of females, whose mean (S.D.) age was 28.8 ± 6.3 yrs.

**Gene analysis**

We found six mutations of SLC22A12 gene in 16/69 (23%) patients with primary gout (Table 2). 11/69 had the C850G mutation. No mutations were found in healthy controls (P = 3 × 10⁻⁸ and 9 × 10⁻⁶, respectively).

Five of these mutations were novel and one was previously reported. One of the mutations occurred within the exon 4 and the others within the exon 5. Five out of the six mutations were heterozygous (one compound heterozygous) and one homozygous. Overall, five of all six are probably de novo mutations.

We found the following molecular defects in the group: (i) one patient showed a novel homozygous single base pair insertion within codon 227 (680insG) producing a premature stop codon; (ii) 11 patients harboured a novel heterozygous missense mutation within codon 284 (C850G) resulting in a substitution of glycine instead of arginine; (iii) two patients had a novel heterozygous missense mutation within codon 305 (T914G) producing a substitution of serine instead of isoleucine and (iv) one patient showed a novel heterozygous missense mutation within codon 297 (C889G) resulting in a substitution of glutamate instead of glutamine (Fig. 1 and Table 2).

Finally, the patient with gout of one family was a compound heterozygote (G868T/G894T) producing a substitution of Cys instead of Gly in codon 290 and Glu instead of Asp in codon 298. Two sisters of this patient had the G894T mutation and her son the G868T mutation (Fig. 1 and Table 2).

There were no mutations in exons 1–3 and 6–10 of the SLC22A12 gene found in the remaining patients. Except for the family described earlier, we did not find mutations in any of the exons (1–10) in the relatives. Neither did we find any mutation in the group of 120 healthy volunteers.

Compared with the group of patients without the mutation, the group of patients with gout having the C850G mutation showed lower mean (S.D.) levels of triglycerides [160 ± 56 mg/dl (1.81 ± 0.63 mM/l)] vs 292 ± 203 mg/dl (3.3 ± 2.9 mM/l).
Patients (n) | Sex | Exon | Nucleotide defect | Codon | Protein effect
--- | --- | --- | --- | --- | ---
11 | M | 5 | C850G | 284 | Arg284→Gly
2 | M | 5 | T914G | 305 | Ile305→Ser
1 | M | 4 | 681InsG | 227 | Ala680→Thr/frameshift
1b | F | 5 | G868T/G894T | 290/298 | Gly290→Cys/Glu298→Asp
Relatives | 2b | F | 5 | G894T | 298 | Glu298→Asp
1b | M | 5 | G868T | 290 | Gly290→Cys

A

C850G

T914G

B

G868T

G894T

C889G

FIG. 1. Electropherograms showing the region of the SLC22A12 (URAT1) gene with the nucleotide changes (underlined). The transversions C850G, T914G (A), G868T, G894T and C889G are shown (B). Heterozygous compound is observed on the left side of B (G868T/G894T).

P = 0.038]; less patients (5/11 vs 45/58; P = 0.05) had hypertriglyceridaemia, and the mean (s.d.) serum uric acid level also were somewhat lower [6.3 ± 1.9 (375 ± 113 μM/l) vs 7.6 ± 2.1 mg/dl (453 ± 125 μM/l)], P = 0.059]. All the other clinical and demographic variables were fairly similar (Table 3). All these differences remained the same after excluding five patients with other mutations.

Discussion

Serum uric acid levels are influenced by genetic and environmental factors. Frameshift and missense mutations in this gene have been described in patients with IRH and in normal populations; homozygous deleterious mutations in the SLC22A12 gene result in IRH [7, 11, 12, 21]. In this study, we analysed all SLC22A12 gene exons in patients with gout, their first-grade relatives and healthy subjects and looked for the association between SLC22A12 gene polymorphisms and serum uric acid levels.

Remarkably, we found five novel mutations located within exons 4 and 5, the gene region that codifies the first part of one of the extracellular loops of the URAT1 protein.

In the molecular analysis of the SLC22A12 gene, we first detected a 1 bp homozygous insertion (680insG) resulting in a frameshift of the open reading frame and in a premature stop codon. A second mutation was observed in a sporadic case who exhibited a novel heterozygous missense mutation (C889G). This mutation produces a substitution of glutamate instead of glutamine, although both are polar amino acids, and the original glutamine presents a positive charge and differs by the presence of an amine group, which confers basic characteristics.
The mutation most frequently found (the C850G mutation) occurred in 11 (16%) patients. This novel heterozygous mutation corresponded to the substitution of glycine instead of arginine. Both amino acids show different polarity and structure. The small glycine is a hydrophobic amino acid that is more frequently present in the intramembrane region than in the cytoplasmatic region. Thus, this change could modify the expression of the ligand in the first part of one of the extracellular loops of the URAT1 protein.

Iwai et al. [12] reported several SLC22A12 gene polymorphisms—most of them in the promoter region or introns—in the healthy Japanese population. One of the limitations of this study was the lack of analysis of SLC22A12 gene promoter, yet we did not find any mutation in the 10 exons analysed in a healthy Mexican population. Although the control group was not age-matched they are comparable, due to the fact that we looked for a genetic marker that was not found in them. If we had found mutations among healthy controls, interpreting the data could be difficult because some of them could develop gout years later.

The G894T mutation that we found in one of the families was previously reported in homozygous state in a patient with IRH. Our patient differed in that he was a heterozygous compound with a G868T/G894T genotype.

The homozygous G774A mutation in exon 4 leads to the substitution of a stop codon for tryptophan. This mutation has been linked to the origin of IRH, but it has also been found in 2.3% of the Japanese healthy population in association with hypouricaemia [12]. The search for G774A mutations in 185 Japanese patients with gout yielded no mutations at all suggesting that the G774A mutation had a rather protective role against the development of gout in men [22]. We did not find the G774A mutation neither in gout nor in healthy subjects.

Interestingly, a recent study [23] reported some polymorphisms of the SLC22A12 gene associated with reduced uric acid excretion and hyperuricaemia, but not specifically gout. Although they did not find any of the mutations described in the Japanese population, the C426T (exon 2) polymorphism was associated with reduced uric acid excretion. In our study, we did not find any mutation in exon 2 in the group of patients with gout.

Unexpectedly, patients with the C850G mutation had lower triglyceride and uric acid levels. These findings suggest an association between some SLC22A12 mutations and other phenotypic characteristics. Several reports coincide that there is a strong association between uric acid and triglyceride levels in patients with gout, nearly 70% of these patients have hypertriglyceridaemia which favours decreased urate renal excretion [14].

Patients with gout have an altered allelic distribution of the apo AI-CIII-AIV cluster [15] which may lead to changes in the lipoproteins levels. Polymorphisms in the same cluster are related to insulin resistance, an event that frequently occurs in gout. It is noteworthy that SLC22A12 and apo AI-CIII-AIV cluster genes are both localized in the short arm of chromosome 11.

Several urate transporters that intercalate urate for organic anions and other substances from the interstitium to intracellular space and to the luminal area have been described. Some of them are located in the apical and others in the basolateral membranes of the proximal tubule. Besides URAT1, there are also other urate transporters as organic anion transporters (OAT1, OAT3 and OAT4), urate transporters (UAT or galectin 9), voltage-driven organic anion transporters (OATV1), a multi-drug resistance protein 4 (MRP4) and urmodulin [1, 2, 5, 6]. Whether mutations in one or more genes, including those codifying these transporters would occur and explain uric acid under-excretion, and the metabolic abnormalities associated with gout is still unknown and needs to be clarified.

Clearly, mutations in the SLC22A12 gene in the Japanese population differ from that in German and Mexican populations. Some of the mutations were found in healthy subjects, but also associated with hypouricaemia and hyperuricaemia. As an explanation to these findings, we could postulate that the mutations in SLC22A12 gene does not always influence the URAT1 activity and, on the other hand, that the activity of URAT1 may depend on alternative splicing or post-translational modifications.

In conclusion, we report five novel mutations in the SLC22A12 gene in patients with gout. None of these mutations segregated in the 240 chromosomes of healthy individuals. All changes were in exons 4 and 5 of the SLC22A12 gene. Four mutations resulted in single amino acid substitutions and one in a frameshift of open reading frame that originates a truncated polypeptide by the introduction of a premature termination codon. The role of these mutations in URAT1 activity needs to be further studied.

The authors have declared no conflicts of interest.

References