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Brief Communication

A novel missense mutation, c.584A > C (Y195S), in two unrelated Argentine patients with hypoxanthine–guanine phosphoribosyl-transferase deficiency, neurological variant

L.E. Laróvere,[a,*] N. Romero,[b] L.D. Fairbanks,[c] C. Conde,[b] N. Guelbert,[a] A.L. Rosa,[b] and R. Dodelson de Kremer[a]

[a] *Centro de Estudio de las Metabolopatías Congénitas, Cátedra de Clínica Pediátrica, Facultad de Ciencias Médicas, Universidad Nacional de Córdoba, Hospital de Niños de la Santísima Trinidad, Córdoba, Argentina*
[b] *Laboratorio de Neurogenética, Instituto de Investigación Médica "Mercedes y Martín Ferreyra" (INIMEC-CONICET), Córdoba, Argentina*
[c] *Purine Research Laboratory, Guy's Hospital, London, UK*

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Abstract

The hypoxanthine–guanine phosphoribosyl-transferase (HPRT) deficiency is an inborn error of purine metabolism, responsible for classic Lesch–Nyhan disease and its neurological and hyperuricemic variants. We report a novel mutation in the *HPRT* gene, c.584A > C (Y195S), in two unrelated Argentine patients affected with the neurological variant with no HPRT activity in lysed erythrocytes. Using PCR plus DNA sequencing and/or restriction enzyme digestion we were able to confirm the diagnosis and identify new cases and potential carriers.

Keywords: HPRT; Hypoxanthine–guanine phosphoribosyl-transferase deficiency; Lesch–Nyhan variant; HPRT mutation

Introduction

The hypoxanthine–guanine phosphoribosyl-transferase (HPRT; EC 2.4.2.8) deficiency (MIM 308000) is an inborn error of purine metabolism associated with a spectrum of disease that may be divided into three overlapping clinical phenotypes, depending on the amount of residual enzyme activity [1]. Lesch–Nyhan disease comprises the most severely affected cases, which display uric acid overproduction, neurological dysfunction, and behavioral abnormalities that include impulsive and self-injurious behaviors. Cases with intermediate severity, referred to as neurological variants, show uric acid overproduction with a neurologic disability that varies from minor clumsiness to disabling neurological dysfunction. Hyperuricemic variant cases are the most mildly affected and demonstrate only marked overproduction of uric acid, with resultant hyperuricemia, nephrolithiasis, and gout [1].

HPRT deficiency is inherited as an X-linked recessive condition, and the *HPRT* gene has nine exons spanning approximately 45 kb at Xq26-27 [2]. The entire *HPRT* gene has been cloned and sequenced [3], and more than 200 mutations throughout the *HPRT* gene have been reported [4,5]. These advances at the molecular level have allowed for the development of rapid and convenient methods for diagnosis, carrier identification, and prenatal testing [1].

In the present study, we report the results of biochemical and molecular analysis in two unrelated Argentine patients with HPRT deficiency, neurological variant.

Case reports and laboratory findings

The two subjects were diagnosed with HPRT deficiency on the basis of clinical symptoms and laboratory findings.

* Corresponding author. Fax: +54 351 4586439.
E-mail address: lauralarovere@hotmail.com (L.E. Laróvere).

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Subject 1 was a 12-year-old male with mestizo ancestry, a race admixture between Amerindian groups and Caucasians. The proband referred episodes of painful inflammatory arthritis of the first left metatarsophalangeal joint with fever and leukocytosis. Serum uric acid was 16.0 mg/dl, and the patient had radiological signs of nephrolithiasis. Neurological involvement includes cognitive aspects—inability to learn, failing memory and low scores in verbal skill tests—mild peripheral neuropathy with low nerve conduction velocity, brisk tendon reflexes, extensor plantar response, and clonus. A maternal uncle has a similar phenotype including gouty nephrolithiasis.

Subject 2 was a 15-year-old male of also a mestizo ethnic origin. The patient showed an accumulation of urate crystals forming tophaceous deposits, radiological signs of nephrocalcinosis, and mild mental retardation. The serum uric acid was 13.8 mg/dl.

The analyses of purine metabolites in urine and HPRT activity in erythrocyte lysates were performed by high-performance liquid chromatography methods [6]. Results are shown in Table 1.

HPRT mutation analysis

Genomic DNA was extracted from total blood using the Wizard Genomic DNA Promega Purification Kit (Promega, Madison, USA). Total RNA was extracted from primary cultured fibroblasts obtained from skin biopsy of subject 1. The entire coding region of *HPRT* was reverse-transcribed from total RNA and amplified by PCR using primers 5′-CTCCGCCTCCTCCTCTGC-3′ and 5′-ACAACAATCCGCCCAAAGGG-3′. Mutation analysis of the *HPRT* gene was performed using direct sequencing of RT-PCR products.

In subject 1, the sequencing analyses showed that the molecular defect in the *HPRT* gene was a novel missense mutation, c.584A > C (Y195S). This mutation creates a new *Hinf*I site, which was amenable for analysis on genomic DNA using PCR (primers 5′-TGCTGGTGAA AAGGACCCC-3′ and 5′-CAAATTATGAGGTGCTG GAAGG-3′) followed by restriction enzyme digestion. DNA of subjects 1 and 2, as well as family members of subject 1, was subsequently examined with this procedure. Members of subject 2's family were not available for the molecular study. The mutation was found in four family members of subject 1: one hemizygote male, the maternal uncle mentioned above; and three heterozygote females (grandmother, mother, and sister). The same mutation was found in subject 2.

Discussion

The present study is the first characterization of the molecular genetic basis of HPRT deficiency, neurological phenotype, in Argentine patients. There is a previous report of another mutation in an Argentine family with Lesch–Nyhan disease, classic phenotype [7].

Four main remarks emerge from this study. First, the clinical presentation in subjects 1 and 2 was consistent with the neurological variant, but it was associated with total HPRT deficiency in the lysate assay employed. Thus, although values of residual enzymatic HPRT activity detected in live cells show a good clinical correlation in HPRT deficiency [1], the HPRT activity assayed in lysate erythrocytes does not define the clinical phenotype. Second, the position of the mutation in *HPRT* described here, c.584A > C (Y195S), is the same as a previously reported mutation, c.584A > G (Y195C); this mutation has been associated with the less severe phenotype of HPRT deficiency [8]. Thus, two pathogenic variants at Y^{195} determine two different phenotypes, both associated with total HPRT deficiency. These phenotypes may be determined by differences of HPRT activity *in vivo*. More interestingly, replacement of Y^{195} by either serine or cysteine may differentially disturb an unknown structural role of the HPRT polypeptide chain which is unrelated to its enzymatic activity. Third, the identification of a novel mutation in *HPRT* in two unrelated Argentine families suggests that this molecular defect may be frequent in our population. Last, although there is not a clear genotype-phenotype relationship for mutations in *HPRT*, the finding of similar phenotypes in subjects 1 and 2 suggests that some predictions might be possible for a subject carrying a mutation found in a previously described case [4].

RT-PCR and DNA sequencing was required to confirm the clinical diagnosis in reported Argentine patients. The developed diagnosis procedure based on PCR followed by *Hinf*I restriction enzyme digestion

Table 1
Urinary purine metabolites and erythrocyte HPRT activities in two Argentine subjects with the neurological variant of HPRT deficiency

Subject	Urinary metabolites (μmol/mmol creatinine)			HPRT activity (nmol/h/mg Hb)
	Uric acid	Hypoxanthine	Xanthine	
1	1700	530	190	<1
2	1800	380	150	<1
Controls	<1300	<45	<43	83–150

provides an effective strategy to widen the possibilities of identification of new cases and potential female carriers when genetic counseling is required.

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