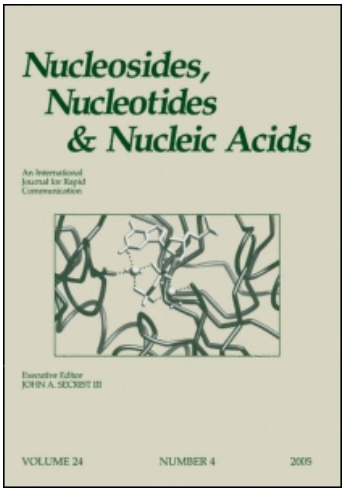

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THE DIAGNOSIS OF HPRT DEFICIENCY IN THE 21ST CENTURY

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□ *We have studied 36 patients with HPRT deficiency, 25 with Lesch-Nyhan syndrome and 11 with partial HPRT deficiency (grades 1 to 3). Patients diagnosed with HPRT deficiency have increased 50% since 2000. The most relevant recent advances have been made in molecular diagnosis. Nevertheless, enzyme determinations are still essential for the diagnosis of HPRT deficiency. Therapy for the neurological manifestations of HPRT deficiency has not advanced. Allopurinol remains the drug of choice to diminish uric acid overproduction, but the optimal allopurinol dose must be established in each patient to prevent xanthine or uric acid urolithiasis, a process aided by sequential determination of urinary oxypurines and uric acid.*

Keywords Lesch-Nyhan syndrome; HPRT; molecular diagnosis; allopurinol; urolithiasis

INTRODUCTION

Deficiency in the activity of the enzyme hypoxanthine-guanine phosphoribosyltranseferase (HPRT, EC 2.4.2.8) is associated with Lesch-Nyhan syndrome and HPRT-related gout. Lesch-Nyhan syndrome (OMIM 300322) was described in 1964.[1] in two affected brothers with hyperuricemia, mental retardation and motor abnormalities, initially described as spasticity and choreoathetosis. In 1967, Seegmiller, Rosenbloom, and Kelley[2] reported a complete deficiency of HPRT activity as the cause of the Lesch-Nyhan syndrome. In 1969, Kelley, et al.[3] described a partial deficiency of HPRT activity, which was associated with gout but no neurological involvement. This partial deficiency was termed Kelley-Seegmiller syndrome or HPRT-related gout (OMIM 300323). It is now recognized that between these two syndromes, a continuous spectrum of neurological involvement may be present in HPRT-deficient patients. Terms such as Lesch-Nyhan variants have been

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introduced to include patients with HPRT-related gout and some degree of neurological involvement but without the complete Lesch-Nyhan syndrome. For classification proposes, the phenotypes associated to HPRT deficiency may be divided into four groups;[4] in the least severe form, partial HPRT deficiency presents as hyperuricemia, hyperuricosuria, nephrolithiasis, and gout without evident neurological manifestations.

HPRT deficiency is inherited as an X-linked recessive disorder. Although at least five affected females have been described, HPRT deficiency largely affects males, with heterozygous females serving as asymptomatic carriers. Molecular diagnosis in the propositus permits carrier and prenatal diagnosis. Human HPRT is encoded by a single structural gene spanning approximately 45 Kb on the long arm of the X chromosome at (Xq26) and consists of nine exons with an overall coding sequence of 654 bp. Documented mutations in HPRT deficiency show a high degree of heterogeneity in type and location within the gene: deletions, insertions, duplications, etc. To date more than 300 disease-associated mutations have been found (www.lesch-nyhan.org). Single point mutations are the major genetic basis of partial HPRT deficiency, whereas Lesch-Nyhan syndrome is caused mainly by mutations that alter the size of the predicted protein.[5] Thus, molecular diagnosis, by means of HPRT cDNA or genomic DNA sequencing needs to be established in each propositus. In 2005, Dawson et al.[6] published a reportof a patient with partial HPRT deficiency in whom no mutation could be found in either the HPRT coding region DNA or the HPRT cDNA.

Increased uric acid synthesis in HPRT deficiency is a consequence of decreased purine base re-utilization for nucleotide synthesis and enhanced de novo purine synthesis due to the excessive availability of the regulatory substrate 5-phosphoribosyl-1-pyrophosphate (PRPP). Uric acid overproduction imparts increased risks for nephrolithiasis, renal insufficiency, gouty arthritis, and tophi. In order to prevent these undesired consequences, treatment with the xanthine oxidase inhibitor allopurinol, hydration, and urine alkalinization is recommended.

PATIENTS AND METHODS

We have studied 36 patients with HPRT deficiency at La Paz University Hospital, Madrid. These patients belonged 30 different Spanish families. Patients were referred as a result of several scientific reports and participation in a network for the Study of Inborn Errors of Metabolism (REDEMETH). Deficiency of HPRT was diagnosed on the basis of:

(a) Clinical signs (clinical symptoms and signs typical of an HPRT-deficient state)

(b) Biochemical abnormalities of purine metabolites: Uric acid and creatinine in plasma and urine were measured in a multi-channel auto analyzer. Urinary hypoxanthine and xanthine were determined by high-performance liquid chromatography[4] while patients were receiving a self-selected diet.
(c) Enzyme deficiency: HPRT and adenine phosphoribosyltransferase (APRT) activities in haemolysates were determined by high performance liquid chromatography.[4] In 26 patients, residual HPRT activity was determined in intact erythrocytes as previously described.[4]
(d) Molecular diagnosis: In 20 families, the genetic defect accounting for the enzyme deficiency was established and carrier diagnosis was performed.[5]

Once the diagnosis of HPRT deficiency was established, allopurinol treatment was initiated and patients were followed-up periodically (every 3, 6, or 12 months). The most commonly administered dose of allopurinol was 5 mg/kg per day.

RESULTS

Clinical, biochemical, enzymatic, and molecular data allowed establishment of the diagnosis of Lesch-Nyhan syndrome (grade 4) in 25 patients. Partial HPRT deficiencies (grades 1 to 3) were diagnosed in 11 patients. Since self-injury does not emerge until 2—3 years of age and can be delayed until late adolescence, patients who did not self-injure were classified as Lesch-Nyhan syndrome if they had non-detectable enzyme levels and a null mutation, and/or another family member diagnosed with Lesch-Nyhan syndrome on the basis of the full neurobehavioral syndrome.

Fifteen Lesch-Nyhan syndrome patients were diagnosed before year 2000. Age at diagnosis in these Lesch-Nyhan patients was >4 years in 8 patients. In these 15 patients, the clinical presentation included: spasticity and choreoathetosis in 10, mental retardation in 8, and self-injurious behavior in 9. Two of them presented with tophi. Ten additional Lesch-Nyhan syndrome patients were diagnosed after year 2000. The age at diagnosis was always $\leq$4 years, with the youngest only 16 days. Only one of these patients showed self-injurious behaviour at diagnosis, and most of them were referred to La Paz University Hospital because of psychomotor delay and hyperuricemia. The motor system abnormalities in Lesch-Nyhan syndrome patients have been reevaluated[7] since the original description, and severe action dystonia, hypotonia, choreoathethosis, and ballismus are considered the major abnormalities.

HPRT activities in erythrocyte hemolysates were undetectable (<0.01 nmol/h/mg haemoglobin) in all patients with Lesch-Nyhan

syndrome. The percentage of ^{14}C hypoxanthine converted into ^{14}C IMP in intact erythrocytes ranged from 0.1% to 1.1%. Genetic studies were performed in 18 families and showed six point mutations: three in a non-coding region causing splice errors and three in coding regions. Of the coding region mutations, two predicted an amino acid change in the translated protein and one a premature stop codon. Three insertions, a double insertion plus deletion, and seven deletions were also found. One Lesch-Nyhan syndrome patient showed no mutation in the HPRT cDNA or in the genomic HPRT coding region and splice sites.

Eleven patients were classified with partial HPRT deficiency on the basis of clinical, biochemical, enzymatic and molecular data. On the basis of their neurological impairments, these patients were classified into groups 1 to 3. Two patients presented a clinical picture resembling Lesch-Nyhan syndrome, but did not show self-injurious behaviour and had normal intelligence. Thus, they were classified into group 3. Erythrocytes studies of these individuals, showed increased APRT activities, and absent HPRT activities both in haemolysates and in intact erythrocytes. In one of them HPRT activity was determined in fibroblasts and a residual enzyme activity could be detected. Three patients were classified into group 2 based on their clinical picture: symptoms of uric overproduction associated with mild neurological symptoms that did not preclude an independent life. HPRT activities in hemolysate were undetectable but APRT activities were increased and a low residual HPRT activity was detected in intact erythrocyte (>1.3%). Group 2 and 3 patients were diagnosed before year 2000. Group 1 patients presented manifestations derived from their purine overproduction but no neurological symptoms, except one patient with a mild obsessive-compulsive disorder. They showed decreased but detectable HPRT activities, which in haemolysates ranged from 0.3 to 9.8 nmol/h/mg Hgb. Increased APRT activity was also found in haemolysates. Erythrocyte HPRT activity was present and ranged from 9.2% of normal to normal values. Two such patients were diagnosed before 2000 and four after, but no significant differences were appreciated with regard to age and clinical characteristics at diagnosis.

Genetic studies were performed in seven families and showed five missense point mutations. In two families, no mutation in the HPRT cDNA or in the genomic HPRT coding region and splice sites was found.

Under baseline conditions, mean serum uric acid levels (sUA) and urinary excretion of uric acid, hypoxanthine, xanthine and total purines were markedly elevated in HPRT-deficient patients. No significant differences were encountered between patients with the Lesch-Nyhan syndrome and partial HPRT deficient patients with the exception of urinary xanthine, which was significantly higher in Lesch-Nyhan syndrome patients.

Allopurinol doses ranged from 50 to 600 mg/day. Treatment with allopurinol significantly modified purine metabolism, normalized serum urate levels in all patients, and decreased uric acid/creatinine ratio to lower than

1.0. Allopurinol-related biochemical changes were similar in patients with either complete or partial HPRT deficiency. Renal function remained stable or improved with treatment. Three patients had urolithiasis during allopurinol treatment. In two patients, xanthine stones were documented, thus, mandating allopurinol dose adjustments aimed at reducing excessive oxypurine excretion rates. No allopurinol hypersensitivity reactions occurred. Neurological manifestations were not influenced by allopurinol therapy.

DISCUSSION

There has been a >50% increase in the incidence of HPRT deficient patients diagnosed at La Paz University Hospital in Madrid, Spain, since the year 2000. In addition, age at diagnosis is now markedly lower than in past years and a precocious diagnosis has allowed earlier allopurinol treatment and carrier diagnosis.

The most relevant advances in recent years have been made in the field of molecular diagnosis. Molecular diagnosis has allowed faster and more accurate prenatal diagnosis and carrier detection. However, in spite of advances in molecular diagnosis, enzyme determinations are still essential for the diagnosis of HPRT deficiency. In this study, analysis of the HPRT coding region sequence failed to detect abnormalities in several patients (one patient with the Lesch-Nyhan syndrome and four with partial deficiency).

Advances in diagnosis have not been accompanied by advances in therapy. Allopurinol is efficacious and generally safe for the treatment of uric acid overproduction in patients with HPRT deficiency. However, xanthine lithiasis may develop as a consequence of allopurinol therapy. The optimal allopurinol dose for HPRT deficient patients has not been established. In our experience and when serum urate is maintained close to its solubility, urate deposition does not occur. Xanthine lithiasis may be prevented by sequential determination of urinary oxypurines and uric acid, which should permit allopurinol dose titration to minimize the risk of either xanthine or uric acid urolithiasis.

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