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# SHORT COMMUNICATION

# Determination of the Mutations Responsible for the Lesch–Nyhan Syndrome in 17 Subjects[1]

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**Hypoxanthine – guanine phosphoribosyltransferase (HPRT) is a purine salvage enzyme that catalyzes the conversion of hypoxanthine to inosine monophosphate and guanine to guanosine monophosphate. Previous studies of mutant HPRT proteins analyzed at the molecular level have shown a significant heterogeneity. This investigation further verifies this heterogeneity and identifies insertions, deletions, and point mutations. The direct sequencing of the polymerase chain reaction-amplified product of reverse-transcribed HPRT mRNA enabled the rapid identification of the mutations found in 17 previously uncharacterized cell lines derived from patients with the Lesch–Nyhan syndrome.** © 1991 Academic Press, Inc.

Hypoxanthine–guanine phosphoribosyltransferase (HPRT) deficiency is an inherited disorder that results in the overproduction of uric acid. Complete deficiency is associated with the devastating Lesch–Nyhan syndrome (Lesch and Nyhan, 1964; Seegmiller *et al.*, 1967). The gene encoding for the HPRT protein is over 44 kb and consists of nine exons and eight introns (Patel *et al.*, 1984). The HPRT mRNA, which encodes for the protein, is about 1400 bp.

Multiple samples of cytoplasmic RNA were isolated from lymphoblast and fibroblast cell lines. The mRNA was reverse-transcribed using standard procedures (Ausubel *et al.*, 1987). The HPRT-specific primers for PCR amplification and direct sequencing were previously reported by our laboratory (Davidson *et al.*, 1989b). The entire coding region was sequenced in both directions and RNase A mapping or direct sequencing of amplified genomic DNA was used to confirm these mutations (Gibbs and Caskey, 1987). HPRT activity was measured in all cell lines using the standard radioisotopic assay (Holden and Kelley, 1978). The activity of all cell lines was virtually undetectable (<0.1% of normal controls).

A total of 17 cell lines representing 16 unrelated individuals were sequenced. We identified 10 point mutations, four deletions, and three insertions (Table 1). The insertions and deletions, three of which have not been described (1266, 1656, and 1650), predict prematurely terminated translation products. The three insertional events in 1266, 1656, and 1650 might be explained by strand slippage, as each of the mutations occurs in repetitive tracts of deoxynucleotides ($d(G \cdot dT)_2$, $d(T)_3$, and $d(G)_7$, respectively). The insertion described in 1650 has been reported previously in a different, unrelated cell line, RJK 866 (Gibbs *et al.*, 1989). Two of the point mutations are nonsense mutations that prematurely terminate translation. The remaining 8 point mutations cause single amino acid changes. The nucleotide changes in 1151, 1265, 1734, and 779 have not been previously described.

Although six of the point mutations cause a C to T (or G to A) change, only the mutations in 754-4 and 1321 are in the CpG context and thus may be indicative of hot spots for mutation in the HPRT gene (Green *et al.*, 1990; Koeberl *et al.*, 1990; Perutz, 1990). Interestingly, these two mutations have been de-

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**TABLE 1**

**HPRT Mutations**

| Cell line | Nucleotide mutation | Amino acid alteration |
|---|---|---|
| Point mutations | | |
| 1151 | $G_3 \rightarrow A$ (1)[a] | $Met_1 \rightarrow Ile$ |
| 1265 | $C_{149} \rightarrow T$ (3) | $Ala_{50} \rightarrow Val$ |
| 754-4 | $C_{151} \rightarrow T$ (3) | $Arg_{51} \rightarrow$ stop |
| 955-2 | $G_{209} \rightarrow A$ (3) | $Gly_{70} \rightarrow Glu$ |
| 1510 | $G_{209} \rightarrow A$ (3) | $Gly_{70} \rightarrow Glu$ |
| 1522 | $C_{222} \rightarrow A$ (3) | $Phe_{74} \rightarrow Leu$ |
| 375 | $T_{389} \rightarrow A$ (5) | $Val_{130} \rightarrow Asp$ |
| 1321 | $C_{508} \rightarrow T$ (7) | $Arg_{170} \rightarrow$ Stop |
| 1734 | $T_{548} \rightarrow C$ (8) | $Ile_{183} \rightarrow Thr$ |
| 779 | $A_{611} \rightarrow G$ (9) | $His_{204} \rightarrow Arg$ |
| Deletions | | |
| 1052 | Exon 4 | Premature termination |
| 1757[b] | Exon 4 | Premature termination |
| 1758[b] | Exon 4 | Premature termination |
| 1423 | ΔATT 80–83[c] (2) | $\Delta Tyr_{28}$ |
| Insertions | | |
| 1650 | $G_{206-211}$[d] (3) | Premature termination |
| 1656 | $T_{435-437}$[e] (6) | Premature termination |
| 1266 | $GT_{511-517}$[f] (7) | Premature termination |

[a] The number in parentheses refers to the exon in which the mutation is found.

[b] Cell lines 1757 and 1758 are derived from siblings.

[c] The exact position of this deletion could be 80–82 or 81–83.

[d] The exact position is unclear; insertion could be at 206 to 211.

[e] The exact position is unclear; insertion could be at 435 to 437.

[f] The exact position is unclear; insertion could be at 511 to 512, 514 to 515, 516 to 517.

scribed previously in the unrelated patients $HPRT_{Shefford}$ and $HPRT_{North\ Mymms}$, respectively (Davidson *et al.*, 1991).

The heterogeneity found in previously published mutant HPRT protein and nucleic acid studies was also shown in this study at the nucleotide level (Wilson *et al.*, 1986, Gibbs *et al.*, 1989, 1990; Davidson *et al.*, 1989a, 1991). Although the mutations identified to date are mostly unique, there is a pattern of clustering. Evidence from previous studies suggests that mutations occur in discrete regions in the coding sequence, including the putative substrate binding sites (Hershey and Taylor, 1986). Review of our compiled data shows strong support for this clustering phenomenon (Davidson *et al.*, 1989a,b, 1991). Of the point mutations found thus far, approximately 40% occur in exon 3 in two regions of only 6 bp. One area contains 38% of all exon 3 mutations, while the other has 30%. Exon 5 has 11% of the mutations and yet encodes for only 3% of the protein. Similarly, these mutations also occur within an 8-base region.

The eight point mutations identified in this report, which predict a single amino acid substitution, are of special interest in the study of the HPRT protein's structural properties required for catalytic competence. These mutations may indicate important areas that either are involved with the tertiary structure of the protein or are required for catalysis. Further protein structure studies are necessary before the exact mechanisms causing enzyme dysfunction can be understood.

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