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The Spectrum of Hypoxanthine-Guanine Phosphoribosyltransferase (HPRT) Deficiency

Clinical Experience Based on 22 Patients from 18 Spanish Families

JUAN G. PUIG, ROSA J. TORRES, FELÍCITAS A. MATEOS, TERESA H. RAMOS, JOAQUÍN M. ARCAS, ANTONIO S. BUÑO, AND PATRICK O'NEILL

Introduction

The enzyme hypoxanthine-guanine phosphoribosyltransferase (E.C.2.4.2.8., HPRT) plays a crucial role in uric acid synthesis and purine metabolism. This enzyme catalyzes the conversion of hypoxanthine and guanine to inosine monophosphate (IMP) and guanosine monophosphate (GMP), respectively, and uses phosphoribosylpyrophosphate (PRPP) as a cosubstrate and as a source of energy (35). The salvage pathway for purine nucleotide synthesis, catalyzed by HPRT, utilizes only 1 mole of ATP (adenosine triphosphate) per mole of product, as compared to 6 moles utilized by "de novo" purine nucleotide synthesis, and thus saves much energy. In 1964, Lesch and Nyhan (19) described 2 brothers with a clinical syndrome characterized by hyperuricemia, hyperuricosuria, and severe neurologic dysfunction including choreoathetosis, mental retardation, and self-injurious behavior. In 1967, Seegmiller et al (34) reported a complete deficiency of the activity of the enzyme HPRT as the cause of the clinical syndrome. Fortunately, not all patients with HPRT deficiency exhibit the dramatic clinical characteristics of Lesch-Nyhan syndrome. Some patients do not suffer neurologic symptoms and show only uric acid overproduction, gout, and nephrolithiasis (17). In other cases, HPRT deficiency is associated with a lesser degree of neurologic impairment that may eventually prevent an independent life but is without the severity of the neurologic symptoms described by Lesch and Nyhan (26).

The prevalence of HPRT deficiency in the population is not accurately known, although an estimate of the minimum frequency of the disorder in Canada is 1 in 380,000 births (5). The HPRT gene is located on the X chromosome in the region q26-q27 (11). Naturally occurring mutations in the HPRT gene are heterogeneous, with most being point mutations or small deletions or insertions, dispersed through all the gene (33). About 30% of the patients have a noninherited mutation (de novo mutation). Most female carriers of HPRT deficiency are healthy, but their somatic cells are mosaics in term of HPRT activity because of random inactivation of the X chromosome (23).

A spectrum of clinical consequences of HPRT deficiency has been recognized in small series of patients and in extensive reviews (8, 10, 17, 18, 26, 29), but the complete spectrum of the neurologic disorder has not been described in a single series of patients examined by the same observers. Since 1984, we have diagnosed 22 patients belonging to 18 different Spanish families with HPRT deficiency. We report here clinical, biochemical, enzymatic, and molecular genetic studies on these patients.

Patients and Methods

Patients

Between 1984 and 1999, we studied, at "La Paz" University Hospital, Madrid, Spain, 22 patients with HPRT deficiency belonging to 18 unrelated Spanish families (Figure 1).

From "La Paz" University Hospital, Divisions of Internal Medicine (JGP), Clinical Biochemistry (RJT, FAM, THR, ASB), and Pediatric Neurology (JMA), Madrid, Spain; University of Vermont, Genetics Laboratory, Burlington, Vermont, USA (PO).

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Address reprint requests to: Juan García Puig, MD, PhD, Servicio de Medicina Interna, Hospital General, Planta 10, Hospital Universitario "La Paz", Paseo de la Castellana 261, 28046 Madrid, Spain. Fax: 34–91-3583717; e-mail: med027514@nacom.es.

Abbreviations used in this article: APRT, adenine phosphoribosyltransferase; ATP, adenosine triphosphate; DNA, deoxyribonucleic acid; GMP, guanosine monophosphate; HPRT, hypoxanthine-guanine phosphoribosyltransferase; IMP, inosine monophosphate; PCR, polymerase chain reaction; PRPP, phosphoribosylpyrophosphate; RNA, ribonucleic acid.

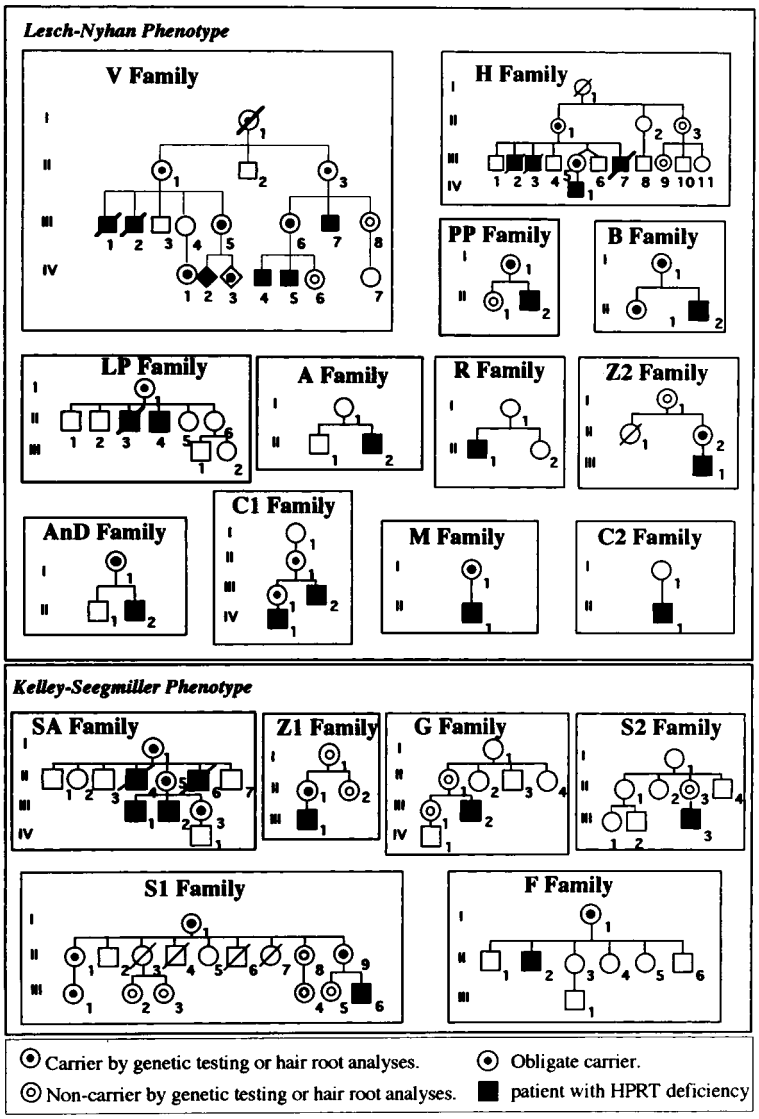

FIG. 1. Genealogic trees of 18 Spanish families with HPRT deficiency.

Diagnostic criteria

HPRT deficiency in the probands was diagnosed on the basis of suggestive clinical symptoms and signs; biochemical results indicative of a severe purine overproduction; and markedly reduced HPRT activity in hemolysates with simultaneously increased adenine phosphoribosyltransferase (APRT) activity.

Biochemical determinations: Purine metabolism

Purine metabolism was examined after all medications known to influence purine metabolism were discontinued for at least 2 weeks. Colchicine (0.5 mg per day) was given as prophylaxis against acute arthritis to patients who had experienced gout. Patients were placed on a weight maintenance, isocaloric, purine-restricted diet (less than 75 mg per day of purines) with a protein content of 10%–15% for 7 days before the studies. Purine metabolism was examined by determining plasma hypoxanthine, xanthine and uric acid concentrations, and the 24-hour urinary excretion rates of these purines (27). Plasma and urinary hypoxanthine and xanthine were determined by high-performance liquid chromatography (21). Uric acid and creatinine in plasma and urine were measured in a multichannel autoanalyzer (Hitachi 704, Hitachi, Tokyo, Japan).

Enzymatic determinations

Activities of the enzymes HPRT and APRT were determined in charcoal-treated hemolysates by a high-performance liquid chromatography-based method (30).

In order to assess for residual HPRT, the percentage of ^{14}C hypoxanthine incorporated into ^{14}C IMP in intact erythrocyte was

also determined (40). In brief, erythrocytes obtained from venous blood were incubated for 40 min at 37 °C in 10 mM HEPES, 125 mM NaCl, 2.6 mM KCl, 5.5 mM glucose, 1 mM $CaCl_2$, 50 mM $MgCl_2$, 18 mM NaH_2PO_4 and 10 μM [^{14}C] hypoxanthine. At the end of the incubation time, radioactive IMP was separated from hypoxanthine by high-performance liquid chromatography, and radioactivity in the IMP peak was measured. HPRT activity was expressed as the percentage of [^{14}C] hypoxanthine transformed into [^{14}C] IMP. Under these assay conditions, normal values for incorporation of [^{14}C] hypoxanthine into [^{14}C] IMP in erythrocytes were 98%–100%.

Molecular analysis

Total cellular ribonucleic acid (RNA) was isolated from nucleated blood cells by the guanidium isothiocyanate method (Ultraspec RNA, Biotecx Lab, Inc, Houston, TX). Genomic deoxyribonucleic acid (DNA) was isolated from patients' samples using the commercial method (Puregene, Gentra Systems, Inc, Minneapolis, MN). Total cellular RNA was reverse transcribed using AMV reverse transcriptase (Promega, USA) and oligo d(T) (15 mer) as primer according to established protocols (31). HPRT transcripts were amplified by polymerase chain reaction (PCR) with HT 3′ and HT 5′ primers as previously described by Davidson et al (6). Both strands of patient cDNAs were sequenced using an automated DNA sequencer (ABI Prism, Model 377). The mutations found in cDNA were verified in genomic DNA. When neither RNA nor HPRT cDNA could not be obtained, genomic DNA was amplified by PCR and the PCR products were sequenced using an automated DNA sequencer according to Gibbs et al (12). Seven families (Z2, H, C2, AnD, C, S2, and Z) were analyzed by use of T-lymphocyte mutant frequency determinations, and cDNA and genomic DNA sequencing as described in Hunter et al (15). The cells from the affected male in family Z did not grow in the presence of 6-thioguanine, while those from the other 6 families did.

Results

Clinical characteristics

Table 1 shows the clinical characteristics of the 22 Spanish patients with HPRT deficiency at presentation to "La Paz" University Hospital. Patients with HPRT deficiency were classified into 2 main syndromes: 1) "classic Lesch-Nyhan syndrome" or "complete HPRT deficiency," characterized by spasticity, choreoathetosis, mental retardation, and self-injurious behavior. Patients classified in this category were indistinguishable from those described by Lesch and Nyhan (19), although some of them did not show self-injurious behavior; and 2) "partial HPRT deficiency," also named "Kelley-Seegmiller syndrome" or "HPRT variants." Patients in this category showed either no neurologic symptoms or mild to severe neurologic manifestations, including different degrees of spasticity and mental retardation. Patients were classified as having "partial HPRT deficiency" if they could perform activities that patients with Lesch-Nyhan syndrome could not (see below).

Fifteen patients, aged 5 months to 31 years, were classified as having Lesch-Nyhan syndrome. At presentation 10 patients showed the typical characteristics of classic Lesch-Nyhan syndrome, including

TABLE 1. Clinical characteristics of 22 Spanish patients with HPRT deficiency

Patient	Family Name	Initials	Age* (yr)	Clinical Characteristics	Group†
"Classic Lesch-Nyhan syndrome"					
1	V	ASV	31	Choreoathetosis, spasticity, self-mutilation, mental retardation, tophi	4
2	V	JJGS	6	Choreoathetosis, spasticity, mental retardation	4
3	V	JGS	5	Choreoathetosis, spasticity, self-mutilation, mental retardation	4
4	LP	FMG	28	Choreoathetosis, spasticity, self-mutilation, mental retardation, tophi	4
5	B	JB	4	Choreoathetosis, spasticity, self-mutilation	4
6	A	JPA	3	Choreoathetosis, spasticity, self-mutilation, mental retardation	4
7	M	AM	6	Choreoathetosis, spasticity, self-mutilation, mental retardation	4
8	R	PR	19	Choreoathetosis, spasticity, self-mutilation, mental retardation	4
9	PP	APP	19 mo	Psychomotor retardation, crystaluria	4
10	AnD	JPR	11 mo	Psychomotor retardation, spasticity	4
11	H	JSSC	5 mo	Psychomotor retardation	4
12	C	ABM	27	Choreoathetosis, spasticity, self-mutilation, mental retardation	4
13	C	RMB	18 mo	Psychomotor retardation, spasticity	4
14	Z2	JCJ	3	Psychomotor retardation, acute pyelonephritis	4
15	C2	MFP	8	Choreoathetosis, spasticity, self-mutilation	4
"Partial HPRT deficiency" (Kelley-Seegmiller syndrome)					
16	F	ACM	30	Gout arthritis	1
17	Z	ASA	5 mo	Hyperuricemia, crystaluria	1
18	G	AGC	13	Acute renal failure, dystonia	2
19	SA	AA	35	Mental retardation, hyperuricemia, dystonic gait	2
20	SA	JMA	33	Mental retardation, hyperuricemia, dystonic gait	2
21	S2	ACHS	14	Severe dystonia, extrapyramidal syndrome	3
22	S	JGS	7 mo	Psychomotor retardation, crystaluria	3

*Age when first seen at "La Paz" University Hospital.
†According to the classification proposed in Table 5.

self-injurious behavior. These patients were unable to walk and were extremely dependent on others for daily activities and personal care. Five patients (Table 1, Patients 2, 9, 10, 11, and 14) when first seen did not show the full clinical characteristics described by Lesch and Nyhan. One patient (Figure 1, V Family, subject IV-4; Table 1, Patient 2) belonged to a family with 2 additional living patients who showed the characteristics of Lesch-Nyhan syndrome, including self-mutilation. In contrast to his living uncle and younger brother, this patient who has been followed for 15 years and is now 21 years old, has never showed self-injurious behavior. Another patient (Figure 1, H Family, subject IV-1; Table 1, Patient 11) was diagnosed at age 5 months as having Lesch-Nyhan syndrome because he had 2 uncles with the typical syndrome. This patient has been followed for 6 years and up to now he has never shown self-mutilation. Three patients (Table 1, Patients 9, 10, and 14) suffered psychomotor retardation, spasticity, and choreoathetosis, but did not show self-injurious behavior at presentation. One of the 3 patients (Figure 1, PP Family, subject II-2; Table 1, Patient 9) tended to bite his fingers for about a year, but was educated to prevent this behavior. We have followed this patient for 8 years and have never seen self-inflicted injuries. At age 3 years this patient is able to propel his wheelchair despite severe spasticity. The other 2 patients (Table 1, Patients 10 and 14) have been followed for the last 6 years. They both have been educated to prevent impulsive behavior and have never self-injured. These 3 patients, who initially did not manifest the full clinical syndrome described by Lesch and Nyhan, when growing up did manifest some of the clinical characteristics of the syndrome, including marked psychomotor retardation. They are absolutely unable to stand up or walk (even with much help), and are totally dependent for their personal care. Thus, although these 3 patients did not show self-injurious behavior, they were diagnosed with Lesch-Nyhan syndrome.

Seven patients were classified as having "partial HPRT deficiency" or "Kelley-Seegmiller syndrome" when first seen at "La Paz" University Hospital. A review of these patients disclosed an extensive spectrum of clinical abnormalities. At 1 end of the spectrum were 2 patients (Figure 1, F Family, subject II-2; Table 1, Patient 16 and Figure 1, Z1 Family, subject III-1; Table 1, Patient 17) with gout and uric acid overproduction, respectively, as the sole manifestations of their HPRT deficiency. At the other end of the spectrum was Patient 21 (Figure 1, S2 Family, subject III-3; Table 1, Patient 21), who was mentally normal but showed severe dystonia and spasticity, which prevent walking. Nevertheless, he was able to stand up and even initiate some steps with much help. In between, there were 4 patients (Table 1, Patients 18, 19, 20, and 22) with a full spectrum of neurologic symptoms, of variable intensity. None of these partial HPRT-deficient patients showed the mental impairment evidenced by Lesch-Nyhan patients. In addition, all were able to perform certain activities related to personal care that could not be carried out by Lesch-Nyhan patients.

In some patients, a precise diagnosis with prognostic implications could not be established when first seen at "La Paz" University Hospital. Four patients (Table 1, Patients 10, 14, 17, 22) with no additional affected family members showed marked heterogeneity with respect to their neurologic symptoms, and, based on clinical grounds and HPRT activity in hemolysates, we could not reliably ascertain whether these patients had Lesch-Nyhan syndrome. This uncertainty prompted investigations to determine whether certain markers could be identified that would help to establish a more accurate prognosis with respect to the neurologic involvement associated with HPRT deficiency.

Biochemical studies

HPRT-deficient patients showed marked purine overproduction. Mean plasma concentrations of urate, hypoxanthine, and xanthine were significantly increased in patients with Lesch-Nyhan syndrome and in patients with the Kelley-Seegmiller syndrome as compared with control subjects (Table 2). The urinary excretion of uric acid, hypoxanthine, and xan-

TABLE 2. Purine metabolism in HPRT-deficient patients

	Controls (n = 20)	Lesch-Nyhan Phenotype (n = 15)	Kelley-Seegmiller Phenotype (n = 7)
Plasma			
Urate (μmol/L)	303 ± 59	643 ± 274*	625 ± 137*
Hypoxanthine (μmol/L)	2.2 ± 1.1	7.0 ± 3.2*	5.9 ± 2.7*
Xanthine (μmol/L)	0.7 ± 0.2	2.7 ± 1.6*	2.1 ± 0.5*
Urine			
Uric acid/creatinine ratio (mmol/mmol)	0.2 ± 0.0	1.9 ± 0.9*	1.3 ± 0.7*
Hypoxanthine (μmol/g creatinine)	47 ± 14	662 ± 368*	476 ± 315*
Xanthine (μmol/g creatinine)	29 ± 9	280 ± 170*	132 ± 85*†

*$p < 0.01$ versus control subjects.
†$p < 0.05$ versus Lesch-Nyhan phenotype.

thine, was also markedly elevated in HPRT-deficient patients. However, these biochemical variables were similarly elevated in patients with the Lesch-Nyhan syndrome and the partial HPRT-deficient phenotype.

Enzymatic studies

All patients showed nondetectable or decreased HPRT activity in the hemolysate. HPRT activity ranged from 0 to 10% of that obtained in control subjects (Table 3). Neither patients with the Lesch-Nyhan phenotype, except patient 4 in whom the result was obtained from the literature (14), nor most patients with clinically partial phenotype showed detectable hemolysate HPRT activity. Mean APRT activity in hemolysates was significantly increased as compared with control subjects (see Table 3). No significant correlation was found between APRT activity and the clinical phenotype.

To better characterize HPRT deficiency at the enzymatic level, enzyme determinations were performed in intact erythrocytes and HPRT activity expressed as a percentage of ^{14}C hypoxanthine transformed into ^{14}C IMP. A positive correlation was found between HPRT activity in intact erythrocytes and neurologic involvement (Figure 2). However, the enzyme activity in intact cells showed overlapping values in 2 patients who actually had very different phenotypes.

Molecular studies

No genetic material could be obtained from the members of 5 families. When we initiated the genetic studies, 1 propositus had died (Figure 1, A Family, subject II-1; Table 1, Patient 6) and we could not obtain samples from families living in the Spanish Baleares Islands (Figure 1, R and M Families) and Canarias Islands (Figure 1, LP and F Families). The mutations found in 13 Spanish families are summarized in Table 4. Among 13 Spanish families we found 3 deletions, 2 insertions, and 8 point mutations (including a double point mutation, and 1 splice site mutation) (39). Mutations from SA Family (HPRT Salamanca) (36) and S Family (HPRT Madrid 1) (3) were identified through international collaboration. Three mutations (HPRT Almodovar, HPRT Andorra, and HPRT Santoña) were previously identified in unrelated families (6, 13, 38). The phenotype of these previously reported patients with partial HPRT deficiency was similar to the observed phenotype in the Spanish patients (Table 1, V Family, Patients 1, 2, and 3; AnD Family, Patient 10; and S Family, Patient 22). Eight mutations were identified at "La Paz" Univer-

TABLE 3. Enzyme activities in hemolysates and in intact erythrocytes of HPRT-deficient patients

Patient/Family	HPRT (nmol/h/mg hemoglobin)	APRT (nmol/h/mg hemoglobin)	% Transformed into ^{14}C IMP*
Lesch-Nyhan phenotype			
1/V	<0.01	80	ND
2/V	<0.01	45	0.1
3/V	<0.01	54	0.1
4/LP	0.14†	80†	ND
5/B	<0.01	45	0.5
6/A	<0.01	108	ND
7/M	<0.01	86	ND
8/R	<0.01	141	ND
9/PP	<0.01	68	0.5
10/AnD	<0.01	52	0.2
11/H	<0.01	60	0.15
12/C1	<0.01	61	0.3
13/C1	<0.01	40	0.6
14/Z2	<0.01	60	0.6
15/C2	<0.01	83	ND
Partial phenotype			
16/F	<0.01	90	ND
17/Z	9.38	45	54.1
18/G	0.28	48	9.2
19/SA	<0.01	41	1.4
20/SA	<0.01	47	1.3
21/S2	<0.01	60	0.65
22/S	<0.01	58	0.2

Abbreviations: ND = not determined.

*HPRT activity in intact erythrocytes was determined as previously described and expressed as the percentage of ^{14}C hypoxanthine transformed into ^{14}C IMP. Normal range, 98%–100%.

†Results reported by Hernandez Nieto et al (reference 14).

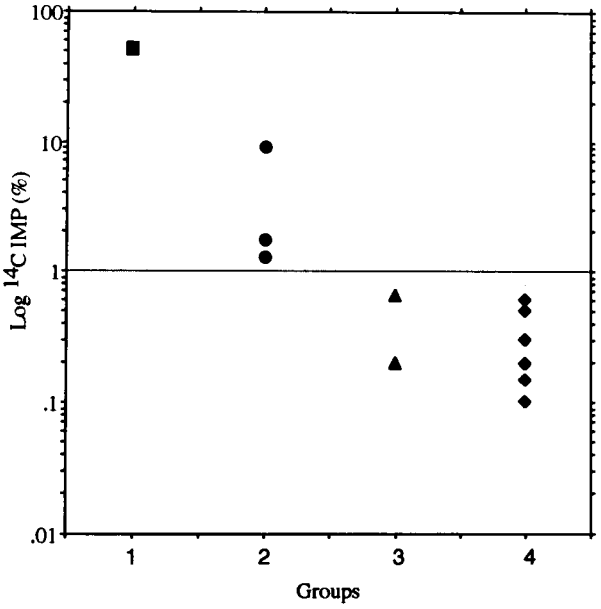

FIG. 2. HPRT activity in intact erythrocytes expressed as percentage of ^{14}C hypoxanthine transformed into ^{14}C IMP. Patients were classified in 4 groups according to their neurologic involvement, dependency on others for self-care, HPRT activity, and molecular defect (see Table 5).

sity Hospital (HPRT Zaragoza 2, HPRT Huelva, HPRT Murcia, HPRT Asturias, HPRT Cartagena, HPRT Sevilla, HPRT Madrid 2, and HPRT Zaragoza 1) and have been reported (39). Among the 13 Spanish families, 7 mutations resulted in truncated, likely nonfunctional proteins and all of them corresponded to classic Lesch-Nyhan patients. No patient phenotypically included as partial HPRT deficiency presented a mutation that altered the predicted protein size. In contrast, among the 6 point mutations, 1 was a Lesch-Nyhan syndrome and 5 corresponded to partial HPRT-deficient patients.

The mutation analysis in this Spanish series allowed carrier diagnosis in 33 women at risk. Twenty-one women were diagnosed as HPRT-deficient carriers and 12 women were noncarriers (see Figure 1). Two of the 13 mutations were new mutations that originated in the proband, since the mother of both patients was found to be a noncarrier. Three pregnant women asked for prenatal diagnosis. This was performed by DNA analysis of the fetuses from a chorionic villus aspirate. These 3 prenatal diagnoses resulted in 2 carrier female fetuses and in 1 nonaffected male fetus. One prenatal diagnosis was performed by biochemical analysis (20) and resulted in 1 HPRT-deficient male fetus (Figure 1, V Family, Subject IV-3).

TABLE 4. Mutations characterized in 13 Spanish families

Name	Family	cDNA*	Genomic DNA†	Protein‡
Lesch-Nyhan phenotype				
HPRT Zaragoza II	Z2	100 ins GG (exon 2) GAA A[GG]GG GTC	14852 ins GG	Frameshift after Arg 34. Stop codon at 42.41 aas
HPRT Huelva	H	118 G → A (exon 2) CAT GGA CTA	14871 G → A	Gly 40 to Arg Normal size
HPRT Murcia	B	333-334 del AG (exon 4) AC[AG]GG GAC	27905-06 del AG	Frameshift after Ser 110. TGA at 120. 119 aas
HPRT Asturias	C2	342 del A (exon 4) AT[A] AAA GTA	27914 del A	Frameshift after Asp 113. TAA at 116. 115 aas.
HPRT Andorra	AnD	319-384 del (exon 4)	Exon 4 deleted	196 aas, no exon 4
HPRT Cartagena	C	405 ins A (exon 6) GAT[A] ATA ATT	34939 ins A	Frameshift after Asp 135 TGA at 138. 137 aas
HPRT Almodovar	V	508 C → T (exon 7) CCA CGA AGT	39838 C → T	Arg 170 to stop codon (TGA), 169 aas
HPRT Sevilla	PP	Exclusion of exon 8	40031 A → G IVS7 −2 A → G tta gTT GTT	Frameshift after Asp 177. TAG codon at 183. 182 aas
Kelley-Seegmiller phenotype				
HPRT Santoña	S2	125 T → C (exon 2) CTA ATT ATG	14877 T → C	Ile 42 to Thr Normal size
HPRT Salamanca	SA	128 T → G (exon 2) 130 G → A (exon 2) ATT ATG GAC	14880 T → G 14882 G → A	Met 43 to Arg Asp 44 to Asn Normal size
HPRT Madrid II	G	143 G → A (exon 3) GAA CGT CTT	16611 G → A	Arg 48 to His Normal size
HPRT Madrid I	S	212 G → T (exon 3) GGG GGC TAT	16680 G → T	Gly 71 to Val Normal size
HPRT Zaragoza I	Z	397 G → A (exon 5) ATT GTG GAA	31629 G → A	Val 133 to Met Normal size

*cDNA: nucleotide positions in cDNA are numbered from the first A of the initiator codon.
†Genomic DNA: the first nucleotide in the genomic sequence is defined as the first G of the *Eco* RI site 5′ to exon 1.
‡Protein: the initiator amino acid methionine is defined as the first amino acid.

Discussion

We have attempted to delineate the spectrum of HPRT deficiency by reviewing our experience with 22 patients belonging to 18 different Spanish families. Patients were studied at the clinical, biochemical, enzymatic, and molecular genetic levels and the results were related to the clinical phenotype. The main results of this study are discussed below.

Clinical spectrum of HPRT deficiency

This study shows that HPRT deficiency may be expressed as very different phenotypes, conforming to a spectrum of disease severity (28). Most patients with HPRT deficiency have been reported as single cases; an extensive review of the literature shows that there is marked heterogeneity with considerable overlap among different phenotypes (8, 10, 17, 26). This has lead to both difficulty and confusion with regard to the categorization of the different HPRT-deficient phenotypes (for example, "intelligent" Lesch-Nyhan syndrome) (4, 22, 32). Thus, a detailed clinical study of an extensive series of patients from a single country, examined by the same observers, with some cultural homogeneity, afforded unique conditions for the study of HPRT-deficient syndromes. From our clinical examination, it is readily apparent that the clinical relevance of HPRT deficiency was determined essentially by the severity of the neurologic disorder, since uric acid overproduction was of a similar intensity in all patients. All patients classified in this study as having Lesch-Nyhan syndrome did show full dependency on others for their personal care, could not stand up even when assisted, and were confined to a wheelchair. However, it was also evident that the neurologic disorder could be markedly modulated by a myriad of environmental factors, among which education was of most relevance. Thus, under conditions of a relatively homogeneous phenotype such as Lesch-Nyhan syndrome, patients showed marked differences, at least with respect to 3 major neurologic symptoms: spasticity, mental retardation, and self-injurious behavior. As shown in this study, mental retardation and self-injurious behavior may be absent in patients with a marked molecular defect (that is, exon deletion, Figure 1, AnD Family, subject II-2; Table 1, Patient 10) that would predict a classical Lesch-Nyhan phenotype. Mental retardation may be due to the learning difficulties associated with the motor deficit and to the intellectual abandonment of these patients, many of them relegated to centers for mentally deficient children (25, 37). Self-mutilation behavior usually starts between 2 and 16 years of age, and in some instances is associated or aggravated by psychologic stress (adolescence, familial conflicts). Some patients did not present self-mutilation behavior, as in the case of the elder brother of a Lesch-Nyhan patient with a brother and 3 uncles with Lesch-Nyhan syndrome (Family V, Figure 1), a fact already well documented (1, 24, 41).

Partial HPRT deficiency or Kelley-Seegmiller syndrome was diagnosed when no neurologic symptoms were apparent, but also when neurologic symptoms were mild or even severe and the patients were able to perform activities precluded in Lesch-Nyhan patients. In this Spanish series 1 patient (Table 1, Patient 21) included in the Kelley-Seegmiller syndrome group could have been diagnosed as an "intelligent Lesch-Nyhan patient" (4, 22), since at age 14 years he has never self-injured and is able to attend school normally seated on his wheelchair. We believe that this patient is better classified as having partial HPRT deficiency with severe neurologic compromise that by no means reaches the severity of classical Lesch-Nyhan patients.

Biochemical, enzymatic, and molecular studies

Purine overproduction appeared to be of a similar intensity in all groups of HPRT-deficient patients. In this study plasma and urinary uric acid, hypoxanthine, and xanthine concentrations did not allow differentiation between patients with Lesch-Nyhan syndrome and the partial HPRT-deficient phenotype. HPRT activity in partial forms ranged from 0 to 10% of control subjects. A residual HPRT activity was only evident in patients with the partial phenotype (with the exception of Patient 4, whose HPRT activity was determined elsewhere [24]). However, a nondetectable HPRT activity did not predict a Lesch-Nyhan syndrome. Patients with nondetectable HPRT activity in the hemolysate can show some degree of HPRT activity in intact erythrocytes, and a correlation has been reported between the HPRT activity and the neurologic involvement (26). The results of our study in 13 patients are consistent with this finding, but HPRT activity in the intact cells showed overlapping values in at least 2 patients who actually had very different clinical phenotypes (see Figure 2).

Previous studies have indicated that there may be a good correlation between the severity of the molecular defect and the clinical phenotype (9, 16, 42). Thus, characterization of the mutation in a HPRT-deficient patient may help to delineate the clinical phenotype. Among the 13 Spanish families in whom the molecular defect could be assessed, 7 mutations resulted in truncated, likely nonfunctional proteins, and all of them corresponded to classic Lesch-Nyhan patients. Jinnah et al (16) recently reviewed all the known HPRT mutations and communicated 75 new cases, totaling 218 different mutations reported in

271 cases. Among these 218 different HPRT mutations (16), most altered the protein size and have been described in patients with the Lesch-Nyhan phenotype. On the other hand, 6 HPRT mutations among the 13 Spanish families did not alter the predicted protein size. Five of these 6 mutations were isolated from partial HPRT-deficient patients. In the 218 different mutations reviewed by Jinnah et al (16), single base substitution accounted for the majority (87.3%) of cases with partial phenotypes. Hence, partial phenotypes are usually related to missense mutations, suggesting that the mutated protein could retain a minimum of enzymatic activity. On the other side, mutations that result in truncated proteins are most often, but not always, accompanied by a complete absence of enzymatic activity and usually correspond to Lesch-Nyhan phenotypes. However, because the nature of the mutation cannot always be used to predict future disease severity, concurrent measurement of HPRT enzyme activity in intact cells may provide valuable additional information.

Among the 13 Spanish families studied, only 3 mutations have been described previously in unrelated HPRT-deficient families (3, 36). Interesting, in these 3 previously reported families (3, 36) the clinical phenotype was similar to that observed in the Spanish patients. From our studies it was evident that mutations in the Spanish HPRT-deficient families are heterogeneous and dispersed all over the HPRT gene. This information allows us to conclude that there is not a prevalent mutation in the Spanish HPRT-deficient population.

From the most extensive review of HPRT-deficient patients reported (16), and our clinical, biochemical, enzymatic, and molecular studies of this series of 22 HPRT-deficient Spanish patients, we believe that a clear-cut classification with 2 distinct groups—Lesch-Nyhan syndrome and Kelley-Seegmiller syndrome—has created up to now much confusion and is of dubious usefulness. We thus propose a classification of HPRT deficiency based on the neurologic symptoms of the patients and their dependency for personal care. When the clinical symptoms do not allow differentiation, determination of the HPRT activity and the molecular defect may be helpful. This classification integrates clinical and laboratory data and considers the 3 variables (clinical, enzymatic, and molecular) that have been used to classify HPRT-deficient patients into complete and partial phenotypes.

According to this classification, our patients with HPRT deficiency could be classified into the following 4 groups (Table 5):

Group 1, normal development with no neurologic symptoms (2 patients). HPRT deficiency in these patients was manifested by asymptomatic hyperuricemia and increased uric acid excretion or renal lithiasis and/or gout. These patients were totally independent for daily activities and carried on normal lives. In these patients HPRT activity was lower than in controls but detectable, both in the hemolysate and in intact erythrocytes. Mutations in these patients did not affect the predicted protein size. The cells of 1 patient included in this group (Figure 1, Z1 Family, subject III-1) did not grow in medium containing 6-thioguanine. There are 2 other patients (16) whose cells did not grow in presence of 6-thioguanine, and they both have only hyperuricemia and kidney problems. These 2 patients and 12 previously reported patients (17) could be included in Group 1.

TABLE 5. Proposed classification of the Spanish HPRT-deficient patients

	Kelley-Seegmiller Syndrome or Partial Phenotype			Classic Lesch-Nyhan Syndrome
	Grade 1	Grade 2	Grade 3	Grade 4
No. of patients	2	3	2	15
Variable				
Psychologic impairment	None	Mild	Evident	Severe
Spasticity and hypertonia	None	Mild	Moderate to severe	Severe
Choreoathetosis and involuntary movements	None	Evident but do not preclude self personal care	Self personal care difficult	Preclude self personal care
Gait	Normal	Alone with some defects	Only with help	Wheelchair
Personal care	Totally independent	Can live alone	Markedly dependent	Totally dependent
HPRT activity in hemolysate*	(+)	(−)	(−)	(−)
HPRT activity in intact erythrocyte†	(+)	(+)	(−)	(−)
Alteration of predicted protein size	(−) Point mutations that do not affect the predicted protein size	(−) Point mutations that do not affect the predicted protein size	(−) Point mutations that do not affect the predicted protein size	(+, −) Predicted protein size can be altered or not

*HPRT activity in hemolysate was determined by HPLC and is considered (−) when <0.001 nmol/h/mg Hb.
†HPRT activity in intact erythrocytes was determined in the text and considered (−) when <1%.

Group 2, mild neurologic symptoms (3 patients). This group included patients with mild neurologic symptoms such as dystonic gait, some degree of spasticity, or mental retardation. These patients were limited by their neurologic symptoms, although they were independent for most activities and could live independently. HPRT activity was undetectable in hemolysate by the routine methods, but a residual HPRT activity could be detected in intact erythrocytes. Mutations found in these patients did not affect the predicted protein size.

Group 3 comprised patients with neurologic symptoms severe enough to preclude an independent life (2 patients). Patients in Group 3 were mentally normal and could feed themselves and fulfill some personal needs but needed a wheelchair. The intelligent patient reported by Catel and Smith (4) in 1959, later diagnosed as suffering HPRT deficiency (2), could have been included in this group. HPRT activity was undetectable in the hemolysate by routine methods and no residual HPRT activity could be detected in intact erythrocytes. Mutations found in these patients did not affect the predicted protein size.

Group 4, classic Lesch-Nyhan syndrome (15 patients), with the typical characteristics of classic Lesch-Nyhan syndrome, including mental retardation, choreoathetosis, spasticity, inability to walk or stand up, and full dependence for daily activities and personal needs. Some of these patients did not show self-injurious behavior. HPRT activity was undetectable in the hemolysate by routine methods and no residual HPRT activity could be detected in intact erythrocytes. Out of the 4 groups, the mutations that affected the predicted protein size were encountered only among these Group 4 patients, although some Lesch-Nyhan patients evidenced mutations that did not affect predicted protein size.

A clinical problem that we faced with 4 patients (Table 1, Patients 10, 14, 17, and 22), may arise when HPRT deficiency is diagnosed in an infant with no previously affected family members and neurologic symptoms overlapping among these 4 categories. In each of these 4 patients, however, enzymatic and molecular studies allowed a definitive classification that helped prognostic indications. Whether this classification of HPRT-deficient patients into 4 groups may be more helpful in terms of accuracy, reproducibility, assessment for treatment trials and prognosis purposes, as compared with the established classification into "complete" and "partial" HPRT deficiency, remains to be determined.

Summary

The enzyme hypoxanthine-guanine phosphoribosyltransferase (HPRT) catalyzes the reutilization of hypoxanthine and guanine to the purine nucleotides IMP and GMP, respectively. HPRT deficiency is an X-linked disorder characterized by uric acid overproduction and variable neurologic impairment. The complete deficiency of HPRT is diagnostic of Lesch-Nyhan syndrome manifested by choreoathetosis, spasticity, mental retardation, and self-injurious behavior. In some HPRT-deficient patients the enzyme defect appeared to be "partial" and the neurologic symptoms mild to severe (Kelley-Seegmiller syndrome). This has prompted the classification of HPRT deficiency in 2 distinct groups: Lesch-Nyhan syndrome and Kelley-Seegmiller syndrome, which has created much confusion. A spectrum of clinical consequences of HPRT deficiency has been recognized in small series of patients, but the complete spectrum of the neurologic disorder has not been described in a single series of patients examined by the same observers.

We analyzed our experience with 22 patients belonging to 18 different families with HPRT deficiency diagnosed at "La Paz" University Hospital in Madrid over the past 16 years. The clinical spectrum of these HPRT-deficient Spanish patients was similar to the different phenotypes occasionally reported in the literature, in some cases diagnosed as Lesch-Nyhan "variants." The clinical, biochemical, enzymatic, and molecular genetic studies on these 22 patients allowed us to delineate a new classification of HPRT deficiency. Based on the neurologic symptoms, dependency for personal care, HPRT activity in hemolysate and in intact erythrocytes, and predicted protein size, patients were classified into 4 groups: Group 1 (2 patients), normal development with no neurologic symptoms, HPRT activity was detectable in hemolysates and in intact erythrocytes, and the mutation did not affect the predicted protein size. Group 2 (3 patients) mild neurologic symptoms that did not prevent independent lives, HPRT activity was detectable in intact erythrocytes, and the protein size was normal. Group 3 (2 patients), severe neurologic impairment that precluded an independent life, no residual HPRT activity, and normal protein size. Group 4 (15 patients), clinical characteristics of Lesch-Nyhan syndrome (some may not show self-injurious behavior), no residual HPRT activity, and in most (7 of 8 patients in whom the mutation could be detected) the mutation affected the predicted protein size.

This classification of HPRT deficiency into 4 groups may be more useful in terms of accuracy, reproducibility, assessment for treatment trials and prognosis. The study of this Spanish series allows us to conclude that HPRT deficiency may be manifested by a wide spectrum of neurologic symptoms; the overall severity of the disease is associated with mutations permitting some degree of residual enzyme activity; and mutation analysis provides a valuable

tool for prognosis, carrier identification, and prenatal diagnosis.

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