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## Human hypoxanthine-guanine phosphoribosyltransferase: a single nucleotide substitution in cDNA clones isolated from a patient with Lesch-Nyhan syndrome (HPRT<sub>Midland</sub>)

(Recombinant DNA; cDNA cloning; secondary structure prediction; hyperuricemia)

Beverly L. Davidson, Thomas D. Paella and William N. Kelley

*Departments of Internal Medicine and Biological Chemistry and the Rackham Arthritis Research Unit, University of Michigan Medical School, Ann Arbor, MI 48109 (U.S.A.)*

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### SUMMARY

We have determined the molecular basis for hypoxanthine-guanine phosphoribosyltransferase (HPRT) deficiency in a patient, J.H., with Lesch-Nyhan syndrome. Radioimmunoassay of lysates of erythrocytes or cultured B-lymphoblasts showed that this patient had no detectable HPRT enzyme activity or HPRT protein. HPRT-specific mRNA levels were normal by Northern analysis.

We created a cDNA library from mRNA isolated from cultured lymphoblasts derived from this patient. Nucleotide sequencing of full-length HPRT cDNA clones revealed a single nucleotide (nt) substitution: a T-to-A transversion at nt 389. We have designated this variant HPRT<sub>Midland</sub>. The predicted amino acid (aa) substitution in HPRT<sub>Midland</sub> is a valine to aspartic acid at aa 130. This substitution is within 2 aa of the amino acid substitution in a previously defined HPRT variant, HPRT<sub>Ann Arbor</sub>. Both mutations are within a highly conserved sequence in the putative 5-phosphoribosyl-1-pyrophosphate-binding domain. The amino acid substitution in HPRT<sub>Midland</sub> causes a significant perturbation in the predicted secondary structure of this region. The HPRT<sub>Midland</sub> mutation affects a different domain of HPRT than the HPRT<sub>Flint</sub> mutation located at 167 nt away.

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### INTRODUCTION

Hypoxanthine-guanine phosphoribosyltransferase (HPRT) is a cytoplasmic enzyme involved in the salvage of purine bases. HPRT catalyzes the for-

mation of IMP and GMP from hypoxanthine and guanine, respectively. Partial deficiency of this enzyme results in overproduction of uric acid leading to severe, precocious gout and nephrolithiasis (Kelley et al., 1967). Virtually complete deficiency of

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*Correspondence to:* Dr. T.D. Paella, Department of Internal Medicine, University of Michigan Medical School, 1150 West Medical Center Drive, 5520 Medical Science Research Building I, Ann Arbor, MI 48109 (U.S.A.) Tel. (313)747-3413.

Abbreviations: aa, amino acid(s); bp, base pair(s); CRM, immunologically cross-reactive material; HPRT, hypoxanthine-guanine phosphoribosyltransferase; *HPRT*, gene coding for HPRT; J.H., patient's initials; mU, milliunits; nt, nucleotide(s); PRPP, 5-phosphoribosyl-1-pyrophosphate.

HPRT causes Lesch–Nyhan syndrome consisting of hyperuricemia, hyperuricaciduria and devastating neurological dysfunction, the hallmarks of which are mental retardation, spasticity, choreoathetosis and compulsive self-mutilation (Lesch and Nyhan, 1964; Seegmiller et al., 1967). The importance of the study of HPRT goes beyond these human diseases. Because of selection systems for both the HPRT<sup>+</sup> and HPRT<sup>-</sup> phenotypes, this protein and its gene have become important tools in genetic research (Szybalska and Szybalski, 1962; Rubin et al., 1971).

Subjects with HPRT deficiency reveal a striking degree of phenotypic and genotypic variety (Yang et al., 1984; Wilson et al., 1986a; Gibbs and Caskey, 1987). However, studies of the *HPRT* gene and mRNA from deficient subjects using Southern- and Northern-blot techniques are normal in most cases (Yang et al., 1984; Wilson et al., 1986a). Thus, more detailed analysis of *HPRT* gene sequences is necessary to understand the molecular bases for enzymatic abnormalities.

Six mutant forms of HPRT from deficient subjects have been characterized previously at the molecular level. The amino acid substitutions in four mutant forms of HPRT were determined by amino acid sequencing of aberrant peptides derived from highly purified erythrocyte HPRT from deficient subjects (Wilson and Kelley, 1983a; Wilson et al., 1983b, 1984). This technique is limited to those HPRT-deficient subjects who express sufficient levels of HPRT protein for purification and amino acid sequence determination. Furthermore, this technique is time consuming and does not necessarily reveal the underlying mutation. More recently, we have exploited the observation that approximately 80% of HPRT deficient subjects have sufficient amounts of *HPRT*-specific mRNA for cDNA cloning in order to define the mutations in two other HPRT variants (Davidson et al., 1988; Fujimori et al., 1988).

Previously identified mutations in HPRT are widely scattered throughout the primary structure. HPRT<sub>Midland</sub>, the mutant described in this study, is due to a single aa substitution located only two aa residues from a previously defined HPRT variant, HPRT<sub>Ann Arbor</sub> (Fujimori et al., 1988). Both mutations fall in the putative PRPP binding domain of HPRT (Argos et al., 1983). Additionally, the region in which these mutations are found is conserved among different species.

## EXPERIMENTAL AND DISCUSSION

### (a) Cell lines

A patient (J.H.) with Lesch–Nyhan syndrome had undetectable levels of HPRT activity in both erythrocytes and cultured B-lymphoblasts (<0.1 mU/mg or <0.7% of control values) (Wilson et al., 1986a). In addition, levels of HPRT protein were undetectable as measured by radioimmunoassay using polyclonal anti-human HPRT antiserum (<1.5 ng CRM/mg or <0.5% of control values) (Wilson et al., 1986a). We have designated HPRT from this patient as HPRT<sub>Midland</sub>.

HPRT<sub>Ann Arbor</sub> was originally described as an HPRT variant on the basis of enzymatic properties (Wilson et al., 1982a). The mutation responsible for HPRT<sub>Ann Arbor</sub> has been defined (Fujimori et al., 1988). In contrast to J.H., this patient had partial HPRT deficiency with 10% of control levels of enzyme activity, and 11% of control values of CRM.

J.H. lymphoblasts were maintained in RPMI medium with 5% fetal calf serum as described previously (Wilson et al., 1982a).

### (b) cDNA cloning and isolation of recombinants

Approximately 1.0 g (wet weight) of HPRT<sub>Midland</sub> B-lymphoblasts was used for extraction of total RNA using guanidium isothiocyanate (Chirgwin et al., 1979) followed by oligo(dT) chromatography to select for poly(A)<sup>+</sup> RNA (Aviv and Leder, 1972). Oligo(dT)<sub>12-17</sub> primed cDNAs were synthesized, using 5 to 20 µg of poly(A)<sup>+</sup> RNA according to established procedures (Okayama and Berg, 1982; Gübler and Hoffman, 1983), and cloned in λgt11 (Young and Davis, 1983).

The resultant cDNA library was screened with an *MspI*-*TaqI* fragment of normal human *HPRT* cDNA which represents 5'-noncoding and coding sequences (Brennand et al., 1983). This 160-bp fragment was labeled with [ $\alpha$ -<sup>32</sup>P]dCTP using hexadeoxynucleotide primers (Feinberg and Vogelstein, 1984).

Two *HPRT*-specific cDNA clones, pHPJH1 and pHPJH7, were isolated. Recombinant phages were purified from 20 ml of phage lysate using standard techniques (Maniatis et al., 1982). The inserts were excised, separated from vector sequences and cloned

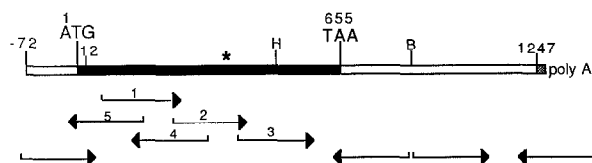


Fig. 1. Sequencing strategy for  $HPRT_{Midland}$  cDNA clones pHPJH1 and pHPJH7. Both pHPJH7, which extends to nt -72 and pHPJH1, which extends to nt 12 were subcloned into the *EcoRI* site of M13mp18 or M13mp19 and sequenced with  $HPRT$ -specific oligodeoxynucleotide primers (arrows 1 through 5) and the M13 universal primer (unlabeled arrows). The nucleotide sequences of the  $HPRT$ -specific oligodeoxynucleotide primers are listed in Table I. The ATG start codon, TAA stop codon and poly(A) tail are indicated. The *HindIII* (H)-*BclI* (B) fragment (255 bp) was subcloned into *HindIII* + *SmaI*-cut M13mp18 and M13mp19 for determination of 3'-noncoding sequences. These subclones were sequenced using the universal primer. The position of the mutation in pHPJH1 and pHPJH7 is indicated by the asterisk. Both clones were sequenced entirely in both directions. Open bar, 5' and 3' noncoding sequences; solid bar, coding sequences; stippled bar, poly(A) tail.

in M13mp18 and M13mp19 for isolation of single-stranded phages (Messing, 1983).

### (c) Sequencing and recombinant analysis

Sequences of pHPJH1 and pHPJH7 were determined using the sequencing strategy described in Fig. 1. Single-stranded M13 recombinant phages were sequenced with [ $\alpha$ - $^{35}$ S]dATP (Biggin et al., 1983) using both the M13 universal primer and  $HPRT$ -specific primers. The sequences of the  $HPRT$ -specific primers are listed in Table I. Normal  $HPRT$  cDNA contains 590 nt of 3'-noncoding sequence, 654 nt of coding sequence, and from 110 to 170 nt of 5'-noncoding sequences (Jolly et al., 1982;

Patel et al., 1986). Clone pHPJH7 consisted of 590 nt of 3'-noncoding sequence, 654 nt of coding sequence, and 77 nt of 5'-noncoding sequence. pHPJH1 lacks 17 nt of coding sequence. Both clones were sequenced in their entirety in both directions (sense and antisense strands).

Recombinant clones pHPJH1 and pHPJH7 differed from normal human  $HPRT$  cDNA at a single nucleotide (Fig. 2): a T-to-A transversion at nt 389. This nucleotide substitution predicts a valine-to-aspartic acid substitution at aa 130 (Fig. 3).

The mutation in  $HPRT_{Midland}$  occurs in a region strongly conserved among human, rodent, and *E. coli* phosphoribosyltransferases (Table II). This was also the case in another  $HPRT$  variant with Lesch-Nyhan syndrome,  $HPRT_{Flint}$  (Davidson et al., 1988). Such regions are probably important for maintenance of normal catalytic and kinetic properties as well as structural integrity. The effect of the valine-to-aspartic acid change in  $HPRT_{Midland}$  and the resultant disruption of this conserved sequence may induce secondary and/or tertiary structural changes which render the protein more unstable, accounting for the lack of detectable activity and protein in this mutant.

The strongly conserved aa 129 through 137 have been implicated as part of the PRPP-binding domain (Argos et al., 1983). However, any catalytic consequence of the mutation in  $HPRT_{Midland}$  cannot be determined because of insufficient residual protein and activity.  $HPRT_{Ann Arbor}$  has an elevated  $K_m$  for PRPP probably due to the isoleucine to methionine change at 132. As in  $HPRT_{Midland}$ , the diminished amount of CRM in  $HPRT_{Ann Arbor}$  may result from disruption of secondary or tertiary structure leading to enhanced proteolysis.

TABLE I

$HPRT$ -specific oligodeoxynucleotides: sequences and sites of priming

Oligodeoxynucleotide	Sequence (5' to 3')	Priming site (nt)	Strand <sup>a</sup>
1	AGTGATGATGAACCAG	31- 47	-
2	CACTGAATAGAAATAGT	251-267	-
3	GATATAATTGACACTGG	403-419	-
4	CCCCTGTTGACTGGTCA	337-321	+
5	AGTCCTGTCCATAATTA	138-122	+

<sup>a</sup> The DNA strand to which the primer anneals is represented as either sense (+) or antisense (-).

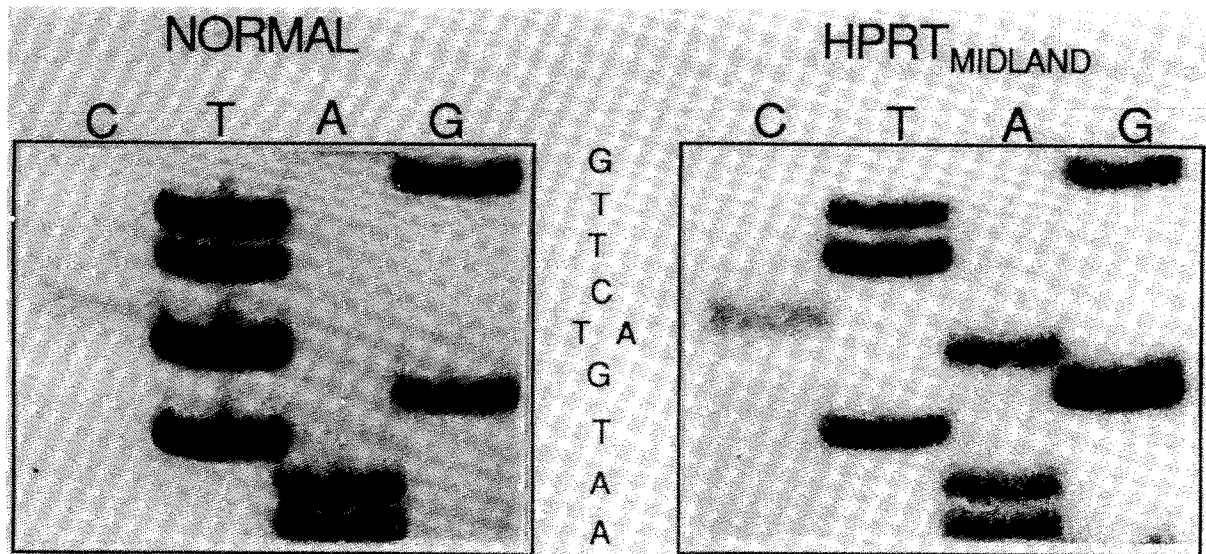


Fig. 2. The nucleotide substitution in  $HPRT_{Midland}$ . The sequencing gels for normal (left panel) and  $HPRT_{Midland}$  (right panel) DNAs are shown from nt 385–393. Single-stranded phages containing these sequences were sequenced using the dideoxynucleotide chain termination method according to Biggin (1983) using  $HPRT$ -specific primer 2 (Table I). One fourth of each sequencing reaction mix was electrophoresed on a 6% polyacrylamide gel containing 8 M urea at constant power (60 W). The gels were then transferred to Whatman paper, dried under vacuum, autoradiographed for 2 to 8 h, and developed. The sequence is shown between the panels with T (normal) transverted to an A ( $HPRT_{Midland}$ ).

TABLE II

The putative PRPP binding domain of various phosphoribosyltransferases<sup>a</sup>

Phosphoribosyl- transferase <sup>b</sup>	Amino acids <sup>c</sup>									Reference <sup>d</sup>
	129	130	131	132	133	134	135	136	137	
Human $HPRT$ (normal) <sup>e</sup>	asn	val	leu	ile	val	glu	asp	ile	ile	A
$HPRT_{Midland}$	asn	asp	leu	ile	val	glu	asp	ile	ile	
$HPRT_{Ann Arbor}$	asn	val	leu	met	val	glu	asp	ile	ile	B
Mouse $HPRT$	asn	val	leu	ile	val	glu	asp	ile	ile	C
Hamster $HPRT$	asn	val	leu	ile	val	glu	asp	ile	ile	D
<i>E. coli</i> XGPRT	—	phe	ile	val	ile	asp	asp	leu	val	E
Human APRT	arg	val	val	val	val	asp	asp	leu	leu	F
Mouse APRT	arg	val	val	ile	val	asp	asp	leu	leu	G
<i>E. coli</i> APRT	lys	val	leu	val	val	asp	asp	leu	leu	H
<i>E. coli</i> OPRT	—	val	met	leu	val	asp	asp	—	val	I
<i>E. coli</i> GlnPRT	asn	val	leu	leu	val	asp	asp	ser	ile	J

<sup>a</sup> This sequence is within a 35 residue stretch of aa predicted to be involved in PRPP binding, as based on secondary structure homology to *E. coli* GlnPRT (Argos et al., 1983).

<sup>b</sup> XGPRT, xanthine-guanine phosphoribosyltransferase; APRT, adenine phosphoribosyltransferase; OPRT, orotate phosphoribosyltransferase; GlnPRT, glutamine phosphoribosylpyrophosphate amidotransferase.

<sup>c</sup> Residue numbers are based on the amino acid sequence of human  $HPRT$  (Wilson et al., 1982b).

<sup>d</sup> A, Wilson et al. (1982b); B, Fujimori et al. (1988); C,D, Konecki et al. (1982); E, Pratt and Subramani (1983); F, Wilson et al. (1986b); G, Dush et al. (1985); H, Hershey and Taylor (1986); I, Poulsen et al. (1983); J, Tso et al. (1983).

<sup>e</sup> Normal sequence for the region from aa 129–137.

HPRT <sub>Midland</sub>	Asn AAT	Asp GATC 389	Leu TTG
Normal	AAT Asn	GTC Val 130	TTG Leu

Fig. 3. The nucleotide and corresponding amino acid substitution in HPRT<sub>Midland</sub>. Nucleotides 385 through 393 and aa 129 through 131 from both normal HPRT and HPRT<sub>Midland</sub> are shown. The nucleotide substitution is boxed.

#### (d) Secondary structure predictions

A computer-assisted analysis of predicted secondary structure perturbations caused by the amino

acid substitutions in HPRT<sub>Midland</sub> was performed (Chou and Fasman, 1978). Hydrophilicity moments were also calculated and overlaid on the secondary structure plots (Hopp and Woods, 1981). The Sequence Analysis Software Package (Genetics Computer Group) for nucleic acid and protein structure analysis was used for calculation and plotting of predicted structures (Wolf et al., 1988). This software is available through the University of Wisconsin Biotechnology Center, Madison, WI 53705. Predicted secondary structures for normal HPRT and HPRT<sub>Midland</sub> are shown in Fig. 4. The predicted secondary structure for normal HPRT from aa 115 to 145 consists of random coil/ $\beta$ -sheet/ $\beta$ -turn/ $\beta$ -turn/ $\beta$ -sheet. Valine, which has the strongest propensity towards forming  $\beta$ -sheet structure is replaced by

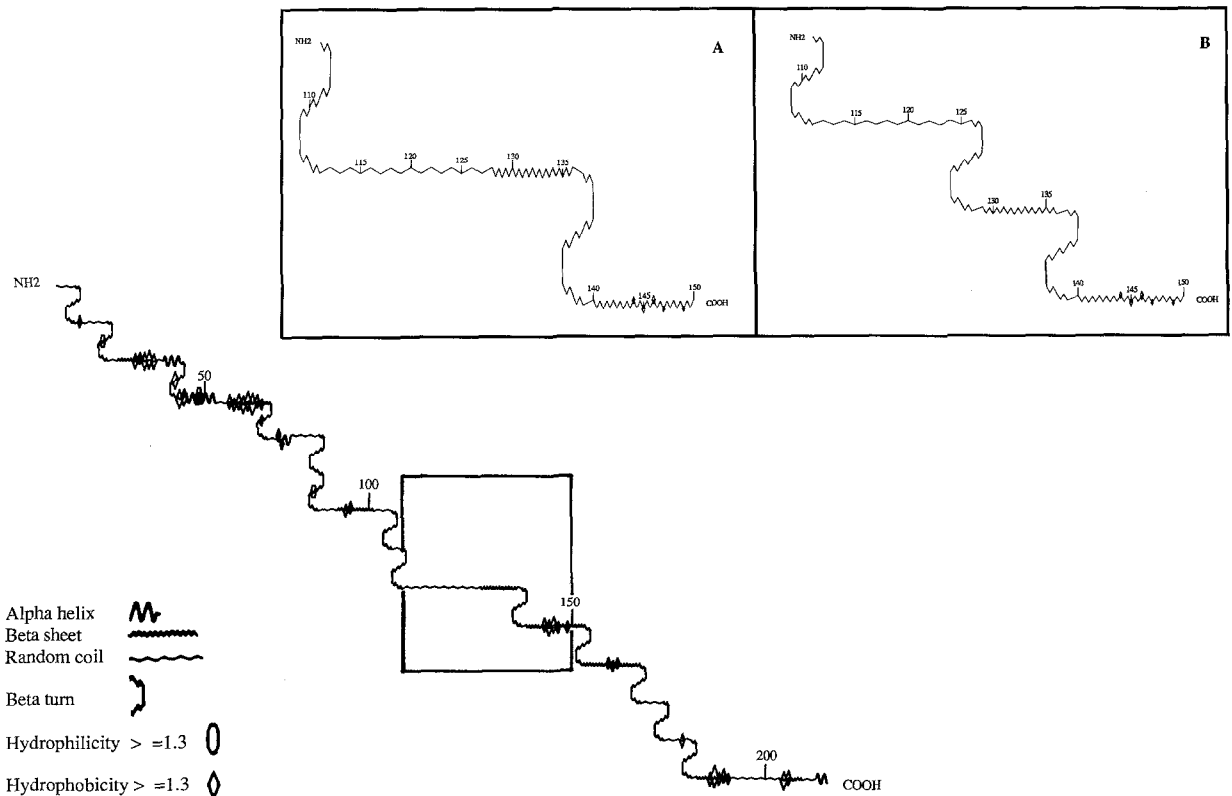


Fig. 4. Secondary structure predictions for normal HPRT and HPRT<sub>Midland</sub>. (Panel A) the predicted secondary structure of normal HPRT. The inset is an enlargement of the region contained within the box. (Panel B) The predicted secondary structure of HPRT<sub>Midland</sub> for the same region as the inset in panel A. The entire amino acid sequence for HPRT<sub>Midland</sub> and normal HPRT were analyzed using the PEPTIDESTRUCTURE and PLOTSTRUCTURE programs of the Genetic Computer Group nucleic acid and protein analysis package. The plot of the Chou-Fasman calculations includes regions of random coil,  $\beta$ -sheet,  $\beta$ -turn, and  $\alpha$ -helix, as illustrated in the figure. The plot of HPRT<sub>Midland</sub> has two additional  $\beta$ -turns compared to that of normal HPRT. The hydrophobicity and hydrophilicity values (Hopp and Woods, 1981) are included in the secondary structure plots as diamonds and ovals, respectively. The size of the character is proportional to the magnitude of the attribute.

aspartic acid in HPRT<sub>Midland</sub>. Aspartic acid has a strong propensity to disrupt  $\beta$ -sheet structure and to form  $\beta$ -turn structure, especially in the context found in HPRT<sub>Midland</sub> (Chou and Fasman, 1978). The predicted result of this mutation on the secondary structure is the replacement of the first region of  $\beta$ -sheet structure by two additional  $\beta$ -turns beginning at or near aa 126 (Fig. 4).

### (e) Conclusions

The genetic basis for enzyme deficiency in HPRT<sub>Midland</sub> has been determined by cDNA cloning. A single nucleotide alteration was determined by sequencing two cDNA clones derived from a subject with Lesch-Nyhan syndrome. The mutation in HPRT<sub>Midland</sub> is near the point mutation identified in HPRT<sub>Ann Arbor</sub>, a variant with partial HPRT activity. The HPRT<sub>Midland</sub> mutation disrupts the putative PRPP binding domain which consists of a region of sequence of 9 aa strongly conserved among human, rodent and bacterial phosphoribosyltransferases. The structural alteration may impair protein stability leading to diminished amounts of CRM in cells derived from this subject. Altered secondary structure patterns are predicted for HPRT<sub>Midland</sub>, consistent with this hypothesis. The HPRT<sub>Midland</sub> mutation is 167 nt away from a previously defined mutant, HPRT<sub>Flint</sub>.

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