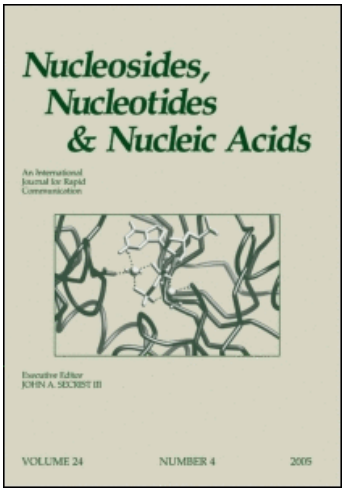

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Clinical and Biochemical Manifestations and Molecular Characterization of the Mutation HPRT Jerusalem

E. Zoref-Shani[a]; Y. Bromberg[a]; J. Hirsch[b]; S. Feinstein[c]; Y. Frishberg[c]; O. Sperling[ad]
[a] Department of Clinical Biochemistry, Tel-Aviv University, -aviv, Israel [b] Department of Biochemistry, Faculty of Life Sciences, Tel-Aviv University, -aviv, Israel [c] Division of Pediatric Nephrology, Shaare Zedek Medical Center, Jerusalem, Israel [d] Felsenstein Medical Research Center, Sackler Faculty of Medicine, Tel-Aviv University, -aviv, Israel

Online publication date: 27 October 2004

To cite this Article Zoref-Shani, E. , Bromberg, Y. , Hirsch, J. , Feinstein, S. , Frishberg, Y. and Sperling, O.(2004) 'Clinical and Biochemical Manifestations and Molecular Characterization of the Mutation HPRT Jerusalem', Nucleosides, Nucleotides and Nucleic Acids, 23: 8, 1165 — 1168
To link to this Article: DOI: 10.1081/NCN-200027436
URL: http://dx.doi.org/10.1081/NCN-200027436

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS
Vol. 23, Nos. 8 & 9, pp. 1165–1168, 2004

Clinical and Biochemical Manifestations and Molecular Characterization of the Mutation HPRT Jerusalem

E. Zoref-Shani,[1,*] Y. Bromberg,[1] J. Hirsch,[3] S. Feinstein,[4] Y. Frishberg,[4] and O. Sperling[1,2]

[1]Department of Clinical Biochemistry, [2]Felsenstein Medical Research Center, Sackler Faculty of Medicine, and [3]Department of Biochemistry, Faculty of Life Sciences, Tel-Aviv University, Tel-Aviv, Israel
[4]Division of Pediatric Nephrology, Shaare Zedek Medical Center, Jerusalem, Israel

ABSTRACT

A novel point mutation (I137T) was identified in the hypoxanthine-guanine phosphoribosyltransferase (HPRT) encoding gene, in a patient with partial deficiency of the enzyme. The mutation, ATT to ACT (substitution of isoleucine to threonine), occurred at codon 137, which is within the region encoding the binding site for 5-phosphoribosyl-1-pyrophosphate (PRPP). The mutation caused decreased affinity for PRPP, manifested clinically as a Lesch–Nyhan variant (excessive purine production and delayed acquisition of language skills). The partial HPRT deficiency could be detected only by measuring HPRT activity in intact fibroblasts (uptake of hypoxanthine into nucleotides).

Key Words: Hypoxanthine-guanine phosphoribosyltransferase; Lesch–Nyhan variant; 5-Phosphoribosyl-1-pyrophosphate.

*Correspondence: E. Zoref-Shani, Department of Clinical Biochemistry, Tel-Aviv University, Tel-Aviv, Israel.

DOI: 10.1081/NCN-200027436
1525-7770 (Print); 1532-2335 (Online)

INTRODUCTION

Various mutations in the HPRT gene, resulting in varying degrees of enzyme deficiency, were reported, manifested in three overlapping clinical phenotypes.[1] Virtually complete enzyme deficiency is associated with the Lesch–Nyhan syndrome (LNS), exhibiting excessive purine production and the resultant clinical manifestations (gout, nephrolithiasis), and characteristic neurological manifestations, including compulsive self-mutilation, choreoathetosis, spasticity and developmental retardation. Patients with partial deficiency represent LNS variants, exhibiting excessive purine production, but varying neurological manifestations. Those with relatively low residual enzyme activity, may exhibit intermediary neurological manifestations, but variants with relatively higher residual activity are free of the neurological manifestations associated with LNS.[1,2] In a small proportion of the HPRT mutants, the enzyme deficiency was shown to be associated with altered affinity of the enzyme for substrate 5-phosphoribosyl-1-pyrophosphate (PRPP). The following is the summary of the clinical and biochemical manifestations[3] and the molecular characterization[4] of a mutation causing partial HPRT deficiency due to decreased affinity to PRPP.

CLINICAL AND BIOCHEMICAL MANIFESTATIONS

The patient was admitted to the hospital at the age of 3.5 y, with acute renal failure due to uric acid nephropathy. Treatment by rehydration, alkalinization of urine and allopurinol resulted in normalization of kidney function within one week.[3] The proband exhibited delayed acquisition of language skills. The cousin of the propositus

Table 1. Parametrs of purine metabolism in erythrocytes and cultured fibroblasts.

Parameter	Control	Propositus
Enzyme activity in erythrocyte lysates[a]		
HPRT	1.3 ± 0.1 (3)	1.55 (1); 1.36 (1)
APRT	0.24 ± 0.03 (3)	0.63* (1); 0.49* (1)
Enzyme activity in fibroblast lysates[a]		
HPRT	2.12 ± 0.89 (4)	1.67 ± 0.57 (4)
APRT	5.42 ± 1.22 (4)	7.05 ± 1.20 (4)
Uptake of purine bases into intact fibroblast nucleotides[b]		
Hypoxanthine	3.0 ± 1.2 (7)	0.7 ± 0.3 (7)*
Adenine	3.9 ± 1.2 (7)	9.1 ± 2.7 (7)*
De novo purine synthesis in intact fibroblasts[c]	25.7 ± 8.9 (4)	191.7 ± 83.9 (3)*

[a]nmol/min/mg protein.
[b]nmol/15 min/mg protein.
[c][^{14}C]formate incorporation (dpm x10^{-3}/2 h/mg protein). Values represent mean ± S.D. Numbers in parentheses represent number of determinations, each performed in duplicates.
*$P < 0.01$, by Students paired t-test.

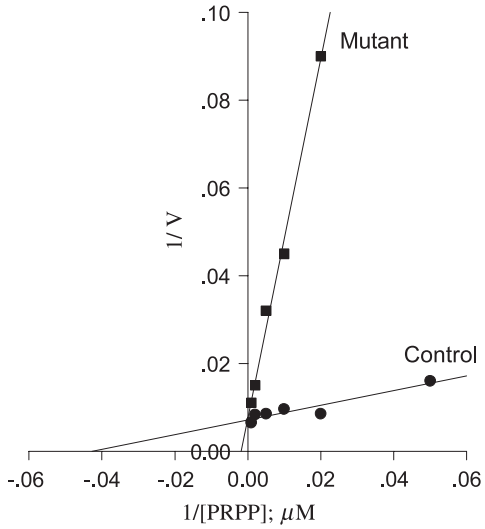

Figure 1. Double reciprocal plots of initial velocity of HPRT activity from fibroblast lysates against PRPP concentrations. V is expressed in nmol/mg of protein/h.

(son of the mother's sister) was recently also diagnosed to be affected with partial HPRT deficiency.[4] Parameters of purine metabolism in the erythrocytes and cultured fibroblasts obtained from the propositus are summarized in Table 1.

HPRT activity in hemolysates was found to be normal, but adenine phosphoribosyltransferase (APRT) activity was 2-fold the control mean activity. In fibroblast lysates HPRT and APRT activities were normal. The rate of incorporation of hypoxanthine into nucleotides in intact cultured fibroblasts, gauging HPRT activity in intact cells, was only 23% of normal, but that of adenine was 2.3-fold of normal. The rate of de novo purine synthesis in intact fibroblasts, was accelerated 7.5-fold. The media of the fibroblast cultures of the patient contained about 10-fold the control amount of hypoxanthine. HPRT activity in fibroblast lysates exhibited a high Km for PRPP (500 μM in comparison to 25 μM in the control cells), but a normal Vmax value, manifested in decreased activity of the enzyme only at low (physiological) PRPP concentrations (Fig. 1). The mutant HPRT exhibited normal apparent Km for hypoxanthine.[3]

MOLECULAR CHARACTERIZATION

Sequencing the amplified cDNA of the HPRTJerusalem gene, identified in both cousins a novel point mutation ATT to ACT at codon 137 (exon 6), indicating a substitution of isoleucine to threonine (I137T) (methionine as the first amino acid with the A in the ATG start codon as the first base). Codon 137 is within the highly conserved PRPP binding motif, which spans over twelve amino acid residues, 130–141.[1,2] The mutation, which was termed HPRTJerusalem,[4] is the fifth point mutation within the PRPP binding region, manifesting clinically as a LNS variant. The

PRPP binding region in HPRT, is comprised of beta strand β6-β6′ and a turn, which is followed immediately by the beginning of alpha helix α5. Residue 137, an isoleucine in the wild type protein, stabilizes the kinked beta strand β6-β6′ and turn that have direct interaction with PRPP. We suggest that in HPRTJerusalem, perturbation by substitution to threonine, a polar residue versus the apolar and bulky isoleucine, may destabilize the trajectory of the polypeptide affecting the affinity to PRPP.

REFERENCES

1. Jinnah, H.A.; De Gregorio, L.; Harris, J.C.; Nyhan, W.L.; O'Neill, J.P. Mutat. Res. **2000**, *463*, 309–326.
2. Jinnah, H.A.; Friedmann, T. Lesch–Nyhan diseaese and its variants. In *The Metabolic and Molecular Bases of Disease*, 8th Ed.; Scriver, C.R., Beaudet, A.L., Sly, W.S., Valle, D., Childs, B., Kinzler, K.W., Vogelstein, B., Eds.; McGraw-Hill: New York, 2001; 2537–2570.
3. Zoref-Shani, E.; Feinstein, S.; Frishberg, Y.; Bromberg, Y.; Sperling, O. Biochem. Biophys. Acta **2000**, *1500*, 197–203.
4. Zoref-Shani, E.; Bromberg, Y.; Hirsch, J.; Feinstein, S.; Frishberg, Y.; Sperling, O. Mol. Genet. Metab. **2003**, *78*, 158–161.