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Severe Gouty Arthritis and Mild Neurologic Symptoms Due to F199C, a Newly Identified Variant of the Hypoxanthine Guanine Phosphoribosyltransferase

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Abstract

A deficiency in hypoxanthine guanine phosphoribosyltransferase (HPRT) activity leads to overproduction of uric acid. According to the degree of enzymatic deficiency, a large spectrum of neurologic features can also be observed, ranging from mild or no neurologic involvement to complete Lesch-Nyhan disease. Herein, we describe a patient with hyperuricemia, juvenile-onset gouty arthritis, nephrolithiasis, and mild neurologic symptoms, attributed to a newly identified variant of the *hprt* gene, c.596T>G, resulting in the amino acid change p.F199C. Residual HPRT activity (8%) protected against severe neurologic involvement in this patient. Modeling of the mutated protein was used to predict the mechanisms that led to partial enzymatic activity. Careful neurologic examination is warranted in juvenile and middle-aged patients with gout, in order to detect mild symptoms that may lead to a diagnosis of HPRT deficiency.

Gout, defined as monosodium urate monohydrate (MSU) crystal–induced arthritis, is usually idiopathic. However, although it happens rarely, in some patients (young men in particular), the disease can be secondary to a deficiency of hypoxanthine guanine phosphoribosyltransferase (HPRT) (EC 2.4.2.8). HPRT catalyzes the synthesis of IMP and GMP from the purines hypoxanthine and guanine, respectively, in a reaction involving phosphoribosylpyrophosphate (PRPP). These monophosphate nucleotides are then used to synthesize the triphosphate nucleotides ATP and GTP, which are components of DNA and RNA and can act as cofactors or substrates for other enzymes. Thus, HPRT allows the recycling of hypoxanthine and guanine into the pool of purines. In the absence of HPRT, these purines are no longer recycled and are metabolized into uric acid, resulting in chronic and severe hyperuricemia. The *hprt* gene encompasses 9 exons that span ~44 kb of DNA at Xq26.1 and is transcribed into a 1.6-kb messenger RNA that includes a 654-bp coding

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Ceballos-Picot had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Ea, Bardin, Jinnah, Lioté, Ceballos-Picot.

Acquisition of data. Ea, Bardin, Jinnah, Aral, Lioté, Ceballos-Picot.

Analysis and interpretation of data. Ea, Bardin, Aral, Lioté, Ceballos-Picot.

region. In humans, complete HPRT deficiency results in Lesch-Nyhan disease (McKusick 30800; Orpha510) (1), which is characterized by mental retardation, dystonia, and dramatic and compulsive self-injurious behavior.

Partial HPRT deficiency leads to Lesch-Nyhan variants, with mild or no neurologic manifestations. Patients with Lesch-Nyhan disease and those with Lesch-Nyhan variants both have MSU crystal formation that leads to gouty arthritis and arthropathy, tophi, nephrolithiasis, and chronic renal disease. HPRT deficiency is an X-linked recessive trait, and thus, usually only affects males (although 5 cases in females have been described previously) (2). The *hprt* gene mutations are heterogeneous, including point mutations in 63% of patients, deletions in 24%, insertions in 7%, and more complex DNA changes in 6% of patients (2). Herein, we describe a patient with Lesch-Nyhan variants who was found to have an *hprt* mutation that has not been previously reported.

CASE REPORT

The patient, a 43-year-old man, had a history of mild developmental delay. He did not walk until he was 3 years old and, for many months, exhibited toe walking, which eventually resolved, although his gait was never entirely normal. He experienced his first gouty attack at the age of 7 years. Recurrent acute gouty attacks occurred approximately every 3 months and involved the feet, ankles, knees, wrists, and hands. The first tophi were noted when the patient was 23 years old. He also had bilateral nephrolithiasis. Treatment with allopurinol was started when the patient was 10 years old, with a dosage of 600 mg/day maintained since he was 35 years old. Nonsteroidal antiinflammatory drugs were used during gouty attacks, since colchicine was not well tolerated. The patient's father and paternal grandfather also had gout, with onset at ages 45 and 28 years, respectively.

When the patient was evaluated at our department, neurologic examination revealed mild dysarthria, with speech that was slightly indistinct and often associated with overflow activation of the frontalis and platysma muscles. He did not have cervical or truncal dystonia, but his hands and arms exhibited slightly slowed and clumsy movements. His arms also showed dystonic posturing when the gait was stressed. At rest, the arm tone was normal. The patient's gait had a heavy and stiff appearance, with reduced knee flexion but no circumduction. He preferred to reach out to hold the walls and used crutches to stabilize his gait. Muscle stretch reflexes were exaggerated throughout his arms and legs. He had bilateral Hoffmann's sign and a crossed hip adductor reflex, but no ankle clonus. When the patient was at rest in the supine position, his limb tone was normal. He scored a total of 22.5 on the Fahn-Marsden dystonia rating scale.

Rheumatologic examination revealed synovitis of the knees, metatarsophalangeal joints, and hand inter-phalangeal joints, along with hand flexor tenosynovitis. Tophi were evident at several sites including the toes, both Achilles tendons, the right wrist, the third fingers on both hands, and the right elbow. The patient's serum uric acid level was high (450 μmoles/liter). Urine uric acid excretion was elevated to 5.20 mmoles/24 hours, with urate clearance of 7 ml/minute, in spite of the allopurinol treatment. Renal function was normal (creatinine clearance 100 ml/minute), and blood testing revealed megaloblastic erythrocytes without anemia. Plain radiography revealed bilateral gouty arthropathy of the feet and hands. Ultrasonography of the hands demonstrated typical MSU deposits within the tendon sheath. Polarized microscopy of a knee joint fluid aspirate showed numerous MSU crystals.

MATERIALS AND METHODS

HPRT enzyme function

HPRT and adenine phosphoribosyltransferase (APRT) (EC 2.4.2.7) enzyme activities were measured in red blood cell lysates using radiolabeled ^{14}C-hypoxanthine and ^{14}C-adenine, respectively, in a chromatographic assay as described previously (3,4). The patient had only 8% residual HPRT activity in erythrocytes (0.16 nmoles/ minute/mg hemoglobin [normal range 2.0–2.9]) with a concomitant increase in APRT (0.86 nmoles/minute/mg hemoglobin [normal range 0.40–0.60]). APRT in erythrocytes from subjects with HPRT deficiency is typically increased about 2–3 fold compared with controls.

Mutation of *hprt*

Genomic DNA was isolated from 5 ml of whole blood using a Wizard genomic DNA purification kit (Promega, Madison, WI). Molecular analysis of the *hprt* gene was performed at the genomic level by polymerase chain reaction (PCR) amplification and sequence analysis of all 9 exons of the *hprt* gene, based on a modified version of a method described previously (5–7). Use of the PCR primer pairs for exons 1–9 also permitted genomic sequence analyses of both intron and exon segments involved in splice sequence mutations. All 9 exons of the *hprt* gene from the patient and a control were amplified on 8 separate DNA fragments having different lengths using specific primer pairs (exon 1 737 bp, exon 2 254 bp, exon 3 572 bp, exon 4 335 bp, exon 5 294 bp, exon 6 443 bp, exons 7 and 8 529 bp, and exon 9 366 bp). The DNA fragments that included exons 2–9 were amplified with the PCR system using Platinum Pfx DNA polymerase (Invitrogen, Carlsbad, CA). For the DNA fragment that included exon 1 (71% GC rich), we used the Accuprime GC-rich DNA polymerase (Invitrogen). The amplified fragments were purified using QIAquick PCR purification (Qiagen, Hilden, Germany) and sequenced directly using the same primers as for the PCR. A novel missense mutation, c.596T>G, was found in exon 8 resulting in the amino acid change p.F199C at the protein level.

Structural analysis

The HPRT structures with bound GMP (Protein Data Bank [PDB] code 1HMP) (8) and a transition-state analog (PDB code 1BZY) (9,10) were downloaded from the PDB. The structures were analyzed and modeled using either RasMol 2.6 or Yasara (www.yasara.org). The complete enzyme is a dimer when the GMP is bound, and a tetramer when the transition-state analog inhibitor is bound. A schematic representation of the protein dimer is shown in Figure 1. The residue involved in dimer interactions may be important for the stability and enzymatic function of the protein. Residues 198–204 are involved in the largest dimer interface. F199 is a fairly highly conserved residue, and its side chain is involved in a number of hydrophobic interactions. The amino acid substitution F199C is distant from the active enzymatic site, but it is adjacent to another (R200) that forms a part of the PRPP binding site.

DISCUSSION

The patient described herein had partial HPRT enzyme deficiency due to a novel mutation in the *hprt* gene, c.596T>G, resulting in the single amino acid substitution, p.F199C, at the protein level. On the basis of the results described in this report and the patient's clinical phenotype, he should be considered as having one of the forms of Lesch-Nyhan variants with mild neurologic symptoms (11–13). Residual enzyme activity (8%) likely prevented the more serious neurobehavioral problems seen in classic Lesch-Nyhan disease. The concept of relating residual activity to severity of phenotype has been addressed in prior studies, with a thorough description of the correlations and putative exceptions (14).

Mutations of *hprt* are responsible for a wide spectrum of disease that can be divided into 3 overlapping clinical phenotypes (2,11). The most mildly affected patients demonstrate only marked overproduction of uric acid, with resultant hyperuricemia, nephrolithiasis, and gout. Patients with intermediate severity exhibit uric acid overproduction, along with neurologic abnormalities that range from only minor clumsiness to disabling neurologic dysfunction. The most severely affected patients display uric acid overproduction, disabling neurologic dysfunction, and behavioral abnormalities that include impulsive and self-injurious behaviors. The most consistent abnormality in the brains of patients who are HPRT deficient is loss of basal ganglia dopamine (11). In HPRT-deficient humans and neuronal cell lines and HPRT-knockout mice, these abnormalities are neuro-chemically selective (15), with little change in most other neurotransmitters. The abnormalities are also neuroanatomically selective since midbrain dopaminergic pathways are affected while other dopaminergic pathways are not. These findings imply that midbrain dopamine neurons are unusually dependent upon HPRT-mediated purine recycling. The most severe form of HPRT deficiency–mediated disease is known as Lesch-Nyhan disease, while the less complete phenotypic manifestations are designated Lesch-Nyhan variants.

Other inherited disorders have also been shown to be responsible for early-onset gout, such as PRPP synthetase overactivity, type I glycogen storage disease (16), and mutations in the uromodulin gene (17). Recently, the urate transporters SLC2A9, URAT1, and GLUT9 were identified. These influence serum urate concentration and urate excretion. SLC2A9 variants and GLUT9 were also associated with low fractional excretion of uric acid and/or with gout (18,19).

Mutations of *hprt* resulting in HPRT deficiency are heterogeneous, and >300 are now known (ref. [2] and www.lesch-nyhan.org). Our own molecular analysis of patients from France confirms this heterogeneity (5,7). Mutations leading to partial HPRT deficiency are associated with less severe phenotypes (2). In Lesch-Nyhan variants that have been described in the literature, missense mutations are nearly universal, with the change at the protein level being relatively conservative and not expected to cause a major change in protein structure. Deletions, stop codons, or major rearrangements are rarely described in patients with Lesch-Nyhan variants with mild phenotypes. For most of the substitutions, the phenotype can be explained as a result of the predicted change in the core structure of the protein, dimer interactions, or ligand binding. In the patient described herein, even though the amino acid substitution p.F199C was not at the active enzymatic site, the substitution of F for C at position 199 may have altered the position of its main chain atoms and indirectly affected the R200 side chain interactions with pyrophosphate or PRPP.

An F199V substitution in patients with Lesch-Nyhan disease has been described in previous reports (20,21). Thus, a mutation at the same codon could lead to very mild or very severe disease, and amino acid substitutions at a single codon could lead to different phenotypic consequences. This phenomenon may be explained by the effect of the substitution on residual enzyme function. Moreover, F199 seems to be implicated in dimer formation; if dimer formation is hindered, a more severe Lesch-Nyhan disease phenotype may be manifested.

In conclusion, assay for HPRT deficiency should be undertaken in young patients with chronic hyperuricemia and gout, as well as in patients with juvenile uric acid nephrolithiasis. Some variant enzymes display residual activity in the erythrocyte assay, often representing >5% of control, making them readily distinguishable from the classic Lesch-Nyhan disease pattern. This difference is particularly important in assessing prognosis in a newly diagnosed young patient. Patients with Lesch-Nyhan variants usually do not exhibit self-injurious behavior, but can have other difficult behaviors and cognitive limitations. Motor disorders

that may be present include dystonia, which can appear as mild clumsiness or can be severely disabling, speech difficulty with dysarthria, and at times, mild corticospinal signs. Careful neurologic examination is warranted in patients with juvenile gout to detect these mild symptoms.

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Biography

Dr. Bardin has received consulting fees, speaking fees, and/or honoraria from Pfizer, Expanscience, and Ipsen (less than $10,000 each). Dr. Jinnah has received consulting fees, speaking fees, and/or honoraria from Savient Pharmaceuticals and Psyadon Pharmaceuticals (less than $10,000 each). Dr. Lioté has received consulting fees, speaking fees, and/or honoraria from Ipsen, Mayoly Spindler, and Genzyme (less than $10,000 each).

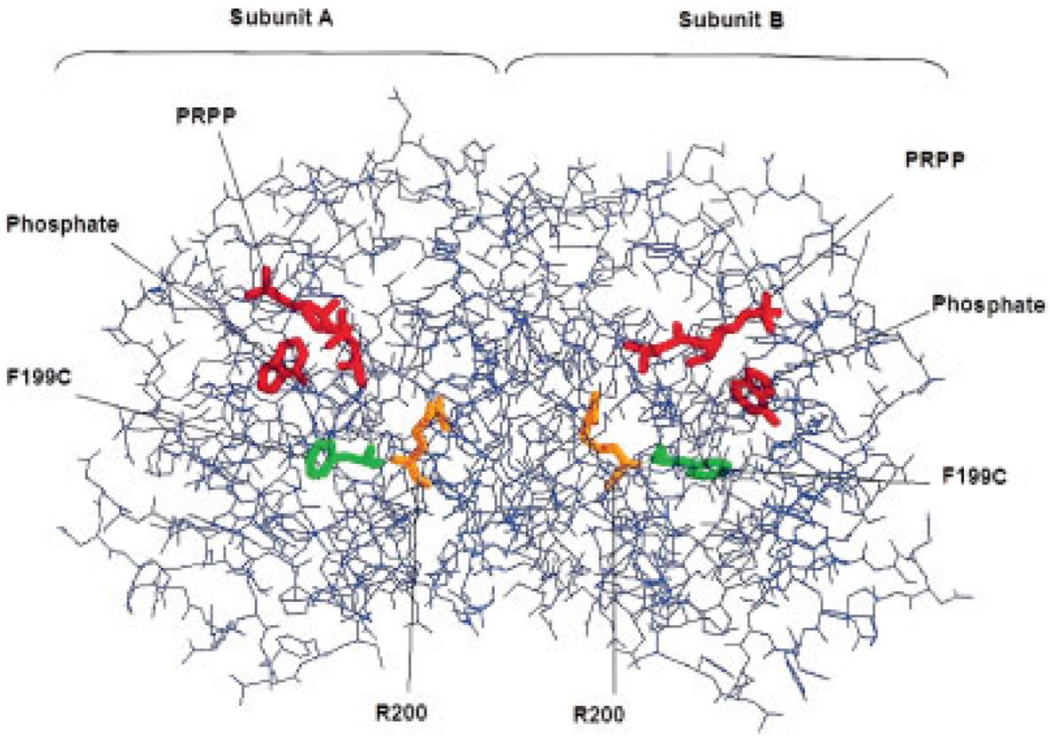

Figure 1.
Representation of the newly identified variant F199C hypoxanthine guanine phosphoribosyltransferase protein. PRPP = phosphoribosylpyrophosphate.