Hereditary pyrimidine 5′-nucleotidase deficiency: from genetics to clinical manifestations

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Summary

Hereditary pyrimidine 5′-nucleotidase (P5′N) deficiency is the most frequent abnormality of the red cell nucleotide metabolism causing hereditary non-spherocytic haemolytic anaemia. The disorder is usually characterised by mild-to-moderate haemolytic anaemia associated with the accumulation of high concentrations of pyrimidine nucleotides within the erythrocyte. The precise mechanisms leading to the destruction of P5′N deficient red cells are still unclear. The pyrimidine 5′-nucleotidase type-I (P5′N-1) gene is localised on 7p15-p14 and the cDNA has been cloned and sequenced; 20 mutations have been identified so far in 30 unrelated families, most of them at the homozygous level. Recently, the comparison of recombinant mutants of human P5′N-1 with the wild-type enzyme has enabled the effects of amino acid replacements on the enzyme molecular properties to be determined and help to correlate genotype to clinical phenotype.

Keywords: pyrimidine 5′-nucleotidase, mutations, erythroenzymopathy, hereditary haemolytic anaemia.

Inherited P5′N-1 deficiency, transmitted as an autosomal recessive trait, is the most frequent enzyme abnormality of the red cell nucleotide metabolism, and is thought to be the most common cause of chronic non-spherocytic haemolytic anaemia after glucose 6-phosphate dehydrogenase (G6PD) and pyruvate kinase (PK) deficiency (Vives Corrons, 2000). Since the first description (Valentine et al., 1974), more than 60 patients with P5′N-1 deficiency have been reported worldwide (Vives Corrons, 2000; Marinaki et al., 2001; Balta et al., 2003; Bianchi et al., 2003; Rees et al., 2003; Kanno et al., 2004; Manco et al., in press), with presumably large numbers undetected (Marinaki et al., 2001). Clinical symptoms occur only in homozygous and compound heterozygous patients. The prevalence of P5′N-1 deficiency is not known.

Structure and function of P5′N-1

The 5′-nucleotidases (5′-NT, nucleoside 5′-monophosphate phosphohydrolase, EC 3.1.3.5) are catabolic enzymes that catalyse the dephosphorylation of non-cyclic nucleoside 5′-monophosphates to their respective nucleosides and inorganic phosphate, thus regulating cellular nucleotide and nucleoside levels. To date, at least seven different 5′-NT with distinctive substrate specificity, distribution, cellular localisation, regulation and amino acid sequences have been identified in mammals. One of them is a membrane-bound form, located on the cell surface via a glycosylphosphatidylinositol anchor (Misumi et al., 1990; Zimmermann, 1992), whereas the other six are intracellular. Among intracellular 5′-NT, five are cytosolic (Rampazzo et al., 2000a; Hunsucker et al., 2001; Sala-Newby & Newby, 2001; Amici et al., 2002; Bretonnet et al., 2005) and one occurs in mitochondria (Rampazzo et al., 2000b). Moreover, some 5′-NT are ubiquitous; others are expressed in a tissue-specific manner (Bianchi & Spychala, 2003; Hunsucker et al., 2005). Most 5′-NT exhibit quite broad substrate specificity and require magnesium for activity (Hunsucker et al., 2005). Furthermore, some 5′-NT display the activity of transferring phosphate groups from nucleotide donors to nucleoside acceptors (Amici et al., 1997; Bretonnet et al., 2005). Analysis of the 5′-NT amino acid sequence demonstrated that all intracellular 5′-NT are characterised by the presence of a DXDX(T/V) motif, a distinctive signature of a number of other phosphatases/phosphotransferases (Collet et al., 1998; Rinaldo-Matthis et al., 2002). This conserved motif is thought to be the enzyme catalytic site in which the first aspartate residue is transiently phosphorylated upon catalysis (Collet et al., 1998; Allegrini et al., 2001; Amici et al., 2005; Bretonnet et al., 2005). All 5′-NT characterised so far, except P5′N-1, exhibit oligomeric structure with identical subunits of about 20–60 kDa. A summary of the advances on the structure, cellular function, and specific properties of individual 5′-NT has been recently reported (Bianchi & Spychala, 2003; Hunsucker et al., 2005).
Two main types of 5′-NT, both active towards pyrimidine nucleotides, have been isolated from red blood cells (Paglia et al., 1984; Hirono et al., 1985, 1987; Amici et al., 1997) (Fig 1). These two enzymes, often considered in the older literature as a single entity, are coded by two distinct genes (Wilson et al., 1986; Marinaki et al., 2001) and display different specificity with respect to the sugar present in pyrimidine nucleotides. P5′N-1 is mainly active towards pyrimidine 5′-ribonucleotides (Amici et al., 1997) whereas P5′N type-2 (P5′N-2), also known as 5′(3′)-deoxyribonucleotidase, is not strictly pyrimidine-specific and is more efficient with deoxyribonucleotides (Rampazzo et al., 2000a).

Both enzymes are ubiquitous, although P5′N-1 is often thought an erythrocyte-specific enzyme, being mainly studied in erythrocytes (Hunsucker et al., 2005).

To date, the haemolytic anaemia caused by P5′N deficiency has been related only to a defective P5′N-1 activity in red blood cells.

The major role of P5′N-1 is found in the catabolism of the pyrimidine nucleotides, mainly resulting from ribosomal RNA degradation during final erythroid maturation (Paglia & Valentine, 1975; Mass et al., 2003; Rees et al., 2003). The pyrimidine nucleotides are not used by mature erythrocytes and need to be transported out of the cells to prevent their accumulation. Differently from pyrimidine nucleotides, the nucleosides can diffuse out of the cells. This fact indicates that P5′N-1 depletes red cells from the excess of nucleotides through a dephosphorylation process (Paglia & Valentine, 1975; Rees et al., 2003). Pyrimidine 5′-nucleotidase type-I activity is much higher in reticulocytes than in old red cells, because of the unique decay pattern during the erythrocyte life span. In fact, contrary to what is observed in the other age-dependent red cells enzymes, the rapid loss of P5′N-1 activity during the first few days of maturation is followed by a further continuous decline throughout the ageing of the cell, as demonstrated by Beutler and Hartman (1985) in transient erythroblastopenia of childhood.

Pyrimidine 5′-nucleotidase type-I, also called cytosolic 5′-nucleotidase III (cNIII) (Bianchi & Spychala, 2003) or uridine monophosphate hydrolase type-1 (UMPH-1) (Swallow et al., 1983), has been recently purified to homogeneity as recombinant form. A thorough characterisation of the biochemical properties of the enzyme is now available (Amici et al., 2005; Chiarelli et al., 2005). It is a protein containing 286 amino acids (TrEMBL accession number Q9P0P5) with no disulphide bridges and no phosphate groups (Amici et al., 1997, 2002). P5′N-1 preferentially catalyses the dephosphorylation of uridine monophosphate (UMP) (k_cat and K_m,1 ≈ 98/s, 0.056 mmol/l respectively) and cytidine monophosphate (CMP) (k_cat and K_m,2 ≈ 14/s and 0.18 mmol/l respectively) and is totally inactive towards purine nucleotides, regardless of the position of the phosphate moiety (Valentine et al., 1974). Interestingly, P5′N-1 also displays phosphotransferase activity.
specific for pyrimidine nucleosides, suggesting an additional role in nucleotide metabolism (Amici et al., 1997). This activity, also present in cytosolic 5’-nucleotidase II (Bretonnet et al., 2003), but absent in P5’N-2 (Rampazzo et al., 2000a), is of clinical interest as pyrimidine nucleoside analogues, such as 3’-azido-2’-deoxthymidine and cytosine arabinoside, are widely used in chemotherapy.

The enzyme is absolutely dependent on magnesium ions (Mg2+) for activity; thus metal chelating reagents, like EDTA, widely used in chemotherapy. Mg2+ cannot be replaced by Mn2+. The enzyme activity is also readily inhibited by heavy metal ions, mostly Pb2+, Hg2+, Ca2+, Ni2+ and Cd2+, and by thiol-reactive reagents, such as N-ethylmaleimide, p-chloromercuribenzoate and 5,5’-dithiobis (2-nitrobenzoic acid) (Valentine et al., 1974; Paglia & Valentine, 1975; Torrance et al., 1977a; Amici et al., 2002). In humans, severe lead poisoning results in P5’N-1 deficiency, which closely resembles the syndrome associated with hereditary deficiency state (Vives Corrons, 2000; Rees et al., 2003).

Pyrimidine 5’-nucleotidase type-I displays optimal activity at pH 7.5–8.0, and is most stable between pH 6 and 7.5 (Valentine et al., 1974; Paglia & Valentine, 1975; Torrance et al., 1977a; Amici et al., 2002). Differently from what is previously reported, P5’N-1 is a rather stable enzyme, retaining full activity after 1 h of incubation at 46°C, as well as over a month storage at 4°C. Nevertheless, it is worth noting that the enzyme becomes less stable when the temperature is lowered to −20°C (activity reduced to 60%) (L.R. Chiarelli, unpublished observations). Unfortunately, the crystal structure of P5’N-1 is not yet available, but far-UV circular dichroism analysis shed light on the secondary structure elements, showing that the enzyme consists of approximately 37% alpha-helices and 26% extended beta-strands (Chiarelli et al., 2005). Unlike other 5’NT, P5’N-1 exhibits monomeric structure, with an apparent molecular mass of 34 kDa.

### Genetic characteristics of pyrimidine P5’N deficiency

The gene encoding P5’N-1 (NT5C3, UMPH-1) is located on chromosome 7p15-p14 (Marinaki et al., 2001) and spans approximately 50 kb. The coding region is split into 11 exons (1, 2, R, 3–10) leading to the production of three mRNA forms by alternative splicing of exons 2 and R (Marinaki et al., 2001; Kanno et al., 2004).

The transcript lacking both exons 2 and R is expressed ubiquitously (Lu et al., 2000), whereas transcripts including exon 2 alone or both exons 2 and R are almost specifically expressed in reticulocytes (Kanno et al., 2004). The biological significance of these various mRNAs found in reticulocytes is at present unknown; among the three expected proteins (285, 286 and 297 amino acids long) only the protein consisting of 286 amino acid residues, identical to the previously identified lupus inclusion protein p36 (Amici et al., 1994, 2000), has been isolated from erythrocytes. It is produced using the initiation codon at the 3’-end of exon 1 and sequentially lengthened from exon 3, both exons 2 and R being spliced out (Marinaki et al., 2001; Kanno et al., 2004).

It should be noted that the amino acid numbering adopted in this review refers to the spliced form of gene for P5’N-1 consisting of 286 residues (Swiss-Prot accession no. Q9H0P0-2; the numbering of nucleotides is referred to cDNA reported in EMBL (coding sequence): accession no. AAF36153).

To date, 20 different mutations in the P5’N-1 gene have been identified in 37 patients from 30 families affected by P5’N deficiency (Table I). Six of them are missense mutations (c.260A → T, c.392T → C, c.469G → C, c.536A → G, c.688G → C and c.740T → C) and do not affect the protein length; two lead to in-frame amino acid deletion (c.159–167del and c.427–429del), the remaining 12 consist of nonsense mutations, deletions, insertions or alterations of the splicing sites, and are expected to generate truncated or aberrant forms of the polypeptide. All the patients but two are homozygous.

Some mutations have been found to be distributed on a regional basis; in particular, c.543delG was found in the homozygous state in three patients originating from a well-defined geographical area of southern Italy (Bianchi et al., 2003), and missense mutation c.688G → C in five families apparently unrelated but all originating from Kyushu island (Kanno et al., 2004). Mutation c.710-711insGG has been reported both in Italy and Turkey (Balta et al., 2003; Bianchi et al., 2003); moreover, in the same nucleotidic position, the insertion of an Alu repetitive element of 330 bp has been described in two unrelated Portuguese patients (Manco et al., in press). It can be assumed that mutation c.710-711insGG originated from the subsequent excision of the Alu sequence.

A codon 81 TAC-TAT polymorphism was found to be linked to a P5’N-1 mutation in a Norwegian family, and a population survey showed that the TAT allele had a frequency of 0.29 in the UK (Marinaki et al., 2001).

### Pathophysiology

The key metabolic abnormality in P5’N-1 deficiency is the accumulation of pyrimidine nucleotides that precipitate, resulting in massive red cell basophilic stippling. However, the precise mechanism(s) leading to the red cells destruction are still unclear (Vives Corrons, 2000; Rees et al., 2003).

It is known that the accumulation of pyrimidine nucleotides has many effects on red cell metabolism (Fig 2). First, it enhances the activity of pyrimidine nucleoside monophosphate kinase, which accounts for the disproportionate increase of the nucleoside triphosphates cytidine triphosphate (CTP) and uridine triphosphate compared with CMP observed in P5’N deficient red cells (Lachant et al., 1987). Moreover, the accumulation of CTP results in a marked increase of cytidine diphosphate (CDP)-choline and CDP-ethanolamine, which are important constituents of the red cell membrane. The abnormal membrane phospholipid composition has been suggested as a possible cause of haemolysis (Swanson et al,
1984), although red cell osmotic fragility is usually not increased in P5'N-deficient patients (Vives Corrons, 2000), with few exceptions (Valentine et al, 1973; Bianchi et al, 2003).

Pyrimidine nucleotides are also known to be strong chelators of Mg$^{2+}$ and may therefore affect the activity of enzymes requiring Mg$^{2+}$ as a co-factor or Mg-ATP as a substrate. In particular, magnesium sequestration by pyrimidine nucleotides is, at least in part, responsible for the reduced activity of phosphoribosyl-pyrophosphate (PRPP) synthetase found in P5'N-deficient erythrocytes (Lachant et al, 1989), which may in turn result in an impairment of the PRPP-

<table>
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<tr>
<th>cDNA nucleotide substitution (Marinaki et al, 2001)</th>
<th>cDNA nucleotide substitution (EMBL AAF36153)</th>
<th>Effect</th>
<th>Exon(s)</th>
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<tr>
<td>c.293A → T</td>
<td>c.260A → T</td>
<td>Asp 87Val</td>
<td>6</td>
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<td>Marinaki et al (2001)</td>
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<tr>
<td>c.429-430del AG</td>
<td>c.396-397del AG</td>
<td>Splice site</td>
<td>8</td>
<td>Italy</td>
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</tr>
<tr>
<td>c.460-462del CAA</td>
<td>c.427-429del CAA</td>
<td>Gln143 del</td>
<td>8</td>
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<td>Unpublished observation</td>
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<td>c.502G → C</td>
<td>c.469G → C</td>
<td>Gly157Arg</td>
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<td>Manco et al (in press)</td>
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<td>c.529C → T</td>
<td>c.496C → T</td>
<td>Gln166 STOP</td>
<td>8</td>
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<td>Marinaki et al (2001)</td>
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<tr>
<td>c.543T → G</td>
<td>c.510 T → G</td>
<td>Tyr170 STOP</td>
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<td>Balta et al (2003)</td>
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<td>c.743-744ins 330bp</td>
<td>c.710-711ins 330bp</td>
<td>ex 9 del</td>
<td>9</td>
<td>Portugal</td>
<td>Manco et al (in press)</td>
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<td>c.773T → C</td>
<td>c.740T → C</td>
<td>Ile247Thr</td>
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<td>Manco et al (in press)</td>
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<td>IVS10+1G → T</td>
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<td>Splice site</td>
<td>IVS10</td>
<td>South Africa</td>
<td>Marinaki et al (2001)</td>
</tr>
</tbody>
</table>

Nucleotide substitutions are numbered according to Marinaki et al (2001) and EMBL accession number AAF36153.

Fig 2. Red cell metabolic abnormalities consequent to pyrimidine 5'-nucleotidase deficiency. CTP, cytidine triphosphate; CDP, cytidine diphosphate; CMP, cytidine monophosphate; UTP, uridine triphosphate; UMP, uridine monophosphate; PN MK, pyrimidine nucleoside monophosphate kinase; NDK, nucleoside diphosphate kinase; PRPP-s, phosphoribosyl-pyrophosphate synthetase; PK, pyruvate kinase; HK, hexokinase; PFK, phosphofructokinase; GSSG, oxidised glutathione; GSH, reduced glutathione; HMS, hexose monophosphate shunt.

Table I. Mutations reported in pyrimidine 5'-nucleotidase type 1 gene associated with chronic haemolytic anaemia.
dependent adenine salvage pathway (Zerez et al, 1986). CTP has also been shown to inhibit in vitro other Mg-dependent enzymes, such as PK and hexokinase (Lachant & Tanaka, 1986), although the activity of these enzymes appears to be normal in P5’N-deficient red cells (Beutler et al, 1980; Dvilansky et al, 1984; David et al, 1991). It has also been suggested that the accumulation of pyrimidine nucleotides may interfere with the glycolytic flux by competing with ATP/ADP-binding sites of the glycolytic enzymes, such as phosphofructokinase and PK (Oda et al, 1984), but no conclusive evidence for a metabolic block was found.

An impaired pentose phosphate shunt activity has also been demonstrated in some patients and related to a decreased intraerythrocytic pH due to the accumulated nucleotides. In particular, G6PD activity is lower in P5’N-deficient red cells (Tomoda et al, 1982; David et al, 1991), especially in young erythrocytes (Tomoda et al, 1982; David et al, 1991). Whether the impairment of the pentose phosphate pathway may contribute to the pathogenesis of haemolysis of P5’N deficiency is not clear. Rees et al (1996) hypothesised that the inhibition of the pentose-phosphate shunt activity in young erythrocytes contributed to the severity of haemolysis in a case of combined HbE and P5’N deficiency.

Finally, increased levels of reduced glutathione have been observed in P5’N deficiency (Valentine et al, 1972; Miwa et al, 1977) and related to the inhibition of outward transport of oxidised glutathione by pyrimidine nucleotides (Kondo et al, 1980, 1987).

Clinical, haematological and diagnostic aspects of P5’N deficiency

Clinical features

Figure 3 summarises the main clinical features of P5’N deficiency, as assessed by the review of the clinical data of 64 patients from 54 families of various origin reported in the literature. The median age at the time of diagnosis was 15 years (range, 3 months to 64 years).

The clinical manifestations comprise the usual hallmarks of lifelong chronic haemolysis. The anaemia was usually mild to moderate, or even fully compensated in rare cases (Kanno et al, 2004). Severe anaemia occurred in 12% of patients. The degree of anaemia is relatively constant during the patient’s life, although occasional exacerbation may occur during acute infections and pregnancy and may require blood transfusions (Vives Corrons et al, 1976; Rechavi et al, 1989). Pregnancy is usually well tolerated and associated with favourable perinatal outcome, although transfusions may be required in some cases (Valentine et al, 1972; Paglia et al, 1980; Ghosh et al, 1991).

Jaundice and splenomegaly are common in P5’N deficiency. Half of the patients were splenectomised, most of them before diagnosis, and one-third developed cholelithiasis.

Overall, 17 patients needed blood transfusions, and three became transfusion dependent (David et al, 1991; Ghosh et al, 1991; Rees et al, 1996). Exchange transfusion was required in one case only.

Iron overload can be a predictable complication of chronic transfusion therapy, but it may also occur in patients with limited or no history of transfusions (Bianchi et al, 2003), similarly to what is described in other non-spherocytic haemolytic anaemias (Zanella et al, 1993, 2001). Iron deficiency secondary to intravascular haemolysis was reported in one case (Ericson et al, 1983).

Delayed development and learning difficulties of variable degree were reported in seven patients, which was not related to the severity of haemolysis (Torrance et al, 1977b; Beutler et al, 1980; Li et al, 1991; Balta et al, 2003). Database searches have shown the presence of P5’N-1 cDNA libraries in brain tissue (Marinaki et al, 2001); however, the significance of the association between mental retardation and P5’N deficiency is hard to assess; possible confounding factors include kernicterus or concomitance of other genetic defects in consanguinous families.

Figure 3. Main clinical data of 64 pyrimidine 5’-nucleotidase-deficient patients reported in the literature.
Aplastic crisis following parvovirus B19 infection (Rechavi et al., 1989) and chronic ankle ulcers (Beutler et al., 1980) were rare.

Pyrimidine 5’-nucleotidase deficiency has been found in association with HbE (Rees et al., 1996), HbD Punjab (unpublished observations) and spectrin deficiency (Bianchi et al., 2003). Only in the former case the associated defect greatly exacerbated the clinical pattern.

**Haematological features**

The haematological features of P5’N deficiency are common to other hereditary non-spherocytic haemolytic diseases, including anaemia, reticulocytosis and biochemical signs of hyperhaemolysis. Figure 4 reports some routine laboratory findings of P5’N-deficient patients, divided into splenectomised and unsplenectomised groups.

The median haemoglobin concentration was 9.5 g/dl (range 2.8–15.2 g/dl) without significant differences between splenectomised and unsplenectomised patients. In seven patients examined before and after splenectomy, surgery resulted in stabilisation of the haemoglobin to higher levels (median Hb increase 3.2 g/dl, range 0.5–5.2).

The reticulocyte count is constantly increased and does not appear to be related to the haemoglobin levels (data not shown). Overall, the reticulocytosis tends to be higher in splenectomised than in unsplenectomised patients (median 15% vs. 8%); in most patients examined before and after splenectomy, surgery resulted in a further increase of reticulocytes. Similar findings have been reported in PK deficiency and referred to a selective sequestration of defective reticulocytes by the spleen (Zanella et al., 2005).

Unconjugated hyperbilirubinaemia is higher in splenectomised than unsplenectomised patients (median 72 µmol/l vs. 47 µmol/l) and was reported to increase after splenectomy in two cases (Buc et al., 1979; unpublished observations).

The blood film is mainly characterised by red cell basophilic stippling (2–12% of erythrocytes) (Ben-Bassat et al., 1976; Marinaki et al., 2001; Bianchi et al., 2003) due to intracellular precipitation of undegraded RNA (Erickson et al., 1983); 5–10% of spherocytes, mostly spiculated, were detected at peripheral blood smear examination in two cases described by Valentine et al. (1974) and Beutler et al. (1980) and in the six patients reported by Bianchi et al. (2003). Red cell osmotic fragility is usually normal with few exceptions (Buc et al., 1979; Paglia et al., 1980; Bianchi et al., 2003).

Iron status parameters have been investigated in eight untransfused P5’N-deficient patients. Three of them, all splenectomised, displayed overt iron overload as assessed by increased iron status parameters (serum ferritin 1620–2400 µg/l; transferrin saturation 61–100%), marked siderosis at liver biopsy and, in one case only, cardiac iron burden at magnetic resonance imaging (Bianchi et al., 2003; unpublished observations). Two of them carried HFE gene mutations (C282Y/C282Y; H63D/wt). All three patients needed iron chelation.

**Diagnostic aspects**

The clinical and haematological features of P5’N deficiency are not distinctive, although the blood film gives a strong clue as to the diagnosis when marked red cell basophilic stippling is seen. Basophilic stippling is a constant but not specific finding in this disease, occasionally occurring in other congenital or acquired conditions, such as β-thalassaemia trait (Vives Corrons et al., 1984; Joishy et al., 1986), some haemoglobin variants (Sozen et al., 2004), sideroblastic anaemia or lead poisoning. The diagnosis ultimately depends upon the demon-

![Fig 4. Routine haematological data and pyrimidine 5’-nucleotidase (P5’N) activity of P5’N-deficient patients reported in the literature (○, unsplenectomised; ○, splenectomised). The horizontal dotted lines delimit the reference range for each parameter. Vertical bars indicate pre- and post-splenectomy values in single subject.](image-url)
stratification of high concentrations of pyrimidine nucleotides and a reduced P5’N-1 activity in red blood cells.

The nucleotides of normal red cells consist largely of purine derivatives (which have an absorption maximum at approximately 260 nm), with very low levels of pyrimidine nucleotides (absorption at 280 nm). In P5’N-1 deficiency, high levels of pyrimidine nucleotides accumulate in erythrocytes, resulting in a decrease in the OD260:OD280 absorbance ratio (Beutler, 1984).

A lower than normal OD260:OD280 ratio was found in all the 28 cases investigated. This test is considered to be specific for P5’N deficiency, and has been recommended by International Committee for Standardisation in Haematology (ICSH) for screening purposes (ICSH, 1989).

There are many different procedures for measuring P5’N-1 activity. They include spectrophotometric determination of the phosphate produced in dephosphorylating reactions (Fiske & Subbarow, 1925; Ames, 1966; Paglia & Valentine, 1975; Zerez & Tanaka, 1985), measurement of radioactive nucleoside liberated from a labelled substrate (Torrance et al., 1977c; Buc & Kaplan, 1978; Ellims et al., 1978), quantification at 254 nm of nucleosides separated by high-performance liquid chromatography (Simmonds et al., 1991; Amici et al., 1994), or by means of micellar electrokinetic chromatography (Zanone et al., 2004). In particular, the last technique seems to meet the standards of performance and reproducibility that are expected of a clinical test.

Residual P5’N-1 activity has been reported to vary from 1% to 64% (Fig 3) and is not correlated with the degree of the haemolysis (data not shown). The high residual activity observed in some patients could be explained by compensatory mechanisms, such as upregulation of other nucleotidases and/or alternative pathways for nucleotide metabolism (Chiarelli et al., 2005). Moreover, P5’N-1 residual activity was not directly correlated with the reticulocyte number (data not shown), supporting the hypothesis that pyrimidine accumulation and metabolic impairment principally occur in the younger erythrocytes of P5’N-deficient patients (David et al., 1991).

Finally is noteworthy that, in patients with transient erythrobastopenia of childhood, an incorrect diagnosis of hereditary P5’N deficiency may be made, due to the very low enzyme activity of the older red cell population (Beutler & Hartman, 1985).

**Relationship between molecular defect and disease severity**

The molecular bases of P5’N-1 deficiency have been recently investigated by studying the biochemical properties of four pathological variants (Asp87Val, Leu131Pro, Asn179Ser and Gly230Arg), obtained by site-directed mutagenesis technology (Kanno et al., 2004; Amici et al., 2005; Chiarelli et al., 2005). All investigated mutant proteins display a reduction of catalytic efficiency, and/or alterations of heat stability. The effects of mutations on the thermal stability of the enzyme and the steady-state kinetics as a function of CMP and UMP are reported in Fig 5, whereas the main parameters produced are summarised in Table II.

Asp87Val enzyme exhibits reduced heat stability and catalytic efficiency especially versus UMP (about 25 times), owing to the increased \( K_m \) values. Thus, the replacement of a charged (Asp) with a hydrophobic (Val) amino acid alters both protein stability and kinetic behaviour, accounting for P5’N-1 deficiency. Moreover, as Asp 87 is a well-conserved amino acid, it is conceivable that the mutation c.260A → T affects a residue involved in the substrate-binding site.

Leu131Pro protein shows a drastic reduction in thermal stability (\( t_{1/2} \) at 46°C about 1 min compared with a fully stable wild type) and kinetic properties slightly altered (\( k_{cat} \) values nearly halved and \( K_m \) three to five times higher) (Table II). Moreover, this mutant was found to be highly unstable even at 37°C, losing 50% of its activity in about 5 min (Chiarelli et al., 2005). Protein stability is particularly important in erythrocytes that lack the ability to synthesise proteins. However, the replacement Lys131Pro is likely to make the enzyme unable to maintain a proper folding, increasing the susceptibility to degradation by ubiquitin–proteasome pathway (Kanno et al., 2005).

**Fig 5.** Characterization of wild-type (●) pyrimidine 5’-nucleotidase and four types of mutants (□, Asp87Val; △, Leu131Pro; ■, Asn179Ser; ▼, Gly230Arg). (A) Steady state kinetics as a function of CMP; (B) steady state kinetics as a function of UMP; (C) thermal stability (residual activity after incubation at 46°C expressed as percentage of the initial activity).
Table II. Kinetic and thermal stability parameters of wild type and pyrimidine 5′-nucleotidase type 1 mutants.

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<td>k&lt;sub&gt;cat&lt;/sub&gt;(s&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>K&lt;sub&gt;m&lt;/sub&gt;(mmol/l)</td>
<td>k&lt;sub&gt;cat&lt;/sub&gt;(s&lt;sup&gt;-1&lt;/sup&gt;)</td>
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<tr>
<td>Wild type</td>
<td>26±14 ± 0·70</td>
<td>0·18 ± 0·02</td>
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<tr>
<td>Asp87Val</td>
<td>23±30 ± 1·20</td>
<td>1·00 ± 0·10</td>
</tr>
<tr>
<td>Leu131Pro</td>
<td>6·39 ± 0·20</td>
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<td>Asn179Ser</td>
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<td>Gly230Arg</td>
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* Maintains more than 90% of activity after 2 h of incubation.

**References**


Amici, A., Caccioli, K., Naponelli, V., Raffaelli, N. & Magni, G. (2005) Evidence for essential catalytic determinants for human erythrocyte occasional transfusions may be required when the anaemia is exacerbated by intercurrent infections, pregnancy or other conditions. Splenectomy does not arrest haemolysis and is generally reported to scarcely improve clinical conditions with a few exceptions (McMahon et al., 1981; Ozsoylu & Gurgey, 1981; Dvilansky et al., 1984; Rees et al., 1996); in particular, two of three regularly transfused patients became transfusion independent after surgery.

Pyrimidine 5′-nucleotidase-deficient patients should be monitored with regard to the iron status, particularly after splenectomy, and screened for the HFE genotype to identify subjects with potential additional risk factors for iron accumulation; repeated courses of iron chelation therapy may be required in these subjects.

**Treatment**

No specific therapy for P5′N deficiency is available, and the treatment of this disease is therefore based on supportive measures; transfusion dependence is very rare, although occasional transfusions may be required when the anaemia is exacerbated by intercurrent infections, pregnancy or other conditions. Splenectomy does not arrest haemolysis and is generally reported to scarcely improve clinical conditions with a few exceptions (McMahon et al., 1981; Ozsoylu & Gurgey, 1981; Dvilansky et al., 1984; Rees et al., 1996); in particular, two of three regularly transfused patients became transfusion independent after surgery.

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of a novel gene family that is conserved through evolution and associated with Lupus inclusions. Development Genes and Evolution, 210, 512–517


