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Homozygous frameshift mutation in the *SLC22A12* gene in a patient with primary gout and high levels of serum uric acid

Idiopathic renal hypouricaemia (IRH), an autosomal recessive disorder, is an uncommon disease that presents with an increase in uric acid excretion. Most patients with IRH have homozygous deleterious mutations in the *SLC22A12* gene that encodes for URAT1 protein. Gout is a multifactorial metabolic disease caused by the deposition of urate crystals. This study describes a patient with high levels of uric acid, primary gout and a novel *SLC22A12* gene mutation, which is associated to IRH. The patient showed a 1 bp homozygous insertion (680insG) that resulted in substitution of threonine instead of alanine and in a premature stop codon. This finding provides information about the influence of environmental and/or epigenetic factors in Mendelian inheritance.

Idiopathic renal hypouricaemia (IRH) is an autosomal recessive disorder characterised by increased uric acid excretion.[1] The main biochemical defect is abnormal uric acid transport at the proximal tubule. URAT protein seems to be the major mechanism regulating blood urate levels.[2 3] Most patients with IRH (Online Mendelian Inheritance in Man 607 096) harbour homozygous deleterious mutations in the *SLC22A12* gene, which encodes for URAT1 protein.[2] The major clinical manifestation of IRH is the presence of uric acid stones in the urinary tract.[4] Around 50% of patients with IRH are Japanese.

Gout is a multifactorial metabolic disease caused by the deposition of urate crystals that produce characteristic joint inflammation. Hyperuricaemia is the most important risk factor for gout and, in 90% of patients, is secondary to renal uric acid under-excretion. *SLC22A12* has been studied in patients with gout, the mutation W258X is observed in a healthy population but in none of the patients with gout; the mutation W258X seems to be a suppressing factor for the development of gout.[5] *SLC22A12* polymorphisms have been associated with reduced uric acid excretion and hyperuricaemia in a German population.[6] In a previous study,[7] authorised by the patients and the ethics committee, we did molecular analysis of the *SLC22A12* gene in patients with primary gout, in first-grade relatives and in healthy controls. We found mutations in 16 patients with gout, 3 in first-grade relatives and none in healthy controls. Most of the mutations were heterozygous with one exception, the subject of this report. This finding provides information about the influence of environmental and/or epigenetic factors in the Mendelian inheritance.

Case report and molecular analysis

A 39-year-old man was referred to our rheumatology department with a history of arthritis in the lower limbs. His parents were healthy and non-consanguineous. The other members of his family (wife and two daughters 8 and 10 years old) were healthy. He had no familial history of gout or hyperuricaemia.

Table 1 Primers used in the PCR analysis of exons 3–6 of the *SLC22A12* gene

Region	Primers	length (bp)
Ex3 F Ex3 R	5′-TGG CAA GCC ACA GAC CCT GC-3′ 5′-AGC CCT GGA GCC TGC ATG GA-3′	250
EX4 F EX4 R	5′-TCC ATG CAG GCT CCA GGG CT- 3′ 5′- GGC AGG ATC TCC TCT GAG GA- 3′	254
EX5 F EX5 R	5′-GCC ACA GGC AAT GAC CCC TC-3′ 5′-ACC TTC TTC CCA GGG AGC TG-3′	163
EX6 F EX6 R	5′-TCA GAG AGG AGG AGG TGC CT-3′ 5′-CCA GGT TCC CCT GTG GAG GT-3′	220

The patient had a 6 year history of podagra, acute attacks affecting the knees and ankles, and tophi. At physical examination his height was 1.70 m, weight 73 kg and waist circumference 83 cm; he had no hypertension, history of lithiasis, or chronic renal failure nor previous treatment with diuretics or salycilates.

Laboratory evaluation reported serum uric acid 11.8 mg/dl (702 μmol/l), urinary uric acid 403 mg/24 h (2.39 mmol/d), and uric acid clearance 3.7 ml/min/1.73 m^2, serum creatinine 0.89 mg/dl (78.8 μmol/l), serum urea 36.4 mg/dl (6.07 mmol/l), glucose 102 mg/dl (5.67 mmol/l), triglycerides 413 mg/dl (4.66 mmol/l), cholesterol 203 mg/dl (5.25 mmol/l) and creatinine clearance 82.8 ml/min/1.73 m^2. The patient had hyperuricaemia and hypertriglyceridemia, but no other criteria for metabolic syndrome according to ATP III criteria.

Gout diagnosis was made according to the American College of Rheumatology criteria. The patient received treatment with allopurinol 300 mg/day, colchicine 1 mg/day and fenofibrate 200 mg/day. Laboratory test of his daughters were within normal limits, but unfortunately it was not possible to determine blood and urinary laboratory values in his siblings because they lived in another city.

As part of our previously mentioned study,[7] *SLC22A12* gene was analysed in this patient. DNA extraction from peripheral blood was performed as described elsewhere.[8] All exons of the *SLC22A12* gene were initially analysed through PCR. Conditions and primers to amplify exons 1–2 and 7–10 are described elsewhere,[9] and conditions to amplify exons 3–6 are shown in table 1. In general, PCR consisted of 30 cycles of denaturation at 94℃ for 60 s, annealing at 62℃ and extension at 72℃ for 60 s. PCR products were purified with a PCR purification kit (Qiagen, Valencia, California, USA). DNA sequence analysis was performed using ABI PRISM 310 genetic analyser (Applied Biosystems, Foster City, California, USA) according to manufacturter's conditions. All procedures were performed twice in patients and healthy controls.

Results and discussion

Hyperuricaemia is defined as serum urate concentration >7 mg/dl in men. Genetic and/or environmental factors such as diuretics, obesity and high alcohol intake are necessary for gout expression.[10] In this study, we describe a patient with primary gout, hyperuricaemia and a novel *SLC22A12* gene mutation. SLC22A12 mutations were first associated with hypouricaemia and IRH.

Our patient showed a 1 bp homozygous insertion (680insG) within exon 4 (fig 1) that resulted in substitution of threonine instead of alanine and in frameshift of the open reading frame; this 1 bp insertion generated a TGA

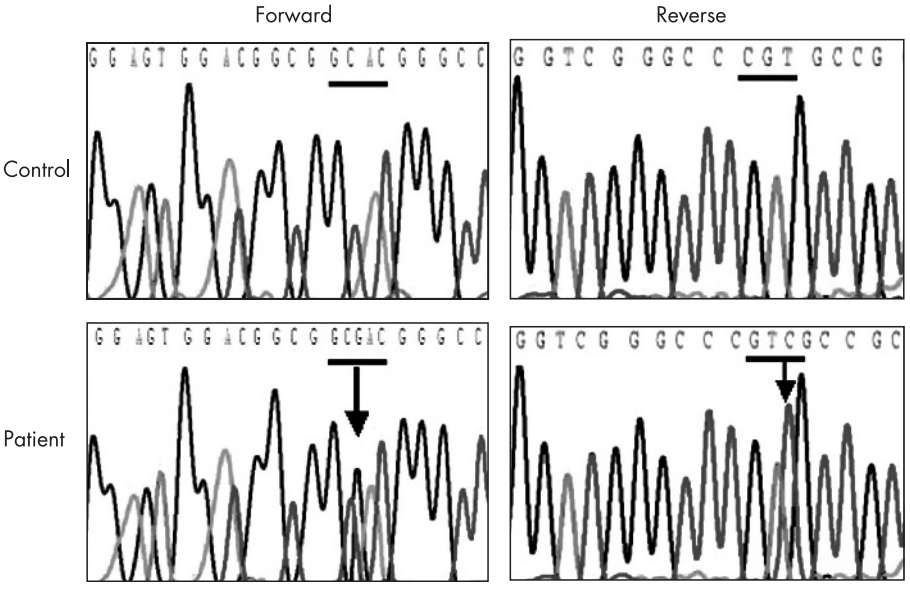

Figure 1 Forward and reverse primers of the partial DNA sequence of exon 4 of the *SLC22A12* gene of the patient with primary gout and the normal control, only a small fragment is shown. Codon 227 with the 1 bp insertion 680Gins is shown underlined.

Take-home messages

- Our patient showed a 1 bp homozygous insertion (680insG) within exon 4 that resulted in substitution of threonine instead of alanine and in frameshift of the open reading frame.
- This 1 bp insertion generated a TGA premature stop codon 91 amino acids downstream from the last original amino acid (Ala 227).
- This mutation 680insG resulted in the subsequent generation of a non-functional short polypeptide (318 amino acids) that lacks its intracellular C-terminal region or well in a null allele.
- The patient had primary gout, hyperuricaemia and a novel *SLC22A12* gene mutation.

premature stop codon 91 amino acids downstream from the last original amino acid (Ala 227). This mutation resulted in the subsequent generation of a non-functional short polypeptide (318 amino acids) that lacks its intracellular C-terminal region or well in a null allele. In this region, URAT1 directly interacts with PDZK1, a PDZ domain-containing protein that interacts with several membrane proteins through its PDZ motif.[11] PDZK1 regulates the functional activity of URAT1-mediated urate transport in the apical membrane of renal proximal tubule.[12] The association of URAT1 with PDZK1 enhances urate transport activities in HEK293 cells (1.4-fold), and the deletion of the patient lacks its PDZ domain. The 680Gins was not present in 240 chromosomes of normal individuals, so it is likely that his abnormal allele represents rare polymorphisms. On the other hand, primary gout phenotype of our patient is not due to this kind of mutation. Our finding that the N-terminus region of the *SLC22A12* gene seems to be associated with reduced renal uric acid is worth mentioning.[6]

Interestingly, the mutation in our patient is similar to that reported for the classic IRH phenotype (W258X); however, our patient had unexpectedly high blood levels of uric acid, primary gout and decreased uric acid clearance. We have no explanation for this finding. We consider that normal levels of uric acid (serum and urinary) and no clinical features in the rest of the members of the family exclude the possibility that the patient has other diseases. We additionally support this because the patient has no other clinical features. The non-penetrant IRH genotype could be attributed to single/multiple Mendelian modifier loci or environmental/epigenetic factors as in other entities.[13–16] It would be interesting to know whether his young daughters will develop gout and hyperuricaemia in the third or fourth decade of life. In conclusion, we report a novel *SLC22A12* deleterious mutation in a patient with high levels of serum uric acid and primary gout. This case illustrates the complexity of the pathogenesis of primary gout and IRH, and possibly of other genetic entities.

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Chronic atrial fibrillation associated with somatic mitochondrial DNA mutations in human atrial tissue

Somatically acquired mitochondrial DNA (mtDNA) mutations have been linked to aging, degenerative diseases, cancer and organ dysfunction. mtDNA alterations were investigated in matched atrial tissues and blood samples from four patients with chronic atrial fibrillation (cAF) and two matched patients without cAF. Nine novel mtDNA mutations were observed in mtDNA control and coding region. Interestingly, two patients with cAF had tissue-specific length heteroplasmic mutations from nucleotide 16184 to 16193 of the polyC tract and CA repeats starting at nucleotide 514. A 9 bp deletion (nucleotides 8271–8279) in the mtDNA COII gene was only found in tissues and blood cells from two patients with cAF. In patients with cAF, mtDNA mutations, including small deletions and tissue-specific length heteroplasmic mutations, occurred in both mtDNA control and coding regions. These findings strongly suggest that mtDNA mutations may play a crucial role in atrial dysfunction in patients with cAF.

Mitochondria are both the power plant of human cells and the target of reactive oxygen species and free radicals. With ageing and excess (ROS) stress, free radicals and (ROS) can overwhelm the antioxidant system and result in damage to cellular constituents such as lipids, proteins and DNA.[1] A few papers have reported that mitochondrial DNA (mtDNA) deletion mutation in human atrial tissue is associated with chronic atrial fibrillation (cAF).[2 3] However, these studies only checked mtDNA deletion in atrial tissue and did not examine the corresponding blood samples. Therefore, we analysed the mtDNA control region and cytochrome c oxidase (CO) I, COII, COIII, ATPase 6 and cytochrome b (Cytb) genes in matched atrial tissues and blood samples from patients with cAF and in two matched patients with normal sinus rhythm and no history of cAF.

Methods

Atrial tissue samples and corresponding blood samples were taken from four patients with cAF and from two matched patients with normal sinus rhythm (see supplementary table 1 available at http://jcp.bmj.com/supplemental). Left atrial appendages were obtained from patients undergoing open heart surgery after institutional review board approval and informed consent had been obtained. They had not taken any drugs that might affect mtDNA mutation.

To amplify and sequence the control region, COI, COII, ATPase 6, COIII and Cytb regions of mtDNA, we used a set of designated primer pairs (supplementary table 2 available at http://jcp.bmj.com/supplemental) and PCR conditions based on a published protocol.[4 5] The mtDNA sequences obtained were compared with the Revised Cambridge Reference Sequence (RCRS) with the Blast2 program and the database search tool, MitoAnalyzer