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### Research Article

In past reports of hypoxanthine-guanine phosphoribosyltransferase (HPRT) deficiency a marked degree of molecular heterogeneity has been noted. We have previously described two apparently unrelated subjects with partial HPRT deficiency, G.S. and D.B., who have a mutant form of HPRT with remarkably similar alterations in physical and kinetic properties. The mutation in G.S. is a serine to leucine substitution at amino acid 110 as determined by amino acid sequence analysis. This mutant enzyme has been designated HPRTL<sub>London</sub>. We have examined HPRT cDNA from D.B. using two different methods to determine if the similar properties of mutant HPRT from these two subjects are the result of a common mutation. HPRT cDNA clones were obtained by routine cloning techniques and by polymerase chain reaction amplification of single-stranded cDNA reverse transcribed from mRNA derived from subject D.B. Dideoxynucleotide sequencing revealed a single mutation, a C to T transition at bp 329 in clones generated by both methods. This mutation in D.B. predicts the identical amino acid substitution described in HPRTL<sub>London</sub>. A C to T nucleotide transition at 329 in D.B. creates an Hpa I site in exon 4 of the HPRT gene. Southern blot analysis of genomic DNA isolated from lymphoblasts derived from G.S. and D.B. revealed that both have this additional Hpa I site, indicating that the similarly altered protein sequence is due [...]

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## Hypoxanthine-Guanine Phosphoribosyltransferase

### Genetic Evidence for Identical Mutations in Two Partially Deficient Subjects

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#### Abstract

In past reports of hypoxanthine-guanine phosphoribosyltransferase (HPRT) deficiency a marked degree of molecular heterogeneity has been noted. We have previously described two apparently unrelated subjects with partial HPRT deficiency, G.S. and D.B., who have a mutant form of HPRT with remarkably similar alterations in physical and kinetic properties. The mutation in G.S. is a serine to leucine substitution at amino acid 110 as determined by amino acid sequence analysis. This mutant enzyme has been designated HPRT<sub>London</sub>.

We have examined HPRT cDNA from D.B. using two different methods to determine if the similar properties of mutant HPRT from these two subjects are the result of a common mutation. HPRT cDNA clones were obtained by routine cloning techniques and by polymerase chain reaction amplification of single-stranded cDNA reverse transcribed from mRNA derived from subject D.B. Dideoxynucleotide sequencing revealed a single mutation, a C to T transition at bp 329 in clones generated by both methods. This mutation in D.B. predicts the identical amino acid substitution described in HPRT<sub>London</sub>.

A C to T nucleotide transition at 329 in D.B. creates an *Hpa* I site in exon 4 of the HPRT gene. Southern blot analysis of genomic DNA isolated from lymphoblasts derived from G.S. and D.B. revealed that both have this additional *Hpa* I site, indicating that the similarly altered protein sequence is due to the identical transition in the HPRT gene.

#### Introduction

Hypoxanthine-guanine phosphoribosyltransferase (HPRT)<sup>1</sup> is a purine salvage enzyme that catalyzes the conversion of hypo-

xanthine and guanine to inosine monophosphate and guanosine monophosphate, respectively. Partial deficiency of HPRT results in hyperuricemia, hyperuricaciduria, uric acid nephrolithiasis, and precocious gout (1). Virtually complete deficiency of HPRT causes the Lesch-Nyhan syndrome (2). In addition to hyperuricemia and hyperuricaciduria, this disease is characterized by growth and mental retardation, spasticity, choreoathetosis, and compulsive self-mutilation (3).

Previous evidence suggests that new and independent mutations sustain these disorders in the population (4). We have previously examined this hypothesis by analyzing 24 unrelated HPRT-deficient patients with regard to HPRT enzyme activity and intracellular concentration, physical and kinetic properties, mRNA levels, and restriction fragment length polymorphisms. Substantial heterogeneity was demonstrated with 67% of cases studied representing different mutations (5).

On the basis of these observations, HPRT-deficient subjects were classified into 16 types (5). 3 of these 16 categories (types I, XI, and XVI) contain more than one mutant. Type I has recently been shown to be heterogeneous by cDNA sequencing (6, 7), and definition of the mutations in type XI at the nucleotide level should result in further subdivision of this group.

Similarities between D.B. and G.S., the two partially deficient subjects with type XI HPRT deficiency, extend beyond DNA haplotype and the presence or absence of HPRT mRNA. Analyses of HPRT from these subjects are similar with respect to enzyme activity, intracellular concentration, kinetic and catalytic parameters, and physical properties. The amino acid substitution in HPRT purified from cultured cells derived from G.S. (HPRT<sub>London</sub>) has been determined to be a serine to leucine substitution at position 110 (8).

The phenotypic similarity of these two mutant proteins raised the question of genotypic identity. Therefore, we have cloned HPRT sequences from mRNA isolated from EBV-transformed lymphocytes derived from subject D.B. Additionally, we have examined the entire coding sequence using the polymerase chain reaction (PCR) to amplify single-stranded cDNA reverse transcribed from D.B.'s mRNA. Dideoxynucleotide sequencing of the HPRT cDNA from D.B. revealed a C to T transition at base position 329 that causes a serine to leucine substitution at amino acid 110. This point mutation creates an *Hpa* I site in the HPRT gene that allows confirmation at the genomic DNA level that both HPRT<sub>London</sub> and HPRT from patient D.B. have identical mutations.

#### Methods

*Cell lines.* Lymphoblastoid cell lines from patients G.S., D.B., and subjects with normal HPRT activity were established and maintained

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1. Abbreviations used in this paper: ds, double-stranded; HPRT, hypoxanthine-guanine phosphoribosyltransferase; PCR, polymerase chain reaction; P<sub>i</sub>, relative probability.

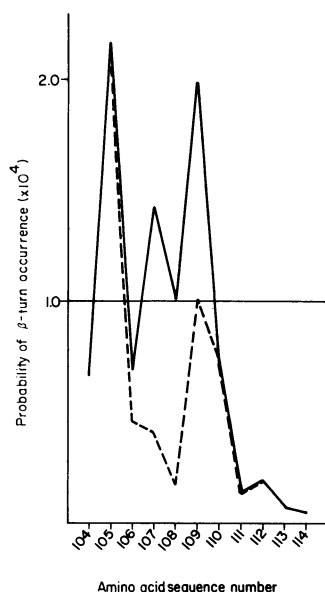
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**Figure 3.** Probability of  $\beta$ -turn occurrence from amino acids 104 to 114 for normal HPRT protein (—) and HPRT from G.S. and D.B. (---). The probability value of a tetrapeptide composed of  $i-i+3$  is plotted against  $i$ , where  $i$  represents the amino acid sequence number. A cutoff value of  $1 \times 10^{-4}$  is represented by the horizontal line.

is an identical mutation within the HPRT coding sequence. In view of the striking heterogeneity of HPRT mutations this genotypic identity is remarkable.

There are three possible explanations for the origin of this mutation in these two individuals: (a) common ancestry of the affected individuals (a single mutational event); (b) independent events at a mutational "hot spot"; and (c) coincidental independent mutational events. Unfortunately we cannot determine if this mutant allele is shared by G.S. and D.B. because of common ancestry since G.S. is adopted. In the case of D.B. there is no family history of gout or kidney stones, suggesting that this mutation arose within a recent generation. Mutational hot spots are most often found in GC-rich areas, with transitions occurring in a CpG or CpC dinucleotide context (24–26). No CpC or CpG dinucleotide exists at the transitional position in HPRT<sub>London</sub> (CAGTCAACA). Thus the classification of codon 110 in the HPRT gene as a hot spot for transitional mutations is unlikely in the context in which nucleotide 329 is found. The frequency of spontaneous mutations occurring at this position can only be addressed by a more extensive survey.

The impact of the serine to leucine substitutions at 110 on enzymatic function is probably a result of steric or polar alterations within the putative hypoxanthine binding site (27). Our calculations predict that proper folding may be impaired due to an alteration in the  $\beta$ -turn structure near the hypoxanthine binding pocket. This may in turn affect protein stability, which could account for the lower than normal amounts of HPRT protein in G.S. and D.B. (5, 28).

cDNA cloning and PCR amplification of HPRT mRNA have been used in this study to determine a point mutation in a patient with partial HPRT deficiency. Since  $\sim 90\%$  of HPRT-deficient subjects show no gross alterations in the HPRT gene, have normal amounts of HPRT-specific mRNA, and have low levels of HPRT protein, these techniques provide a direct means for determination of nucleotide substitutions causing altered protein structure and function. The ease and rapidity with which the PCR technique can be done make it particularly useful for further defining structural mutations in HPRT.

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