Purine nucleoside phosphorylase (PNP) deficiency (OMIM 164050) is a rare autosomal recessive metabolic disorder that results in combined immunodeficiency, neurologic dysfunction and autoimmunity. PNP deficiency has never been reported from Saudi Arabia or in patients with an Arabic ethnic background. We report on two Saudi girls with PNP deficiency. Both showed severe lymphopenia and neurological involvement. Sequencing of the PNP gene of one girl revealed a novel missense mutation Pro146>Leu in exon 4 due to a change in the codon from CCT>CTT. Expression of PNP (146L) cDNA in E. coli indicated that the mutation greatly reduced, but did not completely eliminate PNP activity.

**CASE 1**
A 2-year-old Saudi girl was referred to King Khalid University Hospital with history of recurrent chest infections. She had three chest infections. The first at 11 months of age that was treated with oral antibiotics, while the second and third required prolonged courses of intravenous antibiotics. She also had chronic diarrhea, watery, 3 to 4 times per day, and persistent since she was 9 months of age. There was a history of intermittent anemia and leukopenia; she had received a blood transfusion 3 weeks prior to presentation. The patient’s parents were first cousins. She has two healthy brothers who are 4 years and 8 months of age. One cousin died at 1 year of age after extensive chicken pox infection.

On examination, the patient appeared poorly nourished and growth parameters were all below the third percentile. A BCG scar was absent (the patient received BCG vaccine on day one of life). She had oral thrush and pus draining from the right ear. Chest examination revealed bilateral coarse crackles. CNS exam revealed a global developmental delay corresponding to about 9 months of age and universally decreased muscle tone.
Investigations showed WBCs of 1300/mm$^3$, an absolute neutrophil count of 510/mm$^3$, absolute lymphocyte count 420/mm$^3$, hemoglobin of 11.3 g/dL, and platelets of 506×10$^3$/mm$^3$. Bone marrow aspiration revealed a hypercellular active marrow with no dysplasia. Immunoglobulin levels were all elevated: IgG 1877 mg/dL, IgM 366 mg/dL, and IgA 297 mg/dL. Lymphocyte subset enumeration and proliferation responses are shown in Table 1. Uric acid was 62.6 µmol/L (normal range 130-320 µmol/L). PNP enzyme activity was measured by a radiochemical method, as adapted for measuring activity in eluates of dried blood spots on filter paper and it was 324 nmol/L (normal 1336±441). The low level of PNP activity detected, at least partly, reflected the recent blood transfusion that the patient had received because of her anemia.

**CASE 2**

The second patient was referred to King Faisal Specialist Hospital at 2 years of age because of recurrent chest infections that required multiple hospital admissions for treatment with intravenous antibiotics. Her most recent chest infection was complicated by empyema. There was no history of chronic diarrhea. Her parents were first cousins. She has two brothers and one sister who are alive and well. Another sister died at 3 years of age from extensive chicken pox infection.

On examination, all her growth parameters were below the third percentile. She had delayed motor milestones. Speech was appropriate for age. The tonsils were absent. The remaining systemic examination was normal. Investigations showed WBCs of 4270/mm$^3$, an absolute neutrophil count of 2540/mm$^3$, absolute lymphocyte count 618/mm$^3$, hemoglobin of 9.7 g/dL, and platelets of 643×10$^3$/mm$^3$. Immunoglobulin levels showed IgG 1000 mg/dL (N), IgM 20 mg/dL (low), and IgA 14 mg/dL (normal). Antibody response titer to tetanus was 13.48 IU/mL (protective level>0.17 IU/mL). Lymphocyte subset enumeration and proliferation responses are shown in Table 1. Erythrocyte PNP enzyme activity was undetectable. Unfortunately, material for genotype analysis was not available at the time of diagnosis and the patient was lost to follow-up.

Both patients were vaccinated with BCG, OPV and MMR among other first year killed vaccines (DTP, HIB and Hep B) with no complications.

**Mutation analysis**

Genomic DNA was prepared from blood samples of patient 1 by standard methods. The exons and intron-exon boundaries of the PNP gene were amplified in 6 separate segments spanning exon 1, exon 2, exons 3-5,
Table 1. Immunological investigation data for the two patients.

|                          | Patient 1 | Patient 2 | Normal range  |
|--------------------------|-----------|-----------|---------------|
| Lymphocytes             |           |           |               |
| Subsets enumeration /mm²|           |           |               |
| -CD 3                    | 385       | 314       | 1400-8000     |
| -CD 4                    | 106       | 167       | 900-5500      |
| -CD 8                    | 263       | 18        | 400-2300      |
| -CD 19                   | 311       | 58        | 600-3100      |
| -CD 16/56                | 147       | 231       | 100-1400      |
| Lymphocyte proliferation |           |           |               |
| responses                |           |           |               |
| -PHA (cpm)               | 2343      | 14247     | 90553-117987  |
| -Con A (cpm)             | 1443      | 10350     | 78412-105150  |
| -PWM (cpm)               | 2934      | 8705      | 45008-53536   |
| Enzyme activity          |           |           |               |
| PNP nmol/hr/mg           | 324       | 0         | 1336 (±441)   |

cpm: counts per minute.

**Discussion**

NP deficiency is one of the least common primary immunodeficiency diseases. Seventeen different mutations in 23 unrelated individuals have been described so far. Mutations of C or G nucleotides of Arg at positions 58 or 234 were most common (8/23). The clinical presentation of PNP deficiency is variable, but prognosis is poor, and without restoration of immune function most patients die from complications related to immunodeficiency. There have been no systematic studies of the genotype/phenotype correlation in PNP deficiency, but there is evidence of such correlation in its counterpart, ADA deficiency. The novel P146L missense mutation identified in patient 1 has some residual catalytic activity when expressed in E. coli. By analogy with ADA deficiency, it is possible that this mutant allele might result in a somewhat milder clinical phenotype.
case report

T-cell lymphopenia, recurrent infections, and neurologic findings should raise the suspicion of PNP deficiency, particularly if these are associated with low serum or urinary uric acid level (although the latter is not universally found). The diagnosis is usually established by measurement of PNP enzyme activity in red cells or blood mononuclear cells. Elevated serum or urinary levels of inosine and guanosine are confirmatory.

Although B lymphocyte numbers have been normal in most cases, both of our patients had low B-cell numbers. However, patient 1 had high immunoglobulin levels, which may indicate B-cell dysregulation due to T-cell deficiency, rather than adequate B-cell function. Specific antibody titer to tetanus was normal in patient 2, which is not unusual to find in this disorder.

Neurologically, both patients had motor developmental delay, a frequent feature of the disease. Various neurological abnormalities have been described including spastic diplegia or tetraplegia, ataxia, behavioral difficulties and mental retardation. Autoimmune phenomena occur in about one third of patients, mainly hemolytic anemia, thrombocytopenia and neutropenia. Patient 1 presented with relapsing neutropenia that was most probably autoimmune in nature; patient 2 had no evidence of autoimmunity.

In conclusion, this is the first description of Saudi patients with PNP deficiency, including one with a novel disease-causing mutation in the NP gene in one case. T lymphopenia, recurrent infections, and neurologic findings should raise the suspicion of PNP deficiency, especially with a history of parental consanguinity.

Acknowledgment
This work was supported by NIH Grant DK20902 to M. Hershfield. The case report was presented and published as an abstract in the annual meeting of the American Academy of Allergy, Asthma, and Immunology in 2006. Please see Journal of Allergy and Clinical Immunology. 2006;117(2):S173.

REFERENCES

1. Markert ML. Purine nucleoside phosphorylase deficiency. Immunodef Rev. 1991;3:45-81.
2. Osborne WR, Ochs HD. Immunodeficiency disease due to deficiency of purine nucleoside phosphorylase. In: Ochs HD, Smith CE, Puck JM, eds. Primary Immunodeficiency diseases. A molecular and genetic approach. New York: Oxford University Press; 1999. 140-5 p.
3. Hershfield MS. Combined immunodeficiencies due to purine enzyme defects. In: Stiehm ER, Ochs HD, Winkelstein J, eds. Immunologic disorders in infants and children. 5th ed. Philadelphia: WB Saunders; 2003. p480-504.
4. Giblett ER, Ammann AJ, Wara DW, Sandman R, Diamond LK. Nucleoside-phosphorylase deficiency in a child with severely defective T-cell immunity and normal B-cell immunity. Lancet. 1975;1:1010-1013.
5. Grunebaum E, Zhang J, Rolfman CM. Novel mutations and hot-spots in patients with purine nucleoside phosphorylase deficiency [published erratum appears in Nucleosides Nucleotides Nucleic Acids. 2005;24:303]. Nucleosides Nucleotides Nucleic Acids. 2004;23:1411-5.
6. Arredondo-Vega FX, Kurtzberg J, Chaffee S, Santisteban I, Reisner E, Povey MS, Hershfield MS. Paradoxical expression of adenosine deaminase in T cells cultured from a patient with adenosine deaminase deficiency and combined immunodeficiency. J Clin Invest. 1990;86:444.
7. Arredondo-Vega FX, Santisteban I, Richard E, Ball P, Koleilat M, Loubser M, et al. Adenosine deaminase deficiency with mosaicism for a “second-site suppressor” of a splicing mutation: decline in revertant T lymphocytes during enzyme replacement therapy. Blood. 2002 Feb 1;99(3):1005-13.
8. Arredondo-Vega FX, Santisteban I, Daniels S, Toutain S, and Hershfield MS. Adenosine deaminase deficiency: genotype-phenotype correlations based on expressed activity of 29 mutant alleles. Am J Hum Genet. 1998;63:1049.
9. Hallett RJ, Cronin SM, Morgan G, Duley JA, Fairbanks LD, Simmonds HA. Normal uric acid concentrations in a purine nucleoside phosphorylase (PNP) deficient child presenting with severe chicken pox, possible immunodeficiency and developmental delay. Adv Exp Med Biol. 1994;370:387-389.