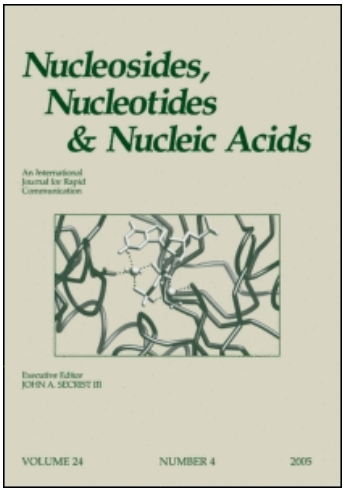


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# Novel Mutations and Hot-Spots in Patients with Purine Nucleoside Phosphorylase Deficiency

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# Novel Mutations and Hot-Spots in Patients with Purine Nucleoside Phosphorylase Deficiency

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## ABSTRACT

Purine nucleoside phosphorylase (PNP) deficiency results in severe immune dysfunction and early death from infections. Lymphopenia, reduced serum uric acid, and abnormal PNP enzymatic activity assist in the diagnosis of PNP-deficient patients. Analysis of the gene encoding PNP in these patients reveals several recurring mutations. Identification of these hot-spots for mutation may allow faster confirmation of the diagnosis in suspected cases.

*Key Words:* PNP; Immunodeficiency; Novel; Mutations; Gene; Hot-spots.

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Other polymorphisms have recently been described (Yu, L., Kalla, K., Guthrie, E., Vidrine, A., Klimecki, W.T.; Genetic variation in genes associated with arsenic metabolism: glutathione S-transferase omega 1-1 and purine nucleoside phosphorylase polymorphisms in European and indigenous Americans. Environ. Health. Perspect. **2003**, *111*, 1421–1427).

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## INTRODUCTION

Children with purine nucleoside phosphorylase (PNP) deficiency suffer from recurrent severe life threatening infections, auto-immune phenomena or malignancies. Many patients also present with neurological manifestations particularly involving the motor system. Because PNP is a crucial enzyme in purine metabolism, its abnormal function hinders the formation of uric acid, which is the end product of purine degradation. Therefore the diagnosis of PNP deficiency is often suspected when lymphopenia is associated with low serum uric acid and reduced PNP enzymatic activity in red blood cells. Bone marrow transplantation has been attempted in few patients with variable degree of success. Other treatments that are currently evaluated include the attachment of modified E-coli PNP to Polyethylene glycol (similar to PEG–ADA) and gene therapy.[1]

Gene analysis in some PNP-deficient children had revealed different mutations, however only few patients were evaluated thereby limiting the ability to assess the presence of hot-spots in the gene.

We present our experience in identifying PNP mutations, including novel mutations and some that appear to occur in CG dinucleotide hot-spots.



## MATERIALS AND METHODS

The PNP gene was evaluated in samples sent to the Canadian Center for Primary Immune Deficiency for PNP gene analysis from Europe, North-America and Asia. In all cases, history of recurrent infection and/or neurological abnormalities and T cell lymphopenia led to the initial suspicion of PNP deficiency. At our center, DNA and total RNA were extracted from peripheral mononuclear cells following Ficoll-Hypaque gradient centrifugation. Isolation of cDNA and gDNA was performed as described before.[2] To examine for genomic mutations the six exons and exon-intron borders of the PNP gene were amplified. All PCR were performed with the ELONGASE® Enzyme (Invitrogen, Canada) utilizing >500 ng of DNA and 1.5 mM $Mg^{2+}$. The reaction mixture was denatured for 1 minute at 94°C and incubated for 35 cycles (denaturing for 30 seconds at 94°C, annealing for 30 seconds at 60–65°C and extension for 1–3 minutes at 72°C). Final extension was continued for 5 minutes at 72°C. The PCR products were purified by QIAquick purification kit (QIAGEN) and sequenced with USB Thermosequenase cycle sequencing kit (Amersham, Pharmacia) according to the manufactures recommendations and separated on an acrylamide gel. The sequence was compared to that published by NCBI (accession number NM_000270). Each mutation was confirmed by repeated PCR amplification and sequencing with both the sense and the anti-sense primers.

## RESULTS

Samples from patients recently diagnosed with PNP deficiency were evaluated in our center for mutations in the PNP gene (Table 1). One of the patients had a novel

homozygous missense mutation, G349A in exon 4, resulting in a putative Ala117Thr, which was not detected in over 100 unrelated individuals. Another patient inherited a previously described[2] C172T mutation from his mother, which results in Arg58stop and a novel C700G mutation, inherited from his father, putatively causing Arg234stop. The patient also carried an A151G substitution putatively resulting in a Ser51Gly change that was described previously as having no effect on PNP activity.[3] The third patient had a novel missense mutation C769G leading to a putative His257Asp on one allele. The His257 is an essential part of the active catalytic site of the enzyme and site-directed mutagenesis of the histidine has demonstrated a 20-fold decrease in the PNP activity.[4] The other allele had a C172T mutation resulting in a putative stop codon. As evident from Table 1, mutations in Arg 58 or 234 occurred in 8 of 13 unrelated patients.[2,3,5–10]

***Table 1.*** Mutation in human purine nucleoside phosphorylase.

| Nucleotide | Mutation type | Exon | # of URI* | Ref.** |
|---|---|---|---|---|
| *Disease causing mutations* | | | | |
| 70 C > T | Arg24Ter (nonsense) | Exon 2 | 2 | [5] |
| 172 C > T | Arg58Ter (nonsense) | Exon 2 | 3 | [2] |
| 181 G > T | Aberrant 5′ splice site. Deletes exon 3 | Exon 2 | | [6] |
| 265 G > A | Glu89Lys (missense) | Exon 3 | 2 | [7] |
| Int. 3 + g > a | Aberrant 5′ splice site, deletes exon 3 | Intron 3 | 1 | [2] |
| Int. 3–18 g > a | New 3′ splice acceptor, 16 bp insert | Intron 3 | 1 | [8] |
| 349 G > A | Ala117Thr (missense) | Exon 4 | 1 | New |
| 383 A > G | Asp128Gly (missense) | Exon 4 | 1 | [3] |
| Del385–387 | Del Ile129 (deletion) | Exon 4 | 1 | [8] |
| 467G > C | Gly156Ala (missense) | Exon 5 | 1 | [9] |
| 520 G > T | Ala174Pro (missense) | Exon 5 | 1 | [8] |
| 569 G > T | Gly190Val (missense) | Exon 5 | 1 | [8] |
| 575 A > G | Tyr192Cys (missense) | Exon 5 | 1 | [8] |
| 700 G > T | Arg234Ter (nonsense) | Exon 6 | 1 | New |
| 700 G > C | Arg234Pro (missense) | Exon 6 | 4 | [3] |
| Del 730 | Asn243Shift (del 1 bp) | Exon 6 | 1 | [10] |
| 769 C > G | His257Asp (missense) | Exon 6 | 1 | New |
| *Polymorphism* | | | | |
| Dup 10 bp-41 | Duplication-silent | 5′ Untranslated | 2 | [3,7] |
| 60 T > C | His20His-Silent | Exon 2 | 1 | [3] |
| 151 A > G | Ser51Gly-silent | Exon 2 | 2 | [3] |
| 171 T > C | Pro57Pro-Silent | Exon 2 | 2 | [3] |
| 649 G > A | Val217Ile-silent | Exon 5 | 1 | [9] |
| Ins + 903 A | Insertion-silent | 3′ Untranslated | 2 | [3,7] |

*URI—unrelated individuals.
**Ref—Reference.

## DISCUSSION

PNP deficiency uniformly leads to death in the first 2 decades of life.[11] Misinterpretations of the immune dysfunction and the neurological abnormalities may result in delayed diagnosis. Therefore, early identification and confirmation of the mutations is essential. Our data emphasize an important feature of the PNP gene. More than half of unrelated patients in whom the PNP gene was analyzed had mutations in the C or G nucleotides of Arginine at positions 58 or 234. Patients from different continents and various genetic backgrounds carried these changes suggesting that these are hot-spots with increased mutation frequency. Interestingly, CpG have been recognized as hot-spots prone to mutations in other primary immune deficiency diseases.[12] Although the data presented here expands the knowledge about mutations in PNP-deficient patients, it is still not enough to conclude upon potential genotype phenotype correlation. However, further accumulation of similar information may eventually allow prediction of the clinical course from the specific genetic defect.

Demonstration of the mutations in the PNP gene is of major assistance for the patients and the treating physicians. It confirms the diagnosis in these patients and provides invaluable tools for future genetic counseling including early intrauterine identification of PNP-deficiency. Identification of mutations in PNP may eventually assist in selecting patients that are more likely to benefit from future gene therapy, particularly if the transformed cells are expected to have a survival advantage over the patient's naïve cells.

In conclusion, by analyzing the genetic abnormalities found in patients with PNP deficiency we were able to identify areas in which mutations occurred frequently. Further studies on larger number of patients will help identify other hot-spots in the PNP gene and may also provide better understanding of the gene itself.



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