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Purine Nucleoside Phosphorylase Deficiency: two case reports from Portugal

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Purine Nucleoside Phosphorylase Deficiency: two case reports from Portugal

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RESUMO

A deficiência da fosforilase dos nucleotídeos purínicos é uma forma rara de Imunodeficiência Primária. A fosforilase dos nucleotídeos purínicos é responsável pela fosforilação de nucleosídeos. Quando ocorrem mutações que comprometem a ação desta fosforilase, fica bloqueada a via das purinas para formação de ácido úrico e de moléculas transportadoras de energia, como a adenosina trifosfato e a guanosina trifosfato. As manifestações da deficiência de fosforilase dos nucleotídeos purínicos surgem pela deposição de metabolitos tóxicos em todas as células do corpo, levando a sintomas neurológicos e imunodeficiência progressiva, e comprometendo o normal desenvolvimento e crescimento das crianças.

Atualmente, menos de 80 casos estão publicados na literatura internacional e, de acordo com o nosso conhecimento, nenhum caso português foi descrito até à data. Serão descritos dois casos desta deficiência em duas crianças portuguesas, uma do sexo feminino e outra do sexo masculino, ambas caucasianas e filhos de pais não consanguíneos. A rapariga é homozigótica para uma mutação *missense* não reportada na literatura. O rapaz é um heterozigótico composto por uma mutação *nonsense*, previamente descrita como benigna, e uma mutação *missense* descrita na literatura como patológica. Estes 2 casos com défice de fosforilase dos nucleotídeos purínicos foram diagnosticados e seguidos no Centro Hospitalar do Porto.

O objetivo principal deste trabalho passa por sensibilizar os profissionais de saúde para os sintomas e sinais de alerta, frequentemente inespecíficos, que constituam manifestação desta doença rara e rapidamente fatal.

A revisão bibliográfica sobre o tema associada à descrição dos casos foi realizada através do motor de busca *Pubmed* e foram considerados todos os artigos escritos em inglês ou português em que, no seu assunto, seja abordado o défice de fosforilase dos nucleotídeos purínicos. As famílias das duas crianças foram informadas sobre o uso das informações e seu fim, e deram o consentimento informado.

ABSTRACT

Purine nucleoside phosphorylase deficiency is a rare form of combined primary immunodeficiency. Purine nucleoside phosphorylase is involved in the phosphorylation of nucleosides. Genetic mutations can cause enzyme defects which block the purine salvage pathway, preventing the synthesis of uric acid and energy source molecules, like Adenosine triphosphate and Guanosine triphosphate. An accumulation of toxic metabolites in the cells ensues, and the resulting clinical presentation includes neurological manifestations, progressive immunodeficiency and compromised growth and development in childhood.

Currently, less than 80 cases of purine nucleoside phosphorylase deficiency have been reported in the international literature and, to the best of our knowledge, no Portuguese case has been reported until now. Here, we present two purine nucleoside phosphorylase deficient Portuguese children, one male, one female, both of whom are Caucasian and have nonconsanguineous parents. The girl is a homozygote for a new missense mutation. The boy is a compound heterozygote who has a nonsense mutation, previously known as a benign mutation, and a pathologic missense mutation also previously reported. Both children were diagnosed and monitored at *Centro Hospitalar do Porto*.

Our main objective is to increase health care professional's awareness of the frequently non-specific symptoms and warning signs of this rare, and often fatal, disease.

A bibliographic review of purine nucleoside phosphorylase deficiency was made through the *Pubmed* search engine and all articles written in English or Portuguese were included. The families of both children were made aware of the information included here and its purpose. Informed consent was given by both families.

KEY-WORDS

Purine Nucleoside Phosphorylase Deficiency; Purine-Pyrimidine Metabolism; Inborn Errors; Immunodeficiency; Uric Acid; Lymphopenia

GLOSSARY

ADA – Adenosine deaminase
ADP – Adenosine diphosphate
ALT – Alanine transaminase
AMA – Anti-mitochondrial antibodies
AMP – Adenosine monophosphate
ANA – Antinuclear antibodies
AST – aspartate transaminase
ATP – Adenosine triphosphate
BCG - Bacillus Calmette-Guerin
CMV – Cytomegalovirus
CNS – Central nervous system
CRP – C-reactive Protein
CSF – Cerebrospinal fluid
dGDP – Deoxy guanosine diphosphate
dGTP – Deoxy guanosine triphosphate
DHL – Lactic dehydrogenase
DTwP – Combined diphtheria, tetanus toxoids, whole-cell pertussis
EEG – Electroencephalography
ERT – enzyme replacement therapy
GDP – Guanosine diphosphate
GMP – Guanosine monophosphate
GTP – Guanosine triphosphate
HGPRT- Hypoxanthine-guanine phosphoribosyl transferase
HIV – Human Immunodeficiency Virus
HSCT – Hematopoietic Stem Cell Transplantation
IMP – Inosine monophosphate
KREC – kappa-deleting recombination excision circles
MCV – Mean Corpuscular Volume
MRI – Magnetic Resonance Imaging
NAD – Nicotinamide adenine dinucleotide
NADP – Nicotinamide adenine dinucleotide phosphate
PCP – Pneumococcal capsular polysaccharide
PCR – Polymerase chain reaction
PEG-ADA – Polyethylene glycol-modified bovine adenosine deaminase
PNP – Purine Nucleoside Phosphorylase
RDW – Red cell Distribution Width
RF – Rheumatoid Factor
SCID – Severe combined immunodeficiency
STR – Striated muscle antibodies
TMS – Tandem Mass Spectrometry
TREC – T-cell receptor excision circles
UDPG – Uridine diphosphate glucose
VASPR – Measles, mumps and rubella vaccine

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INTRODUCTION

Purine nucleoside phosphorylase (PNP) deficiency is an autosomal recessive[1] metabolic disease[2]; a rare form of severe combined immunodeficiency (SCID)[2, 3] caused by mutations in the *PNP* gene[4] (14q11.2)[5, 6].

PNP is expressed at high levels in lymphoid tissue and is involved in the purine salvage pathway (Figure 1), which reversibly converts guanosine to guanine and inosine to hypoxanthine.[7] These bases are either salvaged as precursors for adenosine triphosphate (ATP) and guanosine triphosphate (GTP) or oxidized to uric acid.[7] The enzyme deficit leads to an accumulation of toxic metabolites[1] like inosine, guanine, deoxy inosine (dINO) and deoxy guanosine (dGUO) in all cells, and decreases uric acid production. Deoxy guanosine (dGUO) is converted to deoxy guanosine triphosphate (dGTP) by deoxycytidine kinase specific to lymphocytes, which causes dysfunctional T-lymphocyte development and functioning due to the inhibition of the ribonucleotide reductase and DNA synthesis or repair.[2, 8]

Typical clinical presentation begins after the first year of life with recurrent bacterial infections[8], autoimmune disorders (*i.e.* hemolytic anemia[9, 10], thrombocytopenia, arthritis and systemic lupus erythematosus), and neurological manifestations including ataxia, spasticity, and developmental delay[11].

Biochemically, a PNP deficiency presents with hypouricemia, B- and T-lymphocytopenia (CD4 higher than CD8), normal NK-lymphocytes counts and pan hypogammaglobulinemia, with an absence of vaccine-specific antibodies.[2] At the time of diagnosis, patients typically have elevated inosine, guanine, deoxy inosine and deoxy guanosine in both the blood and urine.[2, 5]

Other forms of SCID should be considered in the presence of these clinical and laboratory presentations. For example, Adenosine deaminase (ADA) deficiency, a prominent T- and B- phenotype SCID, remains the main differential diagnosis when confronted with the clinical symptoms described here.[12] If left untreated, both PNP an ADA deficiency are fatal in childhood, underlining the importance of early diagnosis.[13]

Diagnosis is confirmed through measures of PNP enzyme activity in a red blood cell lysate, blood spots or in white blood cells. Definitive diagnoses can be achieved with genetic analysis of the six exons of the *PNP* gene.[8]

The onset and gravity of PNP deficiencies vary but are often fatal in the first two decades of life. The only approved curative therapy for PNP deficiency is

hematopoietic stem cell transplantation (HSCT)[2, 5], but supportive treatment with immunoglobulin replacement and antimicrobial prophylaxis should be initiated at the time of diagnosis[2].

PNP deficiencies account for 4% of all SCID diagnoses[3, 4] yet less than 80 cases of PNP deficiency have been documented in the international literature[7].

In this report, we describe two cases of PNP-deficient patients, a girl and a boy. These children are from two different Caucasian families, both with nonconsanguineous parents.

CASE REPORT 1

A 19-month Caucasian girl, the first child of young, healthy and nonconsanguineous parents, was admitted to the hospital with fever, hypotonia and obnubilation. The pregnancy had been uneventful. She was born at term, five minutes Apgar score was 10, and birth weight was 3050g (10th Percentile). The neonatal period was normal. She had normal growth (25th Percentile) but Psychomotor Development Retardation (PDR). At 8 months, she had a urinary tract infection and otitis which resolved with oral antibiotic therapy. Retrospectively a severe lymphopenia (600 lymphocytes/mm^3) was already present in the blood workup. There was no history of severe or recurrent infection. She was immunized against BCG, Hepatitis B, poliomyelitis, DTwP and VASPR in accordance with the national vaccination plan and suffered no adverse reactions.

On admission she presented with fluctuating levels of consciousness, normal vital signs, normal pulmonary and cardiac auscultation, normal abdomen with no liver or spleen enlargement, no adenomegalies, severe hypotonia, ataxia, no focal deficits, normal reflexes and absence of meningeal signs.

The initial analysis is shown in Table I and revealed severe lymphopenia, normocytic and normochromic anemia, anisocytosis, thrombocytosis, elevated erythrocytes sedimentation rate (ESR), elevated alpha-fetoprotein, normal reticulocytes, normal serum biochemical ranges and normal Cerebrospinal fluid (CSF). No infectious or toxic etiology was identified (Table I).

Peripheral blood smear examination showed lymphopenia with small size lymphocytes with dense chromatin (Figure 2 – A). Blood marrow smear showed small size and very dense nuclear chromatin lymphocytes, dysmorphic megakaryocytes and platelets in large number (Figure 2 – B). The immunological investigation showed accentuated lymphopenia of all subpopulations, especially T-lymphocytes, with near absence of CD8 T lymphocytes (Table II). There were no abnormal immunophenotyped mononuclear cells and the complement system was normal (Table III). A primary immunodeficiency was suspected. The uric acid measurement was zero, in favor of PNP deficiency (normal range: 2.0–5.5 mg/dL). Samples were sent to the *Purine Research Laboratory* in London and the diagnosis of complete deficiency of Purine Nucleoside Phosphorylase was confirmed - Table IV: absence of PNP in both lysates and intact erythrocytes, erythrocyte ADA was

normal, deoxy GTP/GDP was present in erythrocyte nucleotides, very low GTP, grossly raised Nicotinamide adenine dinucleotide (NAD) and raised Uridine diphosphate glucose (UDPG); plasma uric acid absent and low urinary uric acid; presence of inosine, guanine and dINO in plasma and urine.

A homozygous mutation was identified, in exon 5, c.635T>C+636G>T (p.Leu212Pro). The parents are both carriers of the same mutation (Figure 3).

The child was isolated and received Intravenous Immunoglobulin (IVIG), antibiotic prophylaxis of opportunistic infections, and transfused with irradiated blood products with good clinical response. The patient was proposed for HSCT, but no compatible donor was found.

A progressive encephalopathy with seizures and left hemiparesis emerged. The Magnetic Resonance Imaging (MRI) revealed cerebral and cerebellar atrophy and hyper signals in T2, located in the caudate and lenticular nucleus. She died from sepsis and multi-organ failure at the age of 23 months.

Genetic counseling and prenatal diagnosis were offered to the parents and two unaffected boys were subsequently born. They are now 16 and 20 years old. The younger boy is a carrier of the *PNP* gene mutation and has lower PNP activity, the older boy does not have any mutation. Both brothers are healthy.

CASE REPORT 2

The Second case is a 17-month-old Caucasian boy, the first child of non-consanguineous and healthy parents, hospitalized for fever, prostration, rhinorrhea and bronchospasm. The grandparents are first-degree cousins but there isn't familial history of hereditary diseases. There were no intercurrences during gestation and the birth was a preterm caesarian due to placenta previa. Five minutes Apgar score was 9, birth weight was 2765g (5^{th} Percentile). During the neonatal period, a patent foramen ovale was detected, but closed without intervention. There were no other intercurrences during the neonatal period. The boy experienced normal developmental growth and weight gain. At 9 months, he was diagnosed with PDR due to axial hypotonia and dystonia of the lower limbs, but a normal cerebral MRI was documented at 15 months. He had a history of recurrent wheezing and infections without the need for hospitalization, as was also the case for acute gastroenteritis at 6 months, acute otitis media and perineal candidiasis at 9 months, and perianal fistula at 16 months. He was hospitalized at 12 months for lobar pneumonia (unknown microorganism). By this time, a severe lymphopenia (567 lymphocytes/mm^3) was already present in the blood workup. Vaccinations were administered in accordance with the national vaccination plan, providing immunization against BCG, Hepatitis B, *Haemophilus type B*, poliomyelitis, DTwP, VASPR, *Neisseria meningitidis serotype C* and 1 dose of Pneumococcal Conjugate Vaccine (7-Valent), without any side effects.

On admission, he had a reasonable general appearance, normal vital signs, mucocutaneus paleness, no exanthems or petechia, pulmonary auscultation with rhonchi, normal cardiac auscultation, normal abdomen with no liver or spleen enlargement, no adenomegalies and absence of meningeal signs. Oral and perineal candidiasis and perianal fistula were present.

The initial analysis can be seen in Table I and revealed severe lymphopenia, neutropenia, microcytic anemia, thrombocytosis, elevated reticulocytes, elevated transaminases and lactic dehydrogenase, elevated C-reactive protein (CRP), positive urine culture for *Pseudomonas Aeruginosa* and normal CSF. No toxic etiology was identified (Table I).

The immunological investigation showed absence of T-lymphocytes, B- and NK-cell lymphopenia, hypogammaglobulinemia, normal complement and no auto antibodies or abnormal immunophenotyped mononuclear cells (Table III). A

primary immunodeficiency was suspected. The uric acid measurement was less than 0.2mg/dL, which supports PNP deficiency.

Samples were sent to the *Purine Research Laboratory* in London and a PNP deficiency was confirmed by lack of enzymatic activity.

The genetic analysis identified a compound heterozygosity. The mutations identified are c.171C>T (p.Pro57=), in exon 2, and c.569G>T (p.Gly190Val), in exon 5. The molecular study of the parents confirmed that they are both carriers of different PNP mutations.

The boy was isolated, received IVIG, antibiotic prophylaxis of opportunistic infections, and was transfused three times with irradiated blood products with poor clinical response. Further investigation showed mild proteinuria, new ascites and hepatosplenomegaly, pleural bilateral effusion (exudate) and pericardial effusion.

Although a compatible donor was found for HSCT, the donation was never actualized due to the progressive clinical deterioration which lead to death from sepsis and multi-organ failure at the age of 18 months, one month after hospitalization.

Genetic counseling and prenatal diagnosis were offered to the parents, resulting in the subsequent birth of a baby girl. She is now a healthy 7-year-old girl, despite being a carrier of the *PNP* gene mutation.

DISCUSSION

Characteristically, a PNP deficiency presents with severe T cell defects, and therefore renders the patient susceptible to various life-threatening infections, often from opportunistic pathogens such as *Pneumocystis jiroveci* and *Candida albicans*.[12] The effects on B cells are variable.[7] In case 1, no suspicious infections were reported, but both T and B cell counts were low at presentation. In case 2, the T-cells were absent, and B and NK-cell were low, and there was a history of recurrent infections including a perineal candidiasis and a lobar pneumonia with unknown microorganism, which should have suggested the presence of an immunodeficiency.

Both cases had a fatal outcome because of the late diagnosis, despite previous recordings of severe lymphopenia and developmental delay. Plasma uric acid concentration should be screened for in all children presenting with lymphopenia, and diagnosis of PNP deficiency is suggested when hypouricemia is present. [4, 5, 12] Low levels of plasma uric acid can be found even before the syndrome is fully expressed.[12] Despite the low plasma uric acid levels which are commonly reported with a PNP deficiency , there have been reported cases of PNP deficient patients with normal plasma uric acid concentrations[3]. This suggests that plasma uric acid concentration is not a reliable diagnostic marker.[4] Therefore, in a patient with lymphopenia or neurological manifestation, PNP deficiency should not be excluded from the differential diagnosis in the absence of hypouricemia.[3, 4, 10]

As is the case in more than half of children with a PNP deficiency[1, 11], both children in case studies 1 and 2 showed neurological manifestations of a PNP deficiency in the form of developmental delay, ataxia, spasticity and behavioral changes. They had normal MRI and EEG readings at presentation, but in case 1 a later MRI showed cerebral and cerebellar atrophy. Patients with neurological manifestations and recurrent infections should be systematically evaluated for immunodeficiency.[12] The hypothesis for the etiology of neurologic manifestations in PNP deficiency patients is currently being discussed. These manifestations could be attributed to infection and/or vasculitis, but patients without such prior complications can also show neurologic impairment[10], and some patients have neurologic manifestations before thymic dysfunction[11]. Another hypothesis is the progressive cerebellar atrophy and decreased Purkinje cell mass attributed to toxic purine metabolites, demonstrated in studies of PNP knockout mice[11].

Early research proposed that the *PNP* gene was located at 14q13.1. However, evidence accumulated after 1988 suggests that the PNP locus is positioned centromeric to *TRAC* locus, located at 14q11.2. The *Human Gene Mutation Database (HGMD)* has also concluded that the correct location of the PNP gene is at the14q11.2 locus.[5, 6] Most of the literature reviews, research and reported cases erroneously indicate the PNP locus at 14q13.1[3, 7, 14-17], but the scientific community should be alerted and updated about the confirmed location of the *PNP* gene.

The mutations in our patients lead to a complete absence of the PNP activity and caused both neurological defects and SCID, but no autoimmunity. The homozygous mutation c.635T>C+636G>T (p.Leu212Pro), in exon 5, is a missense mutation that results in the alteration of PNP proteins due to amino acid modification. The compound heterozygosity is formed by a nonsense mutation at position 57 [c.171C>T (p.Pro57=)], in exon 2, and a missense mutation at protein position 190 [c.569G>T (p.Gly190Val)], in exon 5, The mutation found in case 1 is not described in the literature (Table V) or on the *Online Mendelian Inheritance in Man (OMIM)* platform. Also, both parents are carriers of the same mutation despite the negative familial history for consanguinity and lack of previously affected relatives, which is consistent with a pure recessive disease which has remained in silence for many generations. In relation to case 2, the mutation found in exon 2 does not appear associated to any reported cases and is described as benign or likely benign at the *OMIM* platform[18]. The one found in exon 5 has been described twice in the literature as causing partial PNP deficiency,[4] but it is not registered on the OMIM platform. Case 2 is a compound heterozygote: the severity of the disease caused by both mutations depends on their association and determines the absence of PNP activity.

Studies in mice have demonstrated that early treatment with PNP replacement can prevent cerebellar damage, which shows the importance of the early detection and commencement of treatment to reverse the neurological damage.[11] Infants with SCIDs who are receiving HSCT in the first few months after being diagnosed through newborn screening have a higher chance of survival when going to transplant, compared to those identified based on clinical symptoms.[19]

PCR for T-cell receptor excision circle (TREC) and kappa-deleting recombination excision circles (KREC) on dried blood spots, representing newly produced naive T- and B-cells, respectively, are being used during newborn screening to identify immunodeficiency.[20] Low TREC markers provide the earliest possible identification of patients with severe T-cell lymphopenia.[19] KREC is a marker of bone marrow B-

cell used to identify hypogammaglobulinemia. Low KREC levels are associated with the absence of CD19+ cells and lack of immunoglobulin production by the patient.[20] Nevertheless, it is important to remember that TREC and KREC quantitative analysis, when used alone, might fail in identification of some SCID cases.[8] There is the possibility of normal TREC and KREC levels in PNP deficient children if there is a late onset of the immunodeficiency.[7]

There are other methods of screening, such as tandem mass spectrometry (TMS) [5, 8]. Using ADA as a comparison, which has a similar pathogenesis to PNP deficiency, TMS of dried blood spots can easily identify abnormal metabolites, resulting in a highly specific and sensitive method of diagnosis. It is also low cost when included during the routine newborn screening.[8] Studies suggest that metabolite levels tend to remain stable throughout the lifespan and strongly correlate with both genetic variant and enzymatic activity.[8] TMS, but not quantification of TRECs, can identify newborns with delayed onset of the T- and B- cell immunodeficiency.[21]

It has been proposed that SCIDs should be included in newborn screenings, given their low mortality and morbidity rates, the fact that there are curative therapies available and these are more effective the sooner they are implemented, and that specific disease markers are available to identify such disorders. In fact, several countries already include SCIDs in the newborn screening routine.[8]

As seen before, treatment is mandatory and no patient with PNP deficiency has lived longer than twenty years without HSCT.[7] In spite of its associated morbidity and mortality, HSCT is the ideal therapeutic modality where there is central nervous system (CNS) involvement because of an inherited deficiency. HSCT with reduced intensity conditioning regimens are preferred because of lower toxicity in these patients compared to myeloablative regimens.[15]

After transplantation, the blood marrow-derived stem cells can differentiate into blood monocytes which can migrate across the blood brain barrier and have the potential to differentiate into microglial cells, leading to neurological improvement.[13] There is poor evidence that the transplant can reverse the neurological sequelae in PNP deficiency. Some cases have shown that the improvement in neurological status was associated with clearance of *Cytomegalovirus* (CMV) and not directly to the recovery of normal immunity.[2] On the other hand, there are cases of a second transplant where the donor cells act as an enzyme delivery system, allowing reconstitution of host T cells by detoxification.[13]

In ADA deficiency, enzyme replacement therapy (ERT) with PEG-ADA has been considered a practicable therapeutic option but specific enzyme replacement is not available for PNP deficit or absence. This therapy enables immune function by metabolic detoxification. Like HSCT, ERT has its own limitations. The risk of an allogeneic antibody response to pharmacological enzymes by the host immune system is higher in individuals who produce no protein then in individuals who make nonfunctioning enzymes. As well as that, the blood brain barrier limits enzyme delivery to the CNS, contrary to HSCT. [13]

COMMENTS

PNP deficiency is an autosomal recessive and very rare cause of SCID that leads to early death without appropriate management. PNP is an enzyme of the purine metabolism and the partial or total absence is characteristically associated with lymphopenia and heterogenous neurologic involvement. PNP deficiency should be investigated in any child with developmental delay or a neurologic disorder and severe lymphopenia. Uric acid concentration may be a useful indicator for PNP deficiency diagnosis.

TMS measurements for PNP metabolites should be used during routine newborn screening programs, giving the opportunity to confirm PNP deficiency at an early age.

Be aware of these defects is important for the provision of appropriate treatment and genetic counseling. Correct and timely diagnosis avoid the necessity for more costly investigations and genetic counseling, and prenatal diagnosis can be offered to the relatives.

APPENDIX

TABLES

Table I Initial laboratory and imaging investigations of the patients

	Case 1	*Case 2*	*Normal Range*
Hemoglobin (g/d)	7.7	8.9	10.5 – 13.5
Hematocrit (%)	22.1	26.6	33 – 39
MCV (Fl)	77.9	75.4	70 – 86
RDW (%)	25.3		
Leucocyte count (mm^3)	6340	1870	6000 – 17000
Absolut lymphocyte count (mm^3)	300	330	3000 - 9500
Platelets ($x10^3$/mm3)	832	238	200 - 500
Reticulocytes (%)	3	6.28	0.5 – 2.5
CRP (mg/L)	<5	220	0–10
Serum biochemistry	Uric Acid absent	Low Uric Acid	
Alpha fetoprotein (ng/ml)	4,3		<2
CSF	Normal	Normal	
Serologies (HIV1 and 2, measles, Herpes, EBV and CMV)	Negative	Negative	
Metabolic study	↑ pyruvate in blood; Normal urine and CSF	↑ pyruvate in blood; Normal urine and CSF	
Thoracic X-ray	Normal thymus.	Normal thymus.	
Cerebral MRI	No alterations	No alterations	
EEG	Slow waves	Not realized	
Electromyography	Negative for Myasthenia gravis	Normal	

Table II White cells count and mononuclear cells immunophenotyping

	Case 1	Case 2	Normal Range
	WHITE CELLS COUNT		
Leucocytes (/mm³)	6340	1870	6000-17000
Lymphocytes (%)	3.5	17.6	20-45
Neutrophils (%)	83	49.9	40-75
Monocytes (%)	6.5	4.6	2-10
Eosinophils (%)	4.0	14.4	1-6
Basophils (%)	0.5	0.0	0-1
Myelocytes (%)	0.5	7.5	
Metamyelocytes (%)	0.5	6.0	
Lymphocytes variants (%)	1.5	0.0	
	IMMUNOPHENOTYPE		
B-cells			
▪ *CD19 (%)*	44	56	20-30
▪ *CD19 (/mm³)*	154	353	1160-1887
▪ *CD20 (%)*	44	31	
▪ *CD20 (/mm³)*		154	
▪ *CD5 in CD19 (%)*	94	37	
T-cells			
▪ *CD3 (%)*	22	0.0	61-73
▪ *CD3 (/mm³)*	77	0.0	3078-5070
▪ *CD5 (%)*	25	1.5	
▪ *CD5 (/mm³)*	87	9.0	
▪ *CD4 (%)*	16	0.0	41-49
▪ *CD4 (/mm³)*	52	0.0	2105-3457
▪ *CD8 (%)*	2	0.0	15-21
▪ *CD8 (%)*	8	0.0	773-1345
NK-cells			
▪ *CD56 (%)*	31	36.1	5-10
▪ *CD56 (/mm³)*	77	227	236-684

Table III Immunological investigation

	Case 1	Case 2	Normal Range
Complement System (C3, C4)	Normal	Normal	
Autoantibodies	All negative		
▪ *ANA*		Negative	<1/40
▪ *AMA*		Negative	<1/40
▪ *STR*		1/40	<1/40
▪ *TPO (IU/mL)*		<20	0-40
▪ *TG (IU/mL)*		<10	0-35
Immunoglobulins (mg/dL)			
▪ *IgG*	762	332.0	429-1233
▪ *IgA*	68	48.6	11-117
▪ *IgM*	42	26.8	25-182
▪ *IgE*	25	92	2-153
IgG Subclasses (mg/dL)			
▪ G_1	659	230	
▪ G_2	128	168	
▪ G_3	17	12,8	
▪ G_4	12	13,7	
Specific IgG (mg/dL)			
▪ *IgG anti-Tetanus toxoid*	4.23	0.36	>0.79
▪ IgG_1 *anti-Tetanus toxoid*	1.97	0,26	>0.50
▪ *IgG Anti-PCP*	0.66	2,76	>1.54
▪ IgG_2 *Anti-PCP*	<0.11	0,69	>0.54

Table IV Case 1: Purine/Pyrimidine Metabolic Study

URINE

Creatinine 5.0 mmol/L

Endogenous Purines (mmol/L)

- *Total 0.09*
- *Uric acid 0.04*
- *Hypoxanthine 0.044*
- *Xanthine 0.007*
- *Ratio Uric acid/Creatinine 0.02 (↓↓)*

Other endogenous compounds (mmol/L)

- *Inosine 14.92*
- *Guanosine 7.50*
- *Deoxy inosine 7.51*
- *Deoxy guanosine 6.87*
- *Total nucleosides 36.8*
- *Pseudouridine 0.73*
- *Uracil 0.33*
- *Ratio oxypurine/Creatinine 7.4 (↑↑)*

PLASMA

Endogenous Purines (µmol/L)

- *Uric acid <1*
- *Hypoxanthine 2*
- *Xanthine <1*

Other endogenous compounds (µmol/L)

- *Inosine 39*
- *Guanosine 12*
- *Deoxy inosine 4*
- *Deoxy guanosine 1*

ERYTHROCYTE

Nucleotides (µmol/L)

	ATP	ADP	AMP	GTP	GDP	GMP	IMP	NAD	NADP	UDPG
	1316	94	6	11	3	-	-	185	46	52
RN	1570±97	137±35	13±4	66±9	17±3	-	-	69±15	54±12	36±8

Increased levels of components not normally present:

dGTP 5 µmol/L and dGDP 1µmol/L

Enzymes (nmol/mgHb/h)

ADA 104 (NR 40-100)

PNP not detected (NR 3000-7000)

Table V Reported Mutations associated with PNP deficiency

Location	Nucleotide change	Amino acid	No. of cases	Reference
Exon 2	59A>C	p.His20Pro	1	(2)
Exon 2	70C>T	p.Arg24X	1	(4)
Exon2	151A>G	Ser51Gly	1	(22)
Exon 2	171C>T	p.Pro57=	1	Case 2
Exon 2	172C>T	p.Arg57X	3	(4)
Exon 2	181G>T	p.Tyr5AlafsX28	1	(4)
Exon 3	199C>T	p.Arg67X	1	(4, 12)
Exon 3	212G>A	p.Gly71Glu	1	(4)
Exon 3	257A>G	p.His86Arg	2	(4)
Exon 3	265G>A	p.Glu89Lys	3	(4)
Intron 3	285+1G>A	p.Val61GlyfsX30	1	(4)
Intron 3	285G>A	p.IVS3+10G	1	(15)
Intron 3	286-18_286-17ins16	-	1	(4)
Exon 4	349G>A	p.Ala*117*Thr	4	(4, 15)
Exon 4	383A>G	p.Asp128Gly	6	(4, 22)
Exon 4	385_387delATC	p.Ile129del	1	(4)
Exon 4	437C>T	p.Pro146Leu	1	(4, 22)
Exon 5	467G>C	p.Gly156Ala	1	(4)
Exon 5	475T>G	p.Phe159Val	1	(4)
Exon 5	487T>C	p.Ser*163*Pro	1	(4)
Exon 5	520G>C	p.Ala174Pro	1	(4)
Exon 5	569G>T	p.Gly190Val	3	(4); Case 2
Exon 5	575A>G	p.Tyr192Cys	1	(4)
Exon 5	635T>C+636G>T	p.Leu212Pro	1	Case 1
Exon 6	700C>G	p.Arg234X	1	(4)
Exon 6	701G>C	p.Arg234Pro	6	(4, 22)
Exon 6	729C>G	p.Asn243Lys	1	(7)
Exon 6	730delA	p.Lys244ArgfsX17	1	(4)
Exon 6	746A>C	p.Tyr249Cys	1	(7)
Exon 6	769C>G	p.His257Asp	1	(4)
Exon 6	770A>G	p.His*257*Arg	1	(4)

FIGURES

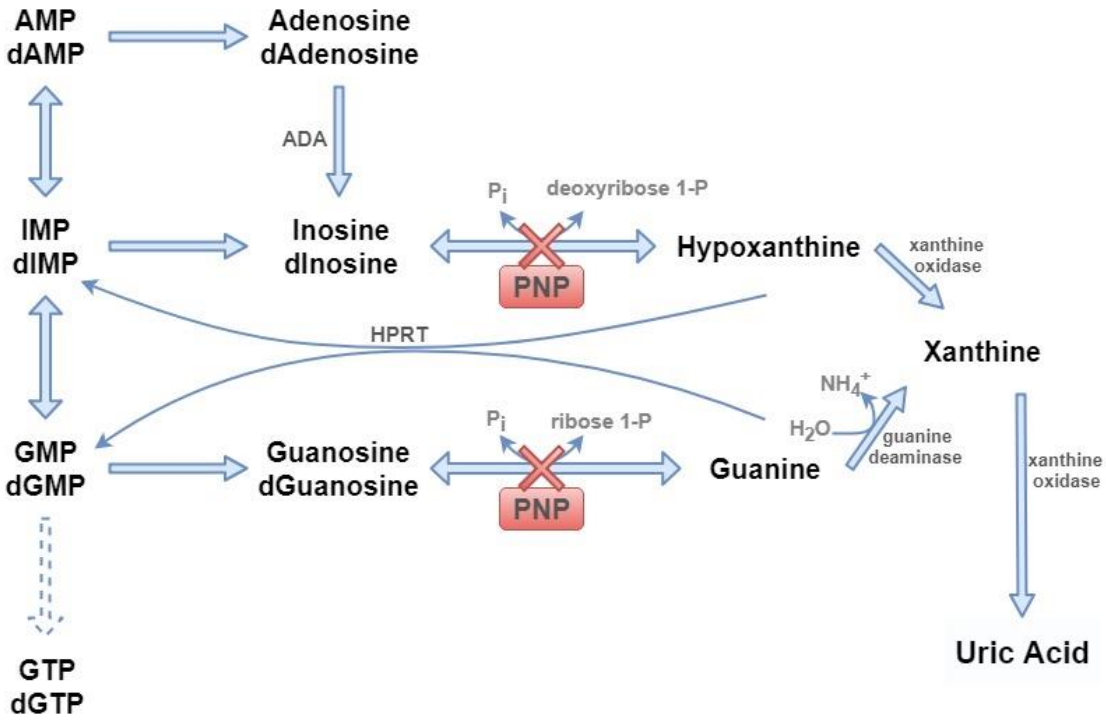

Figure 1 Degradation and salvage pathways of purine nucleosides [Adapted (7)]

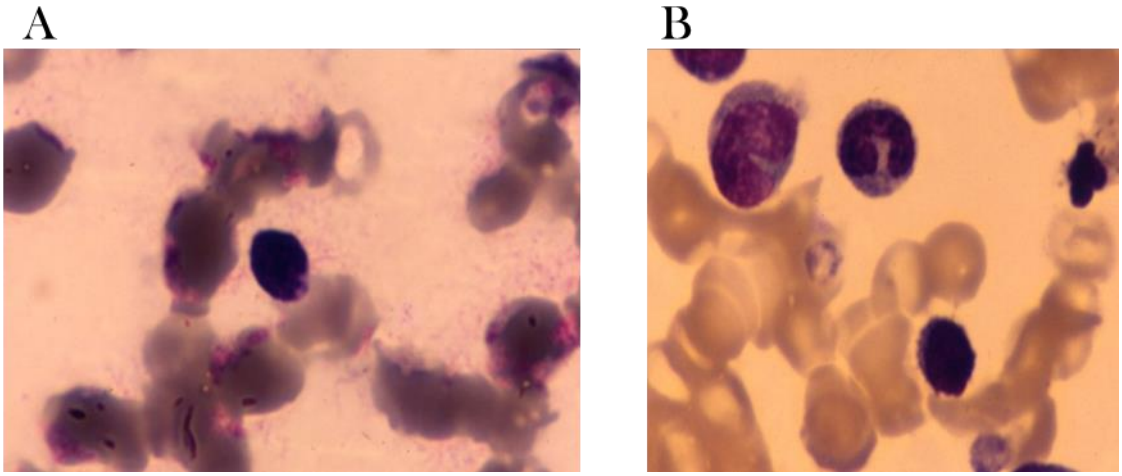

Figure 2 Case 1: Peripheric Blood Smear (A) and Blood Marrow Smear (B)

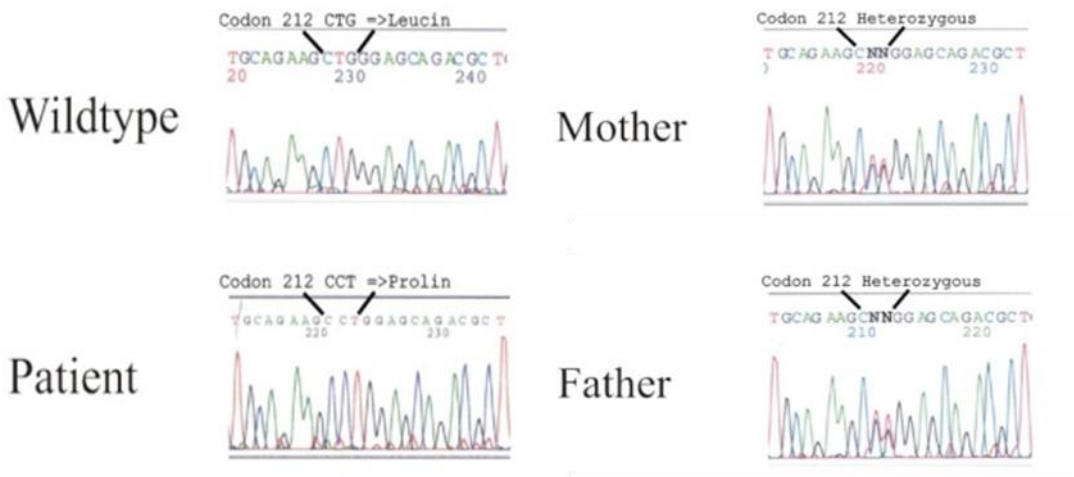

Figure 3 Case 1: Sequencing of the PNP gene coding, exon 5.

CONFLICTS OF INTEREST

The authors alone are responsible for the content and writing of the article and declare that they have no conflict of interest.

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Purine Nucleoside Phosphorylase Deficiency: two case reports from Portugal

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