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RESEARCH

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Purine nucleoside phosphorylase (PNP) deficiency: across-the-board severe combined immunodeficiency

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Abstract

Background Purine nucleoside phosphorylase (PNP) deficiency is a rare, autosomal recessive, inborn error of immunity. It is characterized by progressive immune abnormalities ranging from severe combined immunodeficiency (SCID) to combined immunodeficiency less profound than SCID, neurological abnormalities and autoimmunity. Early detection and diagnosis before the development of life-threatening complications are crucial.

Methods Immune cell subsets were assessed by flow cytometry, serum immunoglobulins and uric acid levels were evaluated, and genetic testing was performed for all patients.

Results Herein, we present six Egyptian PNP deficiency patients from four different families. We describe the patients' clinical phenotypes, their immunological profile as well as their genetic results. Sequence analysis results detected 4 different variants in the *PNP* gene; 1 likely pathogenic frameshift deletion c.452del; p.Asn151MetfsTer20 was found in one family, 1 pathogenic nonsense variant c.172C > T; p.Arg58Ter, and 2 likely pathogenic missense variants c.682G > C; p.Ala228Pro and c.722T > C; pIle2241Thr.

Conclusion In conclusion, PNP deficiency is a variable immunodeficiency and should be considered in various clinical contexts, with or without neurological manifestations. Hematopoietic stem cell transplantation offers a good treatment option, with excellent clinical outcomes, when performed in a timely manner.

Keywords Hematopoietic stem cell transplantation (HSCT), Inborn errors of immunity (IEI), Purine nucleoside phosphorylase deficiency **(**PNP), Severe combined immunodeficiency (SCID)

Introduction

Inborn errors of immunity (IEI) encompass a group of nearly 500 inherited disorders, often due to single-gene mutations, that result in the specific impairment of normal immune development and function. While individually rare, in aggregate, the prevalence of these conditions is approximately 1 in 1000–5 in 1000. The clinical presentation of IEI is variable and includes severe or unusual infections, autoimmune and autoinflammatory diseases, and malignancies [1]. The defects might be autosomal or X-linked, dominant or recessive, with complete or imperfect penetrance [2].

In consanguineous populations, severe primary immunodeficiency diseases are not considered rare diseases [3], mostly reported with an autosomal recessive (AR) mode of inheritance [2].

Purine nucleoside phosphorylase (PNP) deficiency is a rare AR-IEI. It was initially identified in patients with substantial T-cell immunodeficiency in the

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1970s, shortly after discovering a shortage of another purine metabolic pathway enzyme, adenosine deaminase (ADA) enzyme. It is characterized by progressive immune abnormalities ranging from severe combined immunodeficiency (SCID) to combined immunodeficiency less profound than SCID, neurological abnormalities and autoimmunity. T-cell deficiency, varied B-cell abnormalities, low serum uric acid and diminished PNP enzyme activity are noted in such conditions [4].

The estimated incidence of SCID is 1 in 50,000–100,000 live births [5]. PNP deficiency accounts for less than 5% of all SCID patients. The age of onset and the course of this disease are highly variable among patients [6].

The disease occurs due to mutation in the *PNP* gene located at chromosome 14q11.2. *PNP* gene consists of 6 exons and encodes purine nucleoside phosphorylase protein (289 amino acid) which is an enzyme that catalyzes the reversible phosphorolysis of the purine nucleosides and deoxynucleosides inosine, guanosine, deoxyinosine and deoxyguanosine. Deficiency in PNP enzymatic activity leads to the accumulation of its substrates, thus the accumulation of deoxyguanosine triphosphate (dGTP) intracellularly. dGTP may interfere with DNA synthesis or repair, subsequently preventing the cellular proliferation required for an immune response. Low uric acid in plasma was considered a marker for the disease, but being within normal level in some patients, suggests that this marker is not reliable [7].

The disease has a variable clinical presentation; however, neurological abnormalities are the most experienced manifestations in about two-thirds of individuals, meanwhile autoimmune diseases manifest in up to one-third of the patients, most commonly autoimmune hemolytic anemia and immune thrombocytopenia (ITP). Hematological malignancies may sometimes occur [7].

Some infections caused by PNP enzyme deficiency can be prevented with antimicrobial treatment and prophylaxis, yet most patients die from infections, autoimmunity or cancer in infancy and early childhood without a stem cell transplant [8].

Allogenic hematopoietic stem cell transplantation (HSCT) is considered the only curative treatment [8]. Recently developed HSCT protocols resulted in improved outcomes. Although patients may develop a normal immune system after the transplantation, it is noted in the published data that it may not reverse the neurological manifestations [7, 9].

In the literature, 100 cases of PNP deficiency have been reported worldwide until 2022 [10] Later in 2023, a study published PNP deficiency in two Iranian patients and another described the neurological status of 6 Turkish patients before and after HSCT [11, 12].

Herein, we present **6** Egyptian patients from **4** different families who had pathogenic variants in the *PNP* gene. We described the patients' clinical phenotypes and their immunological investigations, aiming to increase awareness of this disease and highlighting the necessity for early diagnosis, as this saved two of our cases successfully transplanted and managed at an early age.

Methods

Study population

This study included 6 patients evaluated in Cairo University Children's Hospital (Cairo, Egypt) between 2019 and 2024 with a SCID/CID phenotype and confirmed *PNP* gene mutation. In accordance with the principles of the Declaration of Helsinki, the patients' parents gave informed consent. This study was approved by the local institutional review board (MD-93–2019).

Inclusion criteria for CID according to the European Society for Immunodeficiencies (ESID) were followed:

- At least one of:
  At least one severe infection (requiring hospitalization), one manifestation of immune dysregulation, malignancy, affected family member.
- AND 2 of 4 T-cell criteria fulfilled:
  Reduced CD3 or CD4 or CD8 T cells (using age-related reference values), reduced naïve CD4 and/or CD8 T cells, elevated g/d T cells, reduced proliferation to mitogen or TCR stimulation.
- AND HIV excluded

Samples

2 ml venous blood was collected in a serum vacutainer for immunoglobulins quantification and uric acid assessment. 3 ml venous blood was collected in ethylene diamine tetra acetic acid (EDTA) vacutainer for complete blood count (CBC), immunophenotyping and DNA extraction. DNA was kept at – 20 °C till the genetic testing.

Methods

Serum uric acid was assessed by an AU chemistry analyzer (Beckman Coulter), and specific immunoglobulins were quantified by automated nephelometry (Omlipo, Goldsite). A differential CBC was done utilizing the Sysmex hematology analyzer.

Initial screening of peripheral blood lymphocytes' subsets by flow cytometry was done within 24 h of sample collection utilizing pan-T marker (CD3) (BD#555,334), T-helper marker (CD4) (BC#IM0448U), T-cytotoxic marker (CD8) (BC#IM0452U), B cells marker (CD19) (BC#IM2643U) and NK cells marker (CD56)

(BC#A51078). Immunophenotyping was done as previously described by Meshaal el al. [13]. Samples were acquired on BD FACS CANTO^{TM}II (BD Biosciences, USA), and the BD FACSDiva$^{™}$ Software was used for data analysis.

For genetic testing, the QIAamp DNA Blood Mini Kit (Qiagen, Germany, Cat. No. 51104) was utilized for silica-membrane-based DNA purification by the protocol indicated by the manufacturer.

Direct Sanger sequencing was done for patients with SCID having neurological/autoimmune manifestations and low uric acid or for target screening for affected family members with previously diagnosed index cases. The amplification of the 6 exons and exon/intron junction of the *PNP* gene was done using primers as described by Moallem et al. [14] and Alangari et al. [15]. Sequencing was performed on an ABI 310 Genetic Analyzer (Applied Biosystems, NY) using the same primers used to amplify the polymerase chain reaction (PCR) fragments. Sequences were compared to the published reference sequences of the National Center for Biotechnology Information (NM_000270.4) and analyzed using the Basic Local Alignment Search Tool (BLAST).

For patients with nonclassic phenotypes, next-generation sequencing (NGS) was performed either by whole-exome sequencing (WES) or by IEI genes panel using a 4 bases PID pro kit (452 genes) according to availability. NGS was performed on the Illumina MiSeq platform. We used Franklin software by Geenox in the genetic data interpretation. Only variants that pass the quality filter: depth > 20 × and quality > 100 were analyzed. The American College of Medical Genetics and Genomics (ACMG) guidelines were used to categorize the identified variants into Pathogenic, Likely Pathogenic, Variant of Uncertain Significance, Likely Benign and Benign. Sequence variants were described according to Human Genome Variation Society (HGVS) recommendations.

Results

Clinical characteristics and laboratory data

Family 1 (Patient 1, 2 & 3)

Patient 1 (P1) was a one-week-old female, 4th child of consanguineous parents, born full-term with a low birth weight of 1700 g. She presented to our clinic for early assessment and screening due to a family history of two previous siblings' deaths: A female sibling died at the age of 15 months with recurrent pneumonia and diarrhea and a male who was diagnosed as T negative B negative SCID (T-B-SCID).

The laboratory tests results showed a low total leucocyte count (TLC) of 1900/mm^3) and absolute lymphocytic count (ALC) 399/mm^3. Immunophenotyping revealed T-B-SCID (CD3:51.4%, CD4: 37.7%, CD8:12%, CD19:5% and CD56:12.4%). All lymphocytes' subsets showed low absolute counts. The immunoglobulin levels showed higher than normal reference range for IgG (1333 mg/dl) and IgM (153.3 mg/dl) while the IgE level was normal (32.8 mg/dl). IgA was not measured as well as serum uric acid.

The patient had normal percentiles and developmental milestones for her age at the time of examination and follow-up; she received monthly intravenous immunoglobulins (IVIG) replacement until she had a successful fully matched related donor (MRD)-HSCT without conditioning at the age of 6 months, and the donor was the only living healthy 5-year-old female sibling. No reported complications during and post-transplantation were detected, only she had a self-limited cytomegalovirus (CMV) reactivation in the first year post-transplant. She is doing great in the follow-up, no Graft versus host disease (GVHD) was reported and no IVIG replacement is needed. She had normal motor and mental development for her age and is now ten years old.

NGS with a panel of IEI genes surprisingly revealed a homozygous likely pathogenic variant in the *PNP* gene: c.452del; p. (Asn151MetfsTer20).

Patient 2 (P2) is the brother of P1, a full-term baby with a birth weight of 2750 g. He presented in the first few weeks of life as well for screening and early evaluation.

The patient was hospitalized at the age of one month with pneumonia. He had normal percentiles for his age; shortly after diagnosis, he was started on prophylactic antimicrobials and IVIG replacement therapy and was planned for HSCT.

His laboratory tests results showed low TLC (5300/mm^3) and ALC (1060/mm^3). Immunophenotyping revealed T-B-SCID (CD3:46.7%, CD4:37.7%, CD8:7.2%, CD19:5.3%, CD56:6%). All lymphocytes' subsets showed low absolute counts. The immunoglobulin levels showed a decrease in IgM (29 mg/dl) while the levels for IgG and IgA were normal (311.7 and 43.6 mg/dl, respectively). P2 serum uric acid was low 0.5 (2–5.5 mg/dl). Genetic testing was done by targeted Sanger sequencing, and it revealed the same mutation as his sister P1.

The patient underwent a full MRD-HSCT without conditioning, same donor of P1; with no reported complications till the meantime, he received regular monthly IVIG replacement for around 6 months post-transplant. He is currently 5 years old, doing well in the follow-up, with normal motor and mental development for his age.

Both P1 and P2 did not receive Bacillus Calmette–Guerin (BCG), and oral polio vaccine (OPV) vaccinations as instructed when having a family history of IEI.

P3 was a previously deceased sibling for P1&P2 who was diagnosed with T-B-SCID but unfortunately, he died before genetic testing. Sanger sequencing results showed

the same mutation as his siblings (P1&P2). The patient had presented to our clinic at the age of 14 months with chronic diarrhea and oral thrush. The onset of his symptoms was at the age of 4 months with unresolving pneumonia, frequent hospitalization due to severe watery diarrhea and dehydration. Unfortunately, we lost our patient due to septicemia.

His laboratory tests results showed normal TLC (9120/mm^3) but there was a marked decrease in the ALC (91.2/mm^3). Immunophenotyping showed picture of T-B-SCID (CD3:7.6%, CD4:5.3%, CD8:1.8%, CD19:1%, CD56:7%). All lymphocytes' subsets showed low absolute counts.

Family 2 (Patient 4)

An eight-month-old male patient, presented at the age of 6 months with severe autoimmune hemolytic anemia with frequent mismatch problems, repeated blood transfusions and poor response to treatment which included steroids, IVIG and immunosuppressive drugs. The patient suffered as well from chronic watery diarrhea and *Cryptosporidium* was isolated. Mild splenomegaly was noted in his abdominal ultrasound imaging.

P4 was born to a nonconsanguineous family, had two male siblings' deaths at the age of 12 and 24 months with infections, and one older healthy female sibling.

The laboratory results showed a decrease in TLC (4300/mm^3) as well as ALC (774/mm^3). Immunophenotyping revealed a decrease in the absolute count of all lymphocyte subsets suggesting the diagnosis of T-B-SCID (CD3:64.3%, CD4:14%, CD8:44%, CD19:16.1% and CD56: 9.6%). The immunoglobulin assessment showed an increase in IgG (2565 mg/dl) and IgM (213 mg/dl). The serum uric acid measured was found to be low (0.5 mg/dl).

Sanger sequencing of the *PNP* gene showed homozygous likely pathogenic missense variant c.682G > C; p. (Ala228Pro) (rs1747682143). Unfortunately, the patient died at the age of one year with septicemia.

Family 3 (Patient 5)

An eleven-month-old female patient, born to consanguineous parents, was referred for immunological evaluation giving the history of recurrent pneumonia since the age of 8 months, followed by the onset of severe autoimmune hemolytic anemia at the age of 12 months. Along the disease course, the patient suffered from frequent mismatch problems, increased frequency of blood transfusions and poor response to treatment including steroids, IVIG and immunosuppressant therapy. The patient later acquired hepatitis C virus and sclerosing cholangitis. She was also to have delayed motor and mental milestones for her age. On examination, weight, length and head circumference were below 3rd centiles for age, microcephaly was noted (-2SD head circumference (HC) on *Z* score), and the patient had mild jaundice, hepatosplenomegaly and hypotonia.

Going in-depth with the family history of P5, she had one living healthy female sibling and two male sibling's deaths at the age of 6 and 13 months with infections and suspected similar features as microcephaly and delayed developmental milestones. Two maternal aunts and one uncle were reported dead at a young age.

Laboratory workup revealed a decrease in TLC (2700/mm^3) as well as the ALC (873/mm^3). Lymphocyte subsets percentages were low (CD3:18%), CD4:12%, CD8:4%, CD19: 9.9% and CD56:3.8%. The absolute counts of all subsets were decreased, with a picture suggestive of T-B-SCID. Immunoglobulins assay revealed a decrease in IgG level (197 mg/dl), an elevated IgM level (238 mg/dl) and normal IgA (119 mg/dl) and IgE (6.2 IU/ml) levels. Low serum uric acid level was also encountered (1.1 mg/dl).

WES was done revealing a previously reported pathogenic nonsense variant c.172C > T, p. (Arg58Ter) (rs104894460) causing severely truncated protein with loss of the active enzyme site. The variant was confirmed by Sanger sequencing.

We lost our patient in the intensive care unit at the age of 30 months with severe refractory AIHA and pneumonia, cultures results showed: Corona virus OC- 43, *Parainfluenza type IV*, Boca virus, *Klebsiella* (ESBL) and *Candida albicans*.

Family 4 (Patient 6)

An eleven-month-old female patient, born to consanguineous parents, presented with severe AIHI and leucopenia. She did an urgent bone marrow (BM) aspirate that revealed: hyper cellular BM, with a mild increase in early forms of the myeloid series and an increase in bone marrow eosinophils. Erythroid series showed hyperplasia and the presence of atypical cells, while megakaryocytes were increased in number with normal morphology.

An immunohistochemistry study was performed on the BM aspirate slides and revealed a marked depression of T lymphocytes with a relative increase in B lymphocytes. The pattern of distribution of positivity of TdT, CD10 and CD 20 is concomitant with increased hematogones. The patient therefore was referred to our clinic for further assessment and immunodeficiency screening.

On examination, her weight, length and HC were below 3rd percentiles. She was microcephalic (-2SD HC on *Z* score) also her mental and motor milestones were delayed for her age, although she had normal muscle tone and power. Her disease course was severe, she suffered from frequent mismatch problems, increased frequency of blood transfusions and very poor response to therapy including steroids, IVIG and immunosuppressant

therapy. Regarding her family history, she had three living healthy female siblings and had no history of previous sibling deaths.

Laboratory workup revealed a decrease in TLC (3790/mm^3) as well as ALC (758/mm^3). Immunophenotyping showed a picture suggestive of T-B + SCID (CD3:9.1%, CD4:8.3%, CD8: 0.3%, CD19:55% and CD56:23%). All lymphocyte subsets absolute counts were decreased. Immunoglobulins assay showed normal IgG level (500 mg/dl), IgM level (97 mg/dl) and IgA (100 mg/dl). Normal serum uric acid level (3.7 mg/dl) was also found.

NGS with IEI genes panel showed a novel homozygous missense likely pathogenic variant in *PNP* gene c.722T > C; p.Ile241Thr.

Unfortunately, we lost our patient in the intensive care unit at the age of 24 months with pneumonia and respiratory failure.

All patients clinical and laboratory data are summarized in Table 1.

Families' pedigree is illustrated in Fig. 1. Sanger sequencing electrogram of the *PNP* gene variants is presented in Fig. 2.

Discussion

PNP deficiency is considered an immunological emergency in childhood with a poor prognosis. Early detection and intervention help to cure not only combined immunodeficiency manifestation but also prevent neurological sequel [4, 16].

The disease had an AR pattern of inheritance and thus has a high incidence among consanguineous families. We report 6 PNP deficiency patients from 4 different families, with positive consanguinity in 3 of them (75%). Family history of similar conditions and siblings' deaths were reported in families 1, 2 and 3 reflecting the high incidence of the disease among consanguineous families as reported in previous studies [17, 18]. Therefore, a family history of unusual or fatal infective complications or unexplained infant death is considered a warning sign that necessitates screening [19]. The early diagnosis allows prompt management of the patients. It was reported that in three neonates/infants who were genetically diagnosed prenatally or early in life because of previous familial index cases, were fortunate enough to have a sequential therapy with immediate erythrocyte transfusions (ET) followed by HSCT thus allowing them to have a better quality of life [16].

P1 and P2 were diagnosed shortly after birth being routinely screened for having a previous sibling diagnosed with SCID, and prior to the onset of any clinical manifestations, for other manifesting patients the mean age of diagnosis was 11 ± 2.4 months. However, almost all patients published in the literature were diagnosed after the age of two years [20, 21]. This may indicate that the lack of awareness of disease phenotype or molecular testing may affect the time of diagnosis.

Newborn screening programs using T-cell receptor excision circles (TRECs) or tandem mass spectrometry (TMS) are sensitive methods to screen PNP deficiency in asymptomatic patients [16, 22]. Unfortunately, in some countries including Egypt, there are no available national newborn screening programs; however, alternative efforts are made according to available resources; families are instructed to avoid live vaccines after birth until immunological workup is done within the first few weeks of life, or they are offered prenatal testing if the molecular genetic defect is known.

The clinical features in our patients regarding infections were similar to SCID presentations; recurrent pneumonia and/or nonresolving pneumonia in 66.6% of the patients (4/6), chronic diarrhea in 50% (3/6) and oral thrush in 50% (2/4). These symptoms were similar to previous published data in literature being the most frequent manifestations leading to classic SCID diagnosis [23–26].

Five of our patients (83%) had immune cytopenia in the form of autoimmune hemolytic anemia requiring frequent blood transfusions, and leucopenia. P5 was diagnosed by BM aspirate due to the presence of bicytopenia; her marrow was hypercellular with erythroid hyperplasia associated with the presence of atypical cells. It was noticed that PNP deficiency has been linked to dysplastic and megaloblastic marrow alterations, as well as autoimmune symptoms such as hemolytic anemia, thrombocytopenia and neutropenia in approximately one-third of individuals. PNP deficiency leads to the accumulation of toxic purine metabolites, which were reported to be associated with dysplastic marrow morphology especially in erythroid series with a picture comparable in severity to those observed in congenital dyserythropoietic anemia, and these marrow changes could be improved with repeated red cell transfusion [27].

Neurological manifestations were found in 2/4 symptomatizing patients (50%). P4 had developmental delay, hypotonic and was noted to be microcephalic, also had 2 siblings with suspected similar features who died at an early age without a diagnosis and P6 also had delayed milestones, and microcephaly but with normal tone and muscle power.

Among SCID types, ADA and PNP deficiency may manifest with central nervous system (CNS) manifestations. Nearly two-thirds of patients have neurological symptoms unrelated to CNS infections or associated sequelae, which are a crucial signal in diagnosing this deadly condition. Before the start of infections, neurological manifestations may arise in the form of

Table 1 Demographic, clinical and laboratory data of PNP deficiency patients

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Gender	♀	♂	♂	♂	♀	♀
Age of onset	1 m	1 m	4 m	6 m	8 m	11 m
Age at diagnosis	Screened age 1 week	Screened age 1 week	14 m	8 m	11 m	11 m
Consanguinity	Positive	Positive	Positive	Negative	Positive	Positive
Affected siblings	♀sibling died at 15 m (pneumonia) ♂ **(P3)** ♂ **(P2)**	♀ sibling died at 15 m (pneumonia) ♂ **(P3)** ♀ **(P1)**	♀ sibling died at 15 m (pneumonia) ♀ sibling **(P1)** ♂ sibling **(P2)**	2♂ siblings died at 12 m and 24 m (infections)	2 ♂ siblings died at 6 m and 13 m (microcephaly, delayed milestones, hypotonic)	None
Clinical presentation	Screened due to family history	Pneumonia	Pneumonia Chronic Diarrhea Oral thrush	Chronic diarrhea (cryptosporidium), AIHA, frequent mismatch, repeated blood transfusions Mild splenomegaly	Failure to thrive Recurrent pneumonia Oral thrush Delayed motor and mental milestones Hypotonic Microcephaly AIHA, frequent mismatch and repeated blood transfusions Hepatosplenomegaly	Failure to thrive Pneumonia Microcephaly Severe AIHA resistant to therapy including steroids, IVIG, immunosuppressive therapy
TLC/mm3	**1900** (7200–18000)	**5300** (7200–18000)	9120 (6400–13000)	**4300** (6400–13000)	**2700** (6400–13000)	**3790** (6400–13000)
ALC/mm3	**399** (3400–7600)	**1060** (3400–7600)	**91.2** (3400–9000)	**774** (3400–9000)	**837** (3400–9000)	**758** (3400–9000)
CD3%	51.4% (3–84%)	**46.7%** (53–84%)	**7.6%** (49–76%)	64.3% (49–76%)	**18%** (49–76%)	**9.1%** (49–76%)
CD3 absolute count	**205** (2500–5500)	**485** (2500–5500)	**6.9** (1900–5900)	**497** (1900–5900)	**151** (1900–5900)	**68.9** (1900–5900)
CD4%	37.7% (35–64%)	37.7% (35–64%)	**5.3%** (31–56%)	**14%** (31–56%)	**12%** (31–56%)	**8.3%** (31–56%)
CD4 absolute count	**150** (1600–4000)	**399** (1600–4000)	**4.8** (1400–4300)	**108** (1400–4300)	**100** (1400–4300)	**62** (1400–4300)
CD8%	12% (12–28%)	**7.2%** (12–28%)	**1.8%** (12–24)%	44% (12–24%)	**4%** (12–24%)	**0.3%** (12–24%)
CD8 absolute count	**48** (560–1700)	**76** (560–1700)	**1.6** (500–1700)	**340** (500–1700)	**33** (500–1700)	**2.2** (500–1700)
CD19%	**5**% (6–32%)	**5.3**% (6–32%)	**1%** (14–37%)	16.1% (14–37%)	**9.9**% (14–37%)	55% (14–37%)
CD19 absolute count	**20** (300–2000)	**56** (300–2000)	**0.9** (600–2600)	**125** (600–2600)	**83** (600–2600)	**416** (600–2600)
CD56%	12.4% (4–18%)	6 (4–18%)	7% (3–15%)	9.6% (3–15%)	3.8% (3–15%)	23% (3–15%)
CD56 absolute count	**49.6** (170–1100)	**63** (170–1100)	**6.3** (160–950)	**74** (160–950)	**32** (160–950)	174 (160–950)
IgG (mg/dl)	1333 (633–1280)	311.7 (217–904)	NA	2565 (217–904)	**197** (424–1051)	500 (424–1051)
IgM (mg/dl)	153.3 (19–146)	**29** (35–102)	NA	231 (35–102)	238 (43–173)	97 (43–173)
IgA (mg/dl)	NA	43.6 (11–90)	NA	NA	119 (14–123)	100 (14–123)
Ig E (IU/ml)	32.8	NA	NA	NA	6.2	NA
Serum uric acid (mg/dl)	NA	0.5 (2–5.5)	NA	0.5 (2–5.5)	1.1 (2–5.5)	3.7 (2–5.5)
Genetic testing	IEI panel NGS	Sanger sequencing	Sanger sequencing	Sanger sequencing	WES	IEI panel NGS

Table 1 (continued)

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Genetic variant	Likely pathogenic Homozygous c.452del p. (Asn151MetfsTer20)	Likely pathogenic Homozygous c.452del p.(Asn151MetfsTer20)	Likely pathogenic Homozygous c.452del p.(Asn151MetfsTer20	Likely pathogenic Homozygous c.682G > C p. (Ala228Pro) (rs1747682143)	Pathogenic Homozygous c.172C > T p. (Arg58Ter) (rs104894460)	Likely pathogenic Homozygous c.722T > C p.Ile241The
Outcome	(MRD-HSCT without conditioning) Alive (10 years)	(MRD-HSCT without conditioning) Alive (5 years)	Died (septicemia)	Died (septicemia)	Died (pneumonia, refractory AIHA)	Died (pneumonia)

Data in bold represents values lower than normal reference range

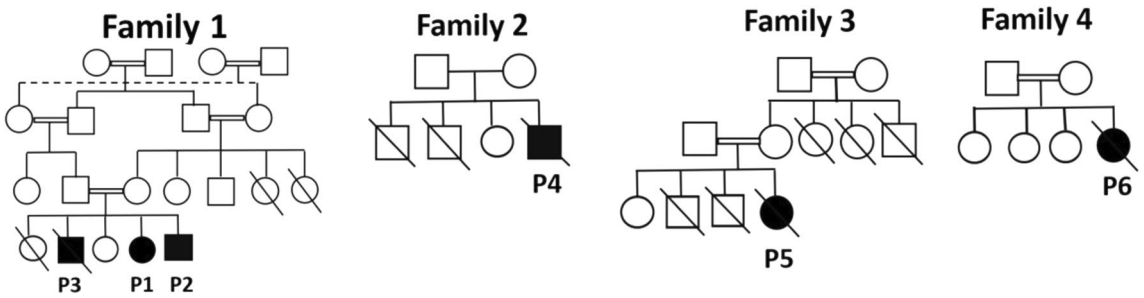

Fig. 1 Patients' family pedigree. Positive consanguinity is indicated by a double line indicates; deceased individuals were indicated by diagonal lines. Black filled squares and circles represent diseased member

developmental delay, hypertonia, spastic diplegia, tremors, ataxia, motor delay and intellectual disability [28].

In a study done on a PNP deficiency mouse model, it was found that PNP knocked-out mice had small cerebellum, corpus callosum and thalamus, and the enzyme deficiency had a direct effect on neural cells as it caused its ex vivo apoptosis that could be corrected by restoring PNP activity in cultures. However, disappointingly the initiation of treatment after 4 weeks of age in the mouse model failed to correct neurological abnormalities most likely due to irreversible brain injury. This agreed with published reports which demonstrated that HSCT beyond the first year of life does not correct neurological defects in PNP deficiency patients [28]. However, there could be no further deterioration of the neurological status post-transplantation [9].

A previous study by Eichinger et al. [16] encouraged the intrauterine ETs in PNP-deficient fetuses to be given as a bridge to HSCT therapy at the earliest possible time point as it might improve the neurocognitive development in the patients.

Clinical manifestations in people with partial PNP deficit may occur late in the third decade of life, with mild-moderate immunological problems and the usual development. With reduced PNP activity, near-normal immunity might be achieved [29].

Regarding the laboratory investigations, all patients included had lymphopenia and a decrease in T and B lymphocytes absolute counts and had variable immunoglobulins levels. This has been also observed in previously reported cases [22, 30].

Serum uric acid level was low in 75% of tested patients (3/4), hypouricemia is more common in PNP-deficient cases than in ADA deficiency (both shares neurological symptoms); sometimes however, the blood uric acid levels can be normal making it a nonreliable test [31].

Regarding the molecular diagnosis, we detected 4 different variants in the *PNP* gene. Three siblings P1, P2 and P3 showed a likely pathogenic homozygous deletion c.452del; p. (Asn151MetfsTer20) causing a frameshift in the protein at position 151 from asparagine to methionine and a truncation of the protein at amino acid 171. This variant is novel and not found in gnomAD exomes entry. P4 had homozygous likely pathogenic missense variant c.682G > C; p. (Ala228Pro). This variant does not have a gnomAD genomes entry and was not previously reported.

P5 had a pathogenic nonsense variant in Exon 2: c.172C > T; p.Arg58Ter causing severely truncated protein. This variant is reported as pathogenic by ClinVar, had a frequency $f = 0.0000438$ in gnomAD exomes entry and was previously reported by Dalal et al. [32] in a PNP-deficient patient.

P6 had a likely pathogenic missense variant, c.722T > C; p.Ile241The. This variant is novel and not found in gnomAD exomes entry. The identified variants in the first 3 families were previously reported in a study of the genetic background of IEI in Egyptian patients from a single tertiary center [33].

Treatment and outcome

PNP deficiency is a fatal disease, patients are generally lost with septicemia, infections with *Candida albicans*, *Pneumocystis jiroveci* and herpes simplex virus which follow a severe course [4]. Regarding the patients' outcome, we lost 4 of our patients mainly due to infections and septicemia. Meanwhile, 33% of the patients (2/6) are alive. The two living patients underwent HSCT without conditioning from a matched related donor (sibling); both patients are doing well in their follow-ups with normal mental and motor development for age. Similarly, previous studies reported that allogenic HSCT is the only curative treatment otherwise it is a deadly condition and patients die from infections, autoimmunity and cancer.

A previous study reported that the CNS requirement for PNP could be targeted indirectly by busulfan-containing HSCT conditioning which allows for donor monocyte

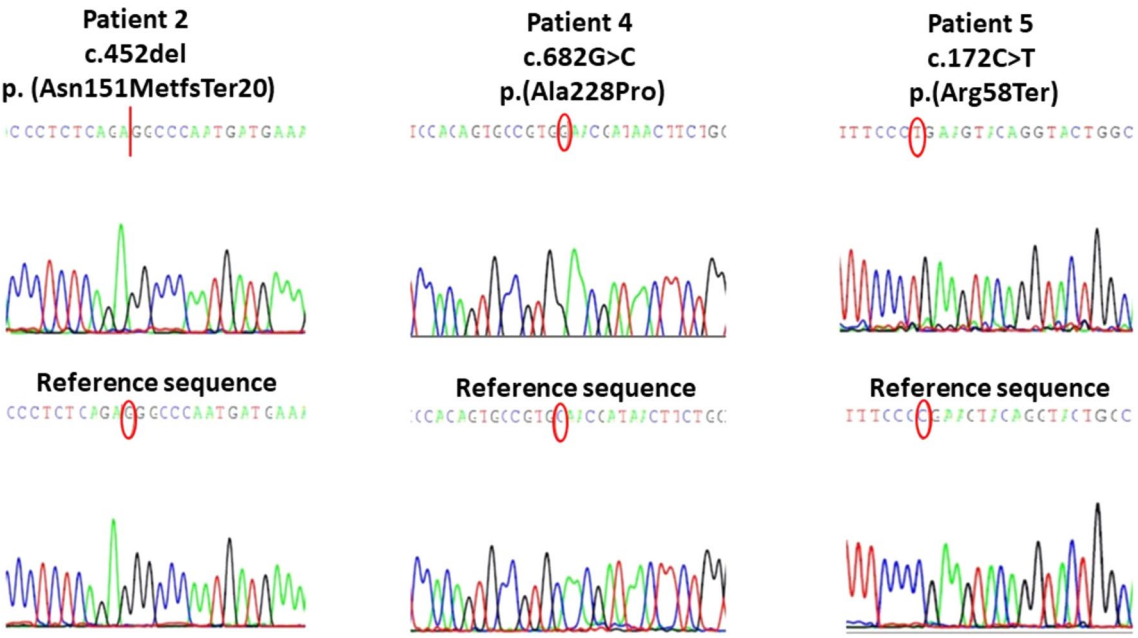

Fig. 2 Patients Sanger sequencing

migration to the CNS and ensuing trans-differentiation into microglia-like cells [9].

Transplant-related complications are still frequently seen, and normal development might not be achieved in all cases. Clearly, larger HSCT series with long-term outcome data are lacking. Moreover, in the preexisting literature, no clear genotype–phenotype correlations were shown for the neurological outcome and the development of the children which might be also influenced by the acquired infections [34].

Conclusion

We want to focus the attention on PNP deficiency which is considered an actual emergency disease of childhood that leads to SCID. The description of newly identified patients and novel pathogenic variants can help in a better understanding of this disease that has a wide range of clinical presentations. We emphasize the importance of early diagnosis and the prevention of diagnostic delay which could make a significant impact on early therapeutic intervention before the occurrence of permanent neurological damage and thus help patients to have a better outcome.

Study limitation

PNP and ADA enzyme activity testing as well as lymphocyte proliferation tests were not available at the time of the study.

Abbreviations

ACMG: The American College of Medical Genetics and Genomics
ADA: Adenosine deaminase deficiency
AIHA: Autoimmune hemolytic anemia
AR: Autosomal recessive
BCG: Bacillus Calmette–Guerin
BM: Bone marrow
CBC: Complete blood count
CNS: Central nervous system
CMV: Cytomegalovirus
dGTP: Deoxyguanosine triphosphate
EDTA: Ethylenediamine tetra acetic acid
ESID: European Society for Immunodeficiencies
ET: Erythrocyte transfusion
GTP: Guanosine triphosphate
GVHD: Graft versus host disease
HC: Head circumference
HIV: Human immunodeficiency virus
HSCT: Hematopoietic stem cell transplantation
IEI: Inborn errors of Immunity.
IVIG: Intravenous immunoglobulins
MRD: Matched related donor
NGS: Next-generation sequencing
OPV: Oral poliovirus vaccine
PCR: Polymerase chain reaction
PNP: Purine nucleoside phosphorylase deficiency
SCID: Severe combined immunodeficiency
TMS: Tandem mass spectrometry
TRECs: T-cell receptor excision circles
WES: Whole-exome sequencing

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Author contributions

All authors contributed to the study conception and design. Material preparation were performed by E.Chohayeb, S.Lotfy, R.El Hawary, S.Meshaal, I.A.Mansour, N.Galal and A.Elmarsafy. Data analysis were performed by R.El Hawary and S.Meshaal**.** The first draft of the manuscript was written by

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Availability of data and materials
The authors confirm that data supporting the findings of the study are available in the article. Raw data were generated in Cairo University Specialized Children Hospital. The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Approval was granted by Cairo University Faculty of Medicine Research Ethics Committee (MD-93–2019). Written informed consent was obtained from the parents of the patients.

Consent for publication
Informed consents were obtained from patients' guardians. The manuscript does not include any patients' pictures or data that may identify them.

Competing interests
The authors declare no competing interests.

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