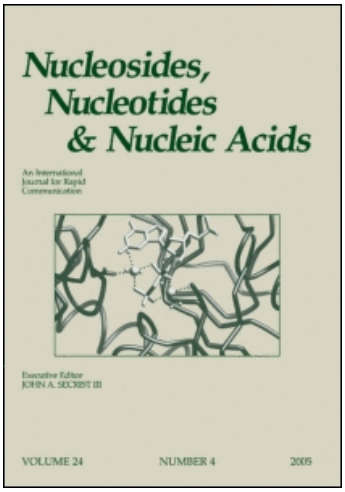

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LESCH-NYHAN DISEASE

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□ *Lesch-Nyhan disease is the most severe or complete phenotype of deficiency in hypoxanthineguanine phosphoribosyltransferase; other variant enzymes are found in patients without abnormality in behavior or mental development, and there are intermediate phenotypes in which enzyme activity is intermediate. A considerable number and variety of mutations in the HPRT gene have been discovered.*

Keywords Lesch-Nyhan disease; HPRT; mutation, hyperuricemia; self-injurious behavior

INTRODUCTION

Lesch-Nyhan disease is an X-linked recessive disorder of purine metabolism which results from deficiency of the activity of the enzyme hypoxanthine guanine phosphoribosyltransferase (HPRT).[1–3] Deficiency of HPRT is the most common of the diseases of purine metabolism and of the hyperuricemias of childhood, and, among patients with HPRT deficiency, the majority have the classic Lesch-Nyhan disease. There is a variety of clinical phenotypes of HPRT deficiency. The most severe is the classic Lesch-Nyhan disease in which neurologic and behavioral features occur, along with clinical manifestations of hyperuricemia that are shared with patients with gout. At the other end of the spectrum are variant or partial variant patients who have only manifestations directly attributable to uric acid overproduction. An intermediate group we have called neurologic variants, have a variety of neurologic manifestations. In the usual neurologic phenotype, the patient is neurologically identical to the classic patient but has no self injurious behavior and intelligence may be normal or nearly so. Another variant we have called $HPRT_{Salamanca}$ is characterized neurologically by spasticity.[4]

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MATERIALS AND METHODS

HPRT activity is most conveniently assayed in erythrocyte lysates. In our early approaches to the assay[5] lysates of erythrocytes or fibroblasts were incubated with 8-^{14}C-hypoxanthine, 5-phosphoribosylpyrophosprite (PRPP) $MgCl_2$ and phosphate buffer, the reaction stopped by chelation of Mg by ethylene-diamine-tetra-acetic acid (EDTA), and the products separated on a cation exchange column and quantified by liquid scintillation counting. In erythrocytes the only radiolabeled product measured over time was IMP, while for the fibroblast assay the sum of radioactivity in IMP and inosine was measured over time. We also developed a simple method suitable for screening in which the reaction products were precipitated with lanthanum chloride and the radioactivity quantified in the supernatant fluid.[6] This assay was adapted for dried blood spots on filter paper as well as heparinized whole blood.

More recently, we have employed a procedure originally developed for the assay of HPRT activity in individual hair roots as a test for heterozygosity.[7] In this assay, the reaction mixture contained the nucleotidase inhibitor $\acute{\alpha}$, β-methyleneadenosine diphosphate. After incubation, lysates were spotted on polyethylene cellulose-coated TLC (PET) sheets and the products separated from substrate and nucleosides by ascending chromatography in methanol/water. The nucleotide spots were cut out and the radioactivity quantified by liquid scintillation counting.

Our current practice is to assay blood spots routinely. APRT activity is measured simultaneously as a control for sample integrity and the vagaries of shipment. APRT activity is elevated in patients with HPRT deficiency, and this serves as an indicator that a deficiency of HPRT is not factitious. Samples are shipped at room temperature. Following incubation for 10 minutes, with 8-^{14}C-hypoxanthine or adenine and PRPP in HEPES buffer at pH 7.2, reactions are terminated with $HClO_4$ and neutralized with KOH.

Supernatant fluids are chromatographed on PET cellulose plates. The spots at the origin are cut out and their radioactivity determined by liquid scintillation counting. Activities are reported in μmoles of nucleotide/3 mm blood spots (Figure 1). Our normal values for HPRT are 16.88 $\pm$ 3.47, and for APRT 3.72 $\pm$ 1.64. In a series of 18 patients with Lesch-Nyhan disease studied from 9/22/04 to 4/20/07, the mean activity was 0.02 $\pm$ 0.04 for HPRT while APRT mean was 7.49 $\pm$ 4.63; the range for APRT was 2.6 to 22.3.

In the intact cell method[8] cultured fibroblasts are incubated with ^{14}C-hypoxanthine; the products are separated by high performance liquid chromatography (HPLC); and the total numbers of picomoles of isotope incorporated into purine products are expressed per nanomole of total purine compound present. Cells are grown in roller bottles, harvested and

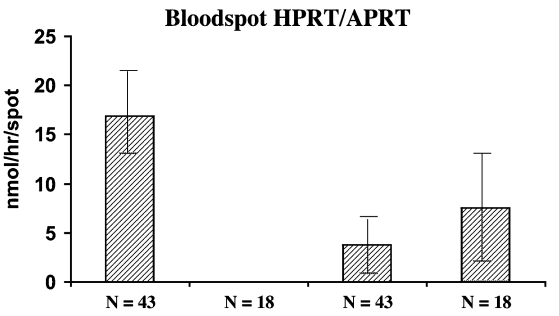

FIGURE 1 The bar on the left represents HPRT in 43 controls; the next, HPRT activity approximating 0 in 18 patients with Lesch-Nyhan disease. Those on the right represent APRT activity in the 43 controls and 18 patients.

suspended in phosphate buffered saline with glucose and human serum albumin in concentration of 4×10^6 cells/2ml; and separate 2 ml aliquots are incubated with 8-^{14}C-hypoxanthine, guanine, and adenine at 37°C for 2 hours. After centrifugation and removal of supernatant fluid, the reaction is stopped with $HClO_4$, neutralized with KOH and centrifugation. Samples are separated on HPLC with a UV detector and a scintillation flow detector. Results are reported in pmol/100 nMol UV for HPRT, GPRT, and APRT.

RESULTS

The Au: Pls. provide cite. figure 1 in text. classic Lesch-Nyhan disease patient usually has an enzyme activity close to 0. Patients with partial HPRT deficiency may have values between 0% and 60% of control. Thus a value of 0 in a patient with the classic clinical presentation is diagnostic. In the intact fibroblast assay, cells of patients with the classic Lesch-Nyhan phenotype have displayed activity below 1.2% of control. Classic partial variants have been over 10%, and the neurologic variants have been intermediate.[6] In $HPRT_{Salamanca}$ activity was 7.5 % of control.[4]

DISCUSSION

A preliminary diagnosis of classic Lesch-Nyhan disease can be made on the basis of the phenotype. Dystonia and spasticity in combination with the typical self-injurious behavior is virtually diagnostic. In the variant partial HPRT deficiencies, the clinical phenotype may be misleading. The numbers of patients even with the classic phenotype carrying a diagnosis of cerebral palsy well into childhood indicate that indices of suspicion may be low. The presence of hyperuricemia and increased excretion of uric acid are important clues, but it is worth remembering that uric acid is not part of routine chemistry panels in children's hospitals.

A serum concentration of uric acid in a child of more than 4–5 mg/dl and a urine uric acid to creatinine ratio of 2 to 4 are strongly supportive, but a definitive diagnosis requires analysis of HPRT enzyme activity. There are pitfalls in the analysis of uric acid too. Some patients are so efficient in excreting uric acid that serum concentrations are normal. Others are thought to be normal because the laboratory determined its normal ranges on adult males in whom hyperuricemia is common. Uricosuria is more reliable, but purines are so readily metabolized by contaminating microorganisms at the bedside that these values may be factitiously normal even in classic patients. Particularly bad is the collection of a 24-hour urine sample in a bottle at room temperature at the bedside. Accordingly, we recommend instead a spot collection promptly frozen or analyzed. We do collect 24-hour samples, but the collection bottle is kept in the freezer and each voiding sample is added as passed.

Activities in erythrocytes of patients with variant forms of HPRT are readily distinguished from normal. Some have quite a lot of demonstratable activity, but most do not. The problem is distinguishing them from patients with the classic Lesch-Nyhan phenotype; because the assay often reveals no activity. Fibroblast lysates are the same. Structurally abnormal enzymes are often unstable and lose activity rapidly once cellular barriers are broken. This issue is of enormous importance in counseling parents of a very young infant found to have HPRT deficiency. What parents want to know is if the prognosis for neurologic and behavioral disability or are the problems simply those of hyperuricemia. One day mutational analysis may give the answer, but so far it usually does not. Currently the gold standard for predication of phenotype remains the intact cell assay developed by Bohdan Bakay.[8]

The gene for HPRT is located on the X chromosome and mapped to the distal part of the long arm at position Xq2.6–Xq2.7. The disease is almost exclusively in male hemizygotes but seven affected females have been reported.[9,10] The HPRT gene contains 44kb of DNA in 9 exons. The mRNA is 1.6kb in length but the actual reading frame is smaller. It codes for protein of 217 amino acids in a subunit which is assembled in a tetrameric enzyme protein. A wide spectrum of mutations is known. The most recent compilation is of 302, and virtually every family has had unique mutation in the HPRT gene.[11,12] Every possible type of mutation has been described within this gene.

In general, major disruptions such as deletions, large insertions, splice mutations; nonsense mutations or amino acid substitutions that are not conservative are associated with the classical Lesch-Nyhan phenotype. Deletions have been described for exon 4, and exon 7, while larger pieces of the gene have also been found, for instance deletion of exon 4 to 9 or even the whole gene. Genotype-phenotype corrections are emerging in this expanding data base.

Among point mutations found in variants harboring some residual activity there is often a conservative amino acid substitution. At any given position in the gene conservative change leads to variant, a nonconservative change leads to the classical Lesch-Nyhan phenotype. For instance, in exon 2, there is normally a glycine at position 16; if it is changed to serine, a conservative change, the phenotype is variant; if it changes to aspartic acid, a nonconservative change, the patient has the classic Lesch-Nyhan phenotype. In another example, at position 194 the acidic amino acid aspartic when changed to glutamic, a conservative transition, the phenotype is a variant; a change to tyrosine or histidine would be very non-conservative, and the phenotype in these patients was Lesch-Nyhan.

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