

Letter to the Editor

T- and B-cell defects in a novel purine nucleoside phosphorylase mutation

To the Editor:

Two autosomal recessive genetic disorders of the purine salvage pathway, including adenosine deaminase and purine nucleoside phosphorylase (PNP) deficiencies, are associated with immunodeficiency.¹ In PNP deficiency, the hallmark pathophysiology is the accumulation of deoxyguanosine triphosphate toxic metabolites, particularly in lymphocytes. Consequently, T-cell development is impaired, and there is variable B-cell dysfunction. Increased risk of autoimmune disorders (eg, hemolytic anemia, thrombocytopenia, neutropenia, and thyroiditis) is a characteristic of PNP deficiency, and it is seen in approximately one third of the cases.² Autoimmune features may be recognized clinically before the onset or identification of immunodeficiency. Characterization of the B-cell immunodeficiency in PNP deficiency is still incomplete, and it is mainly attributed to impaired T-cell function resulting in autoimmune phenomena.³ Serum immunoglobulin levels can be normal to near-normal, but the production of specific T-cell-dependent antibodies is reduced.³ A similar combination of effects is likely to be operative in patients with delayed onset or partial adenosine deaminase deficiency who also show variable residual B-cell function and autoimmunity. The diagnosis of PNP is usually suggested by a low level of serum uric acid, supported by reduced PNP activity and confirmed by molecular gene analysis.⁴ Normally, T-cell maturation in the thymus and B-cell maturation in the bone marrow progress through distinct stages that are defined phenotypically by the expression of their specific receptors (T-cell receptors [TCRs] and B-cell receptors [BCRs], respectively). This process, which involves DNA rearrangement, results in the formation of normal numbers of functional and mature cells that carry coding joints in their rearranged fragments. Stable by-product circles, known as TCR excision circles (TRECs) or the kappa-deleting recombination excision circles (KRECs), are created during the excision of the DNA fragments in T and B cells, respectively. These signal joint circles, which are present solely in newly derived thymic or bone marrow cells and not in replicating cells during mitosis, allow indirect quantification of thymic and bone marrow output. In contrast, cells containing only coding joints may reflect the peripheral replication history of the lymphocyte subsets.⁵ Taken together, the ratio between genomic coding joints and signal joints in these cells reflects both peripheral cell divisions in response to antigen exposure and the export of new T cells from the thymus and B cells from the bone marrow. Simultaneous quantification of TRECs and KRECs, which contain the required signal joint rearrangement in patients with primary immunodeficiency after bone marrow transplantation, was recently proposed for monitoring the appearance of newly derived thymic and bone marrow cells.⁶ The amounts of TRECs and KRECs are determined by real-time quantitative PCR. Reactions are performed by using genomic DNA extracted from patients' PBMCs and PCRs that contain specific primers and probes as previously described in detail.⁷

Herein, we present 2 sisters with a novel nonsense PNP mutation causing combined immunodeficiency. Patient 1 was born after a normal pregnancy and delivery to parents who are

TABLE I. Clinical and laboratory characteristics of the PNP-deficient patients

	Patient 1	Patient 2
Age at diagnosis (mo)	12	30
Hemolytic anemia	+	+
Infections	+	—
Uric acid	UD	UD
PNP activity (IU) (20-30)	0	0
Lymphocyte count/mm ³ (2,500-10,000)	920	495
CD3/mm ³ (60%-85%)	32%	12%
CD3 ⁺ CD4 ⁺ /mm ³ (36%-63%)	9%	7%
CD3 ⁺ CD8 ⁺ /mm ³ (15%-40%)	23%	5%
CD19 ⁺ /mm ³ (5%-25%)	22%	4%
CD3 ⁺ CD56 ⁺ /mm ³ (6%-30%)	38%	79%
IgM (mg/dL) (50-280)	69	UD
IgG (mg/dL) (680-1,560)	836	34
IgA (mg/dL) (0.4-3.7)	UD	UD
IgE (IU/mL) (0-15)	289	UD
HBV IgG antibodies	Negative	Negative
Varicella IgG antibodies	ND	Negative
Polio-type IgG antibodies	Positive*	Negative
Measles IgG antibodies	ND	Negative
Mumps IgG antibodies	ND	Negative
Rubella IgG antibodies	ND	Negative
CMV IgG antibodies	Positive	Negative
Isohemagglutinin IgM antibodies	Negative	Negative
PHA mitogenic response (patient/control, CPM)	2,397 of 26,107	ND
αCD3 mitogenic response (patient/control, CPM)	4,327 of 45,557	ND
Signal joint TRECs/0.5 μg DNA†	UD	UD
Coding joint KRECs/0.25 μg DNA‡	12.6	300
Signal joints KRECs/0.25 μg DNA‡	UD	UD

Normal levels are presented in parentheses.

CMV, Cytomegalovirus; CPM, counts per minute; HBV, hepatitis B virus; ND, not done; UD, undetectable.

*Anti-poliovirus serum type 1, more than 1 in 512; type 2, more than 1 in 128; and type 3, more than 1 in 128.

†The normal value is more than 400 for signal joint TRECs based on 100 age-matched normal individuals for whom the diagnosis of primary T-cell immunodeficiency was excluded.

‡The normal value is more than 1500 for KRECs and more than 250 for KREC coding joint and KREC signal joint, based on 5 age-matched normal individuals for whom the diagnosis of primary immunodeficiency was excluded.

first-degree cousins. She presented at the age of 1 year with acute respiratory illness and marked lymphopenia (920/mm³). Her history was remarkable for hypotonia, hemolytic anemia, and developmental delay. Her serum uric acid was undetectable, suggesting the diagnosis of PNP deficiency. The patient's eldest sister, who was 2½ years old at that time, was incidentally found to have lymphopenia (495/mm³). Her medical history was also significant for hemolytic anemia, hypotonia, and developmental delay. No PNP enzymatic activity was detected in either patient (Table I), and genetic sequence analysis of the PNP gene revealed a novel homozygous mutation in exon 3: a C-to-T transition at nucleotide 244, putatively resulting in premature stop codon (Q82X). Both parents were found to be carriers for this mutation. A thorough immune workup of both sisters revealed significantly low levels of T cells, severely depressed lymphocyte proliferation to various mitogens, and undetectable signal joint TREC levels (Table I).

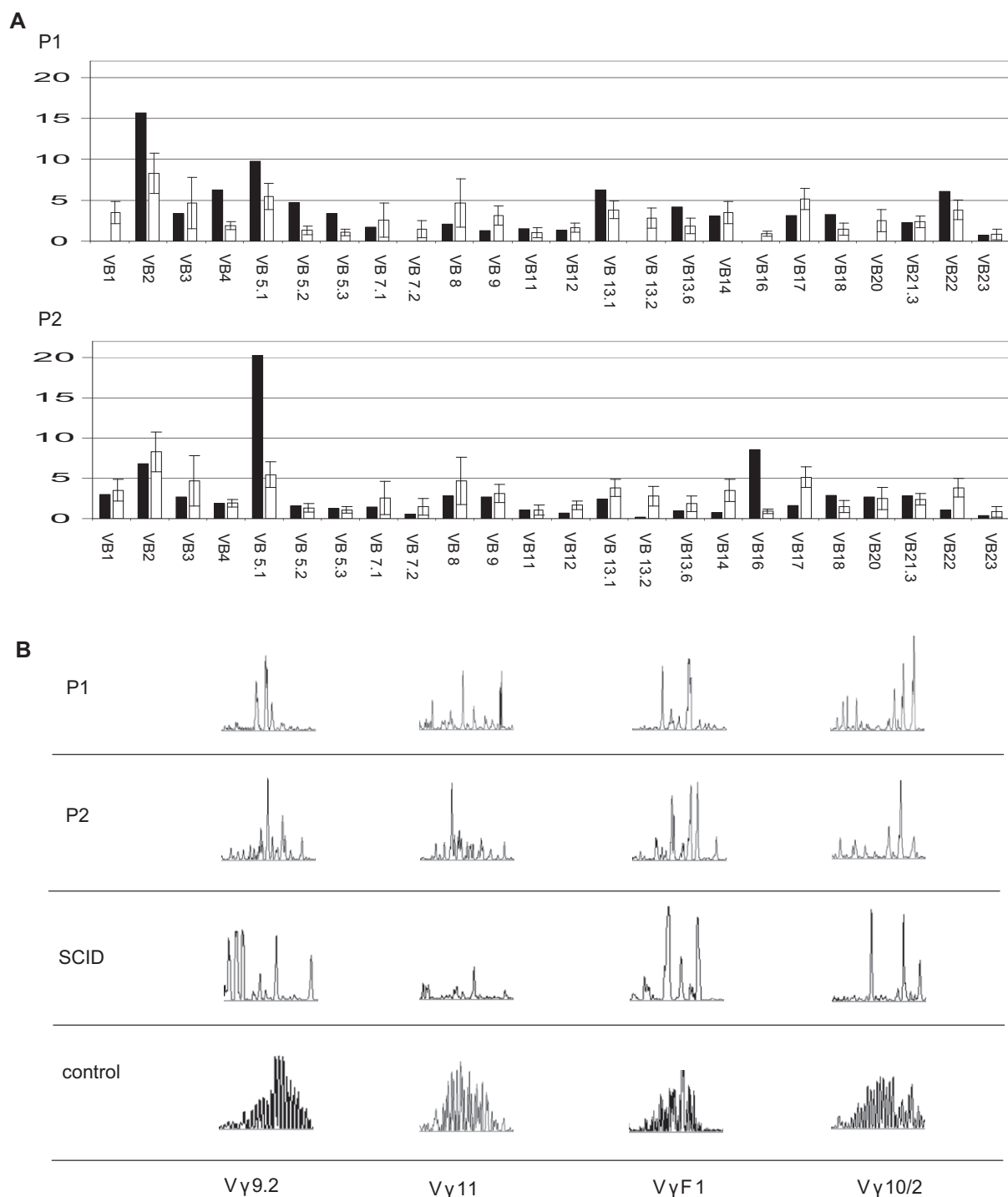


FIG 1. Skewed TCR and BCR repertoires in PNP-deficient patients. **A**, Relative expression of the various V β families detected by fluorescence-activated cell sorting (black bars) in PBMCs obtained from patient 1 (upper panel) and patient 2 (lower panel) compared with $n = 85$ normal controls (white bars). **B**, Four different TCRV γ gene rearrangements (V γ 9.2, V γ 11, V γ F1, and V γ 10/2) were amplified by PCR followed by Gene Scan analysis in patient 1 (I), patient 2 (II), a RAG deficiency control (III), and a normal healthy control (IV). **C**, Two different IgH BCR gene rearrangements (FR2 and FR3) were amplified by PCR followed by Gene Scan analysis in patient 1 (I), patient 2 (II), a RAG deficiency control (III), and a normal healthy control (IV). P1, Patient 1; P2, patient 2; RAG, recombination activating gene; SCID, severe combined immunodeficiency.

Unexpectedly, significantly low levels of B cells were detected in the older patient, while there were no signal joint KREC levels and barely detected coding joint KREC levels in both patients.

These findings suggest that their B cells originated mainly from proliferation and not from newly derived bone marrow cells (Table I). Both TREC and KREC values were significantly

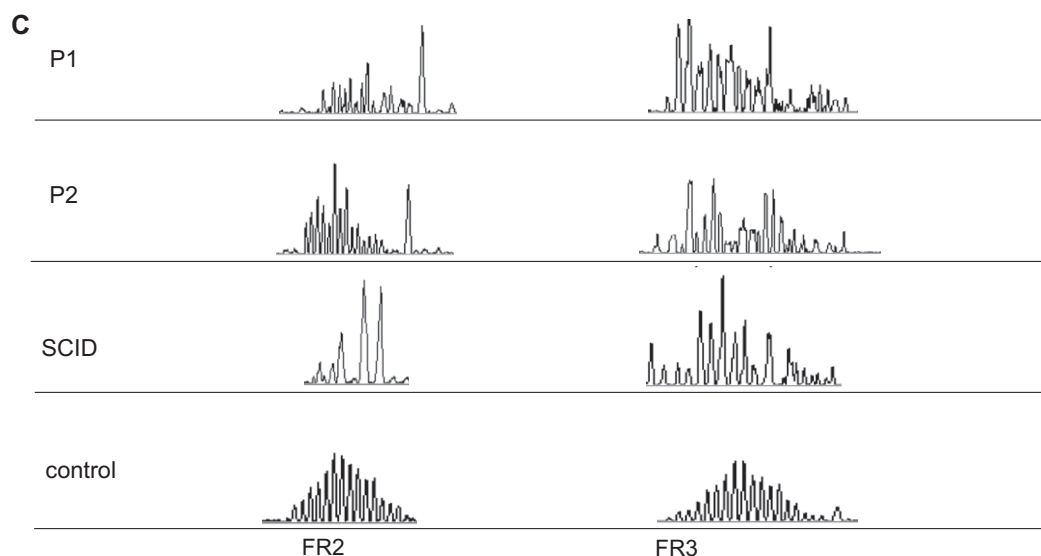


FIG 1. (continued).

different from age-reference normal controls and similar to those of 10 patients affected by recombination activating gene mutations that severely disrupt T- and B-cell development.⁷

T- and B-cell receptor repertoires were analyzed for clonality to determine whether the 2 PNP-deficient patients also had increased peripheral cell proliferation. Representatives of 24 specific TCRV β families were detected and quantified by flow cytometry (Becton-Dickinson, Franklin Lakes, NJ) and referred to the Quick Reference Card according to the manufacturer's instructions (Beckman Coulter, Immunotech, Marseille, France). TCRV β repertoires were restricted in both patients (low V β 1, V β 7.2, V β 13.2, V β 16, and V β 20 in patient 1 and V β 7.2, V β 13.2, V β 14, V β 17, V β 22, and V β 23 in patient 2) with the presence of a few dominant clones (V β 2 and V β 5.2 in patient 1 and V β 5.1 and V β 16 in patient 2) (Fig 1, A). Spectratyping amplification by PCR using 4 fluorescence-labeled V γ chain primers was used to better delineate the clonality. Profiles were characterized as oligoclonal if they contained 4 or fewer peaks, as polyclonal skewed if they had more than 4 peaks but were not Gaussian-like, and as polyclonal Gaussian if they had more than 4 peaks in a Gaussian-like distribution.⁸ Both patients had a polyclonal skewed profile, similar to the pattern of a typical patient with disrupted T- and B-cell immunity (Fig 1, B). The BCR profiles that resulted from spectratyping amplification by PCR with 2 fluorescence-labeled V immunoglobulin heavy chain primers were also polyclonal skewed. This result was similar to a typical severe combined immunodeficiency with clonal expansion and in strong contrast to the Gaussian-like distribution of healthy control cells (Fig 1, C). Taken together, these polyclonal skewed TCR and BCR profiles may possibly be reflecting abnormal selection or expansion of some families in response to a stimulus. Another line of evidence to support direct variable B-cell immunodeficiency in our patients came from the finding that immunoglobulin levels were normal in patient 1 who had some specific antibodies (eg, polio and cytomegalovirus), while, in marked contrast, specific antibodies were completely absent in patient 2 whose immunoglobulin levels were reduced. These findings are suggestive of a profound defect in B-cell immunity. Moreover, both patients were found to have undetectable isohemagglutinin titers. The lack of these

antibodies, directed against blood group A and B antigens, is a clear indication of the direct effect that the PNP deficiency has on B-cell immunity, since these antibodies are normally produced in a T-cell-independent manner.

Herein we present 2 siblings with a novel nonsense PNP mutation causing PNP deficiency. Both patients had low and dysfunctional T cells and profoundly impaired T-cell production. Similar to the PNP^{-/-} mice, which are known to have a reduced number of thymocytes and abnormal T-cell rearrangement as well as reduced peripheral T cells,⁴ our patients exhibited restricted TCR repertoires. This is probably due to a massive apoptotic event in the thymus. It was recently shown that PNP deficiency preferentially causes apoptosis of double-positive thymocytes in mice and that the accumulation of deoxyguanosine triphosphate in cases of PNP deficiency leads to mitochondria-initiated apoptosis of these thymocytes.⁹ B cells are marginally affected in PNP deficiency and are even completely normal in number and function in some patients.¹⁰ In contrast, we describe 2 sisters whose PNP deficiency had a variable effect on B-cell function, including reduced B-cell numbers, lack of B cells containing signal joint circles, reduced immunoglobulin levels, absence of specific antibodies, and abnormal BCR repertoires. Some of these abnormalities cannot be explained by the known T-cell defect of patients with PNP deficiency alone, and they may be attributed to a direct effect on B-cell immunity. This observation widens the impact of B-cell immunodeficiency in cases of PNP deficiency and is probably explained by the complete lack of PNP enzyme activity due to the reported nonsense mutation. Numerous symptomatic mutations, mostly missense, of the *PNP* gene have been identified. These mutations produce proteins with variable degrees of enzymatic activity, which correlate with the accumulation of nucleoside substrates and, to some degree, with the clinical course. It is the progressive accumulation of deoxyguanosine triphosphate in PNP deficiency that leads to progressive disease. Interestingly, similar to the progressive T-cells toxicity caused by PNP deficiency during the first years of life, the more severe B-cell defect observed in our older patient also suggests accumulating effects of PNP deficiency on B cells. Our results concur with several recent *in vitro* and *in vivo* studies describing

B-cell death following PNP inhibition with excess deoxyguanosine/deoxyguanosine triphosphate concentrations, albeit at a reduced frequency compared with T-cell apoptosis.¹¹ Additional studies, including longitudinal studies, are still required to determine whether B-cell function, like T-cell function, deteriorates over time in PNP-deficient patients. In normal peripheral blood B cells, the addition of deoxyguanosine leads to an inhibition of proliferation and differentiation. This effect was found to be independent of deoxyguanosine accumulation.¹² Complete lack of PNP (as observed in our patients) triggers accumulation of deoxyguanosine, thereby disrupting B-cell development, the consequence of which is more profound with time, as indeed was found in the older sister. Another line supporting the hypothesis that the accumulation of deoxyguanosine results in global cell toxicity and that it is not restricted to T cells alone came from the use of purine and pyrimidine nucleoside analogs to treat certain malignancies, including B-cell malignancies.¹³ For example, forodesine, a PNP inhibitor, converts deoxyguanine to deoxyguanosine triphosphate and causes general apoptotic cell effect, similar to what is seen in PNP deficiency.¹⁴

We conclude that the variable effect on B-cell function that is observed in PNP deficiency may be an independent event of T-cell dysfunction and that it is subject to the severity of the deficiency and the duration of the disease.

Raz Somech, MD, PhD^{a,b,c}
Atar Lev, MSc^{a,b,c}
Amos J. Simon, PhD^a
Suhair Hanna, MD^d
Amos Etzioni, MD^d

From ^athe Cancer Research Center and ^bthe Pediatric Department B North and Immunology Service, Edmond and Lily Safra Children's Hospital, Tel Hashomer, affiliated to the ^cSackler Faculty of Medicine, Tel Aviv University, Tel Aviv, and ^dMeyer's Children Hospital, Rappaport Faculty of Medicine, The Technion, Haifa, Israel. E-mail: raz.somech@sheba.health.gov.il.

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REFERENCES

1. Hirschhorn R. Inherited enzyme deficiencies and immunodeficiency: adenosine deaminase (ADA) and purine nucleoside phosphorylase (PNP) deficiencies. *Clin Immunol Immunopathol* 1986;40:157-65.
2. Notarangelo LD. Primary immunodeficiencies (PIDs) presenting with cytopenias. *Hematology Am Soc Hematol Educ Program* 2009;139-43.
3. Hirschhorn R, Candotti F. Immunodeficiencies due to defects of purine metabolism. In: Ochs HD, Smith CIE, Puck JM, editors. *Primary immunodeficiency diseases*. Oxford University Press: 2nd ed; 2007. p. 169-96.
4. Grunebaum E, Zhang J, Roifman CM. Novel mutations and hot-spots in patients with purine nucleoside phosphorylase deficiency. *Nucleosides Nucleotides Nucleic Acids* 2004;23:1411-5.
5. Serana F, Airò P, Chiarini M, Zanotti C, Scarsi M, Frassi M, et al. Thymic and bone marrow output in patients with common variable immunodeficiency. *J Clin Immunol* 2011;31:540-9.
6. Sottini A, Ghidini C, Zanotti C, Chiarini M, Caimi L, Lanfranchi A, et al. Simultaneous quantification of recent thymic T-cell and bone marrow B-cell emigrants in patients with primary immunodeficiency undergone to stem cell transplantation. *Clin Immunol* 2010;136:217-27.
7. Lev A, Simon AJ, Bareket M, Bielora B, Hutt D, Amariglio N, et al. The kinetics of early T and B cell immune recovery after bone marrow transplantation in RAG-2-deficient SCID patients. *PLoS One* 2012;7: e30494.
8. Markert ML, Alexieff MJ, Li J, Sarzotti M, Ozaki DA, Devlin BH, et al. Complete DiGeorge syndrome: development of rash, lymphadenopathy, and oligoclonal T cells in 5 cases. *J Allergy Clin Immunol* 2004;113:734-41.
9. Papinazath T, Min W, Sujitha S, Cohen A, Ackley C, Roifman CM, et al. Effects of purine nucleoside phosphorylase deficiency on thymocyte development. *J Allergy Clin Immunol* 2011;128:854-63.
10. Dalal I, Grunebaum E, Cohen A, Roifman CM. Two novel mutations in a purine nucleoside phosphorylase (PNP)-deficient patient. *Clin Genet* 2001;59:430-7.
11. Homminga I, Zwaan CM, Manz CY, Parker C, Bantia S, Smits WK, et al. In vitro efficacy of forodesine and nelarabine (ara-G) in pediatric leukemia. *Blood* 2011;118:2184-90.
12. Scharenberg JG, Spaapen LJ, Rijkers GT, Duran M, Staal GE, Zegers BJ. Functional and mechanistic studies on the toxicity of deoxyguanosine for the in vitro proliferation and differentiation of human peripheral blood B lymphocytes. *Eur J Immunol* 1986;16:381-7.
13. Bantia S, Parker C, Upshaw R, Cunningham A, Kotian P, Kilpatrick JM, et al. Potent orally bioavailable purine nucleoside phosphorylase inhibitor BCX-4208 induces apoptosis in B- and T-lymphocytes—a novel treatment approach for autoimmune diseases, organ transplantation and hematologic malignancies. *Int Immunopharmacol* 2010;10:784-90.
14. Robak T, Lech-Maranda E, Korycka A, Robak E. Purine nucleoside analogs as immunosuppressive and antineoplastic agents: mechanism of action and clinical activity. *Curr Med Chem* 2006;13:3165-89.

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