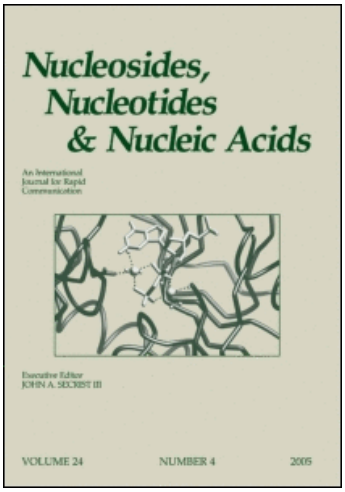

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R. J. Torres[a]; M. G. Garcia[a]; J. G. Puig[a]
[a] Divisions of Clinical Biochemistry and Internal Medicine, La Paz University Hospital, Madrid, Spain

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PARTIAL HPRT DEFICIENCY PHENOTYPE AND INCOMPLETE SPLICING MUTATION

R. J. Torres, M. G. Garcia, and J. G. Puig
Divisions of Clinical Biochemistry and Internal Medicine, La Paz University Hospital, Madrid, Spain

□ *Deficiency of hypoxanthine-guanine phosphoribosyltransferase (HPRT) activity is an inborn error of purine metabolism associated with uric acid overproduction and a continuum spectrum of neurological manifestations depending on the degree of enzyme deficiency. The complete deficiency causes Lesch-Nyhan syndrome (LNS). Partial HPRT-deficient patients can show a variable degree of neurological manifestations. Both diseases have been associated with mutations in the HPRT1 gene. Documented mutations in HPRT deficiency show a high degree of heterogeneity in type and location within the gene. In fact, more than 300 disease-associated mutations have been described. Splice mutations accounts for more that 16% of HPRT mutations and in most cases cause a complete LNS phenotype. A 16 year-old boy consulted to La Paz University Hospital because of hyperuricemia (9.4 mg/dL). At age one year he was given a diagnosis of dystonic cerebral palsy. Although he usually employs a wheelchair, under certain circumstances, he is able to stand up and walk by himself. He has never showed self injurious behavior. This patient presented a splice mutation (NM_000194.2: c.552 -2 A > G) causing exon 5 exclusion. An exon-5 specific PCR was designed, and a minor amount of normally spliced HPRT mRNA was found. Normally spliced HPRT mRNA was quantified by real-time PCR in this patient, in control subjects, and in two Lesch Nyhan patient with splice mutations excluding exon 4 (patient B) and exon 8 (patient C) who had clinically a Lesch Nyhan disease phenotype. A minor amount of normally spliced HPRT mRNA was found in all the patients. No correlation was found between the percentage of the normally spliced HPRT mRNA and the phenotype. We conclude that the partial HPRT deficient phenotype of this patient can not be explained by the finding of a minor amount of normally splice HPRT mRNA. It is possible that the amount of normally splice mRNA vary among different tissues.*

Keywords Lesch-Nyhan syndrome; HPRT; molecular diagnosis; splicing

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Address correspondence to Rosa J. Torres, La Paz University Hospital, Biochemistry Laboratory, P° de la Castellana 261, Madrid, 28046 Spain. E-mail: rtorres.hulp@salud.madrid.org

INTRODUCTION

Complete deficiency of enzymatic hypoxanthine-guanine phosphoribosyltransferase (HPRT) activity (OMIM 300322) is associated with the classical Lesch Nyhan syndrome phenotype:[1] uric acid overproduction, severe action dystonia, choreatetosis, defects in attention and executive functions, and self injurious behavior.[2] Partial deficiency of HPRT (OMIM 300323) is characterized by excess uric acid synthesis and a continuum spectrum of neurological manifestations.[3,4] Both diseases have been associated with mutations in the HPRT1 gene, located in the long arm of the X chromosome at Xq26. Documented mutations in HPRT deficiency show a high degree of heterogeneity in type and location within the gene: deletions, insertions, duplications, etc.[5] To date more than 300 disease-associated mutations have been found (www.lesch-nyhan.org). Missense point mutations are the main cause of partial HPRT deficiency, whereas mutations that alter the size of the predicted protein usually caused Lesch-Nyhan syndrome.[6,7] Splice mutations causing exon exclusion accounts for more that 16% of HPRT mutations described to date, and in most cases cause a complete Lesch Nyhan syndrome phenotype.

PATIENTS AND METHODS

Patient

A 16-year-old boy consulted to La Paz University Hospital because hyperuricemia (9.4 mg/dL). At age one year he was given a diagnosis of dystonic cerebral palsy. He lived with his parents under a university-education environment. Although he usually employs a wheelchair, under certain circumstances he is able to stand up and walk by himself (Figure 1). He has never showed self injurious behavior. After a detailed history and physical exam he was given a provisional diagnosis of partial HPRT deficiency with severe HPRT related neurological dysfunction (HRND, grade 3). HPRT activities in the haemolysate and in intact erythrocytes of the patient were both undetectable. This patient presented a splice mutation (NM_000194.2: c.552 -2 A > G) causing exon 5 exclusion.

Exon 5 Specific PCR

Total RNA was isolated from peripheral blood using the QIAamp RNA Blood Mini Kit (QIAGEN GmbH, D-40724, Hilden, Germany). A first-strand cDNA template was generated using the ImProm-II Reverse Transcriptase system (Promega, Promega Corporation, Madison, WI, USA) and oligo (dT) as a primer for RT-PCR. The entire coding region of the HPRT cDNA was

FIGURE 1 HPRT deficient patient is able to stand up and walk in the beach.

amplified from the single strand cDNA by PCR (Figure 2; PCR A). Exon 5-containing HPRT cDNA was amplified from the single strand cDNA by the use of an exon5-specific forward primer which anneal to exon 5 (Figure 2; PCR B).

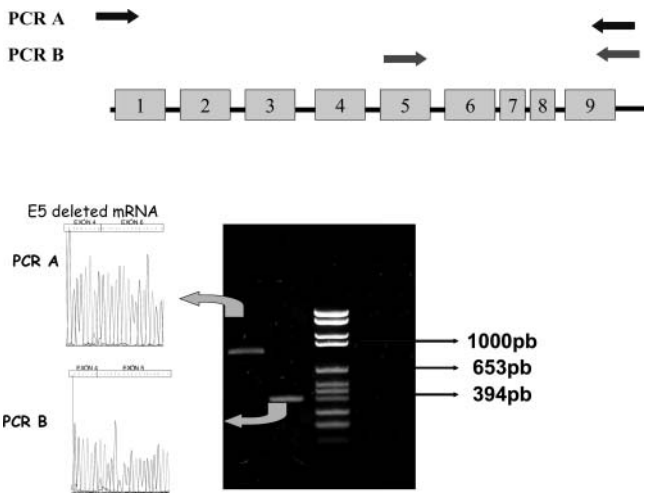

FIGURE 2 A) Agarose gel showing exon 5 deleted mRNA obtained in PCR A and normally spliced mRNA obtained in PCR B.

Real-Time HPRT Expression Quantification

HPRT mRNA expression was quantified by real-time PCR with the use of a relative quantification method. In addition to the studied patient, we also examined exon 4 and exon 8-containing HPRT expression from two Lesch Nyhan patients with the complete phenotype and a splicing mutation accounting for exon 4 (patient B) and exon 8 (patient C) exclusion, respectively. Exon 5, exon 4, and exon 8-containing HPRT expression was also quantified in 6 control subjects. In all subjects, total RNA was isolated and first-strand cDNA template was generated as previously described.

We employed a housekeeping gene such as β-Actin as a reference gene and the results were expressed as a relative ratio of the HPRT to β-Actin expression measured in the same sample material. Serial dilutions of cDNA were employed to construct standard curves for human β-Actin and exon5, exon 4, and exon8-specific mRNA expression. The calibrator sample was assigned a value of 100. Real-time PCR was performed in a Roche LightCycler using SYBR Green Premix Ex-Taq (Takara Bio Europe, Saint Germain en Laye, France) with 2 μl of cDNA as the template and specific exon 5, exon 4, or exon 8 specific forward primers. A melting curve analysis was used to determine the melting temperature (Tm) of the amplified products so as to ensure its specificity. The mean of the control subject ratio of exon5, exon 4 and exon8-specific HPRT mRNA/β-Actin expression was considered 100% expression.

RESULTS

Selective amplification of patient's HPRT cDNA including exon 5 was confirmed by agarose gel electrophoresis (Figure 2; PCR B). Quantified exon 5-specific HPRT mRNA expression showed a 0.44% expression as compared to control subjects. Patient B and C showed an exon 4-specific and exon 8-specific HPRT mRNA expression of 0.19% and 3.0%, respectively, as compared to control subjects.

DISCUSSION

In this study, we present an HPRT deficient patient in which no residual enzymatic activity could be detected. He presented a splice mutation causing exon 5 deletion in HPRT cDNA. The in-frame exon 5 deletion predicted 6 amino acids excluded protein, with the rest of the sequence normal. Two additional HPRT deficient patients with a splice mutation causing exon 5 exclusion have been described.[7,8] Both of them were primarily described as Lesch Nyhan syndrome[7,8] but none of them presented typical self-injurious behavior.[9,10] The diagnosis of a variant form of LND required evidence for

an HPRT gene mutation or reduced HPRT enzyme activity in a male patient without the self-injurious behavior typical of Lesch Nyhan patients.[9] Thus, our patient, who has never showed self injurious behavior, can be clinically diagnosed as a variant or partial HPRT deficiency with severe HPRT related neurological dysfunction (HRND, grade 3). To date, at least 5 variant patients with splicing mutations, which are known to be "leaky" and permit variable residual activity have been described,[9] so the aim of the present study was to asses if a minor amount of normally spliced HPRT mRNA could account for the less severe phenotype and the absence of self-injurious behavior. As we expected, a minor amount of normally spliced HPRT mRNA was found in our patient. However, by real-time PCR a minor amount of normally spliced mRNA was also detected in two Lesch Nyhan patients, with a splice mutation, who showed self-injurious behavior. When we quantified the normally spliced HPRT mRNA in the three patients we found that there was no correlation between the percentage of the normally spliced HPRT mRNA and the phenotype. However, we have measured HPRT mRNA in blood cells and it has been reported that the amount of normally spliced mRNA may vary between different tissues.[11] So, there remains the possibility that these patients, with a severe partial HPRT deficiency phenotype and splice mutations, may have some HPRT activity in the brain.

REFERENCES

1. Lesch, M.; Nyhan, W.L. A familial disorder of uric acid metabolism and central nervous system function. *Am. J. Med.* **1964**, 36, 561–570.
2. Jinnah, H.; Visser, J.E.; Harris, J.C.; Verdu, A.; Larovere, L.; Ceballos-Picot, I.; Gonzalez-Alegre, P.; Neychev, V.; Torres, R.J.; Dulac, O.; Desguerre, I.; Schretlen, D.J.; Robey, K.L.; Barabas, G.; Bloem, B.R.; Nyhan, W.; De Kremer, R.; Eddey, G.E.; Puig, J.G.; Reich, S.G. Lesch-Nyhan Disease International Study Group. Delineation of the motor disorder of Lesch-Nyhan disease. *Brain* **2006**, 129, 1201–1217.
3. Kelley, W.N.; Rosenbloom, F.M.; Henderson, J.F.; Seegmiller, J.E. A specific enzyme defect in gout associated with overproduction of uric acid. *Proc. Natl. Acad. Sci. USA* **1967**, 57, 1735–1739.
4. Puig, J.G.; Torres, R.J.; Mateos, F.A.; Ramos, T.H.; Arcas, J.M.; Buno, A.S.; O'Neill, P. The spectrum of hypoxanthine-guanine phosphoribosyltransferase (HPRT) deficiency. Clinical experience based on 22 patients from 18 Spanish families. *Medicine (Baltimore)* **2001**, 80, 102–112.
5. Jinnah, H.A.; De Gregorio, L.; Harris, J.C.; Nyhan, W.L.; O'Neill, J.P. The spectrum of inherited mutations causing HPRT deficiency: 75 new cases and a review of 196 previously reported cases. *Mutat. Res.* **2000**, 463, 309–326.
6. Torres, R.J.; Mateos, F.A.; Molano, J.; Gathoff, B.S.; O'Neill, J.P.; Gundel, R.M.; Trombley, L.; Puig, J.G. Molecular basis of hypoxanthine-guanine phosphoribosyltransferase deficiency in thirteen Spanish families. *Hum. Mut.* **2000**, 15, 383.
7. Jinnah, H.A.; Harris, J.C.; Nyhan, W.L.; O'Neill, J.P. The spectrum of mutations causing HPRT deficiency: an update. *Nucleosides Nucleotides Nucleic Acids* **2004**, 23, 1153–60.
8. Mak, B.S.; Chi, C.S.; Tsai, C.R.; Lee, W.J.; Lin, H.Y. New mutations of the HPRT gene in Lesch-Nyhan syndrome. *Pediatr. Neurol.* **2000**, 23, 332–335.
9. Jinnah, H.A.; Ceballos-Picot, I.; Torres, R.J.; Visser, J.E.; Schretlen, D.J.; Verdu, A.; Laróvere, L.L.; Chen, C.J.; Cossu, A.; Wu, C.H.; Sampat, R.; Chang, S.J.; Dodelson de Kremer, R.; Nyhan, W.; Harris,

J.; Reich, S.G.; Puig, J.G. for the Lesch-Nyhan Disease International Study Group. Attenuated Variants of Lesch-Nyhan Disease. *Brain* **2010**, 671–689.

10. Jinnah, H.A.; Visser, J.E.; Harris, J.C.; Verdu, A.; Larovere, L.; Ceballos-Picot, I.; Gonzalez-Alegre, P.; Neychev, V.; Torres, R.J.; Dulac, O.; Desguerre, I.; Schretlen, D.J.; Robey, K.L.; Barabas, G.; Bloem, B.R.; Nyhan, W.; De Kremer, R.; Eddey, G.E.; Puig, J.G.; Reich, S.G. Lesch-Nyhan Disease International Study Group. Delineation of the motor disorder of Lesch-Nyhan disease. *Brain* **2006**, 129, 1201–1217.
11. Guiraud-Dogan, C.; Huguet, A.; Gomes-Pereira, M.; Brisson, E.; Bassez, G.; Junien, C.; Gourdon, G. DM1 CTG expansions affect insulin receptor isoforms expression in various tissues of transgenic mice. *Biochim. Biophys. Acta.* **2007**, 1772, 1183–1191.