PGK deficiency

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Summary

Phosphoglycerate kinase (PGK) deficiency is one of the relatively uncommon causes of hereditary non-spherocytic haemolytic anaemia (HNSHA). The gene encoding the erythrocyte enzyme *PGK1*, is X-linked. Mutations of this gene may cause chronic haemolysis with or without mental retardation and they may cause myopathies, often with episodes of myoglobinuria, or a combination of these clinical manifestations. Twenty-six families have been described and in 20 of these the mutations are known. The reason for different clinical manifestations of mutations of the same gene remains unknown.

Keywords: anaemia, haemolytic, HNSHA, myopathy, rhabdomyolysis.

A deficiency of the glycolytic enzyme phosphoglycerate kinase (PGK) is one of the uncommon causes of hereditary non-spherocytic haemolytic anaemia (HNSHA). There are several aspects of PGK deficiency that distinguish it from the other glycolytic defects that produce HNSHA. It is the only enzyme in the direct glycolytic pathway that is encoded by an X-linked gene. PGK is the first glycolytic enzyme of the red cell in which specific amino acid substitutions were identified by direct protein sequencing, a considerable achievement before the era of molecular cloning and sequencing made the identification of mutations commonplace. PGK deficiency is of interest to the haematologist because it is an uncommon cause of haemolysis, but it has also gained the attention of physicians in other fields of medicine because a deficiency may produce rhabdomyolysis, mental retardation, and various neurological disorders in some patients.

The enzyme

Phosphoglycerate kinase catalyses the transfer of the high-energy phosphate from the 1-position of 1,3 diphosphoglycerate (1,3-DPG) to ATP, converting the 1,3-DPG to 3-phosphoglyceric acid (3-PGA):

$$1,3\,DPG\,+\,ADP \leftrightarrow 3\,PGA\,+\,ATP$$
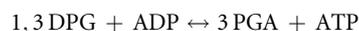
PGK competes with the enzyme diphosphoglycerate mutase (DPGM) for the substrate 1,3 DPG in the reaction.

$$1,3\,DPG \rightarrow 2,3\,DPG \rightarrow 3\,PGA\,+\,P_i$$

The end product of the two competing reactions is the same except for the important fact that the PGK reaction preserves the high energy phosphate in the form of ATP. This alternative routing of glycolytic intermediates has been very aptly called the 'energy clutch' of glycolysis (Keitt & Bennett, 1966). The position that this key reaction occupies in the glycolytic pathway is illustrated in Fig 1.

Red cell enzymes may not be evenly dispersed in the cytosol, and some of the steps of glycolysis may be carried out by enzyme complexes assembled on the red cell membrane. Under these circumstances PGK localised to the membrane could preferentially provide ATP to the sodium/potassium pump, and some experimental data have been adduced to suggest this may be the case (Parker & Hoffman, 1967; Proverbio & Hoffman, 1977). The ATP generated in the pyruvate kinase step, in contrast to that generated in the PGK reaction, would then be available for the phosphorylation of glucose. However, using an isotope labelling technique, it has not been possible to show that there is a separate pool of ATP utilised for glucose phosphorylation(Beutler *et al*, 1978). This does not rule out definitively the existence of a small membrane-localised ATP pool, and the question of whether PGK is metabolically docked to the membrane to form an ATP-generating complex is unresolved.

History

The first reported case of PGK deficiency (Kraus *et al*, 1968) was detected, ironically for an X-linked defect, in a woman without relatives available for study. The only clinical manifestation in this patient appeared to be long-standing HNSHA. In the same year, Valentine *et al* (1968, 1969) described a large kindred of Chinese origin manifesting HNSHA, in which several apparently affected males died during infancy. The surviving children were mildly mentally retarded. Mild haemolysis in affected females and the pattern of genetic

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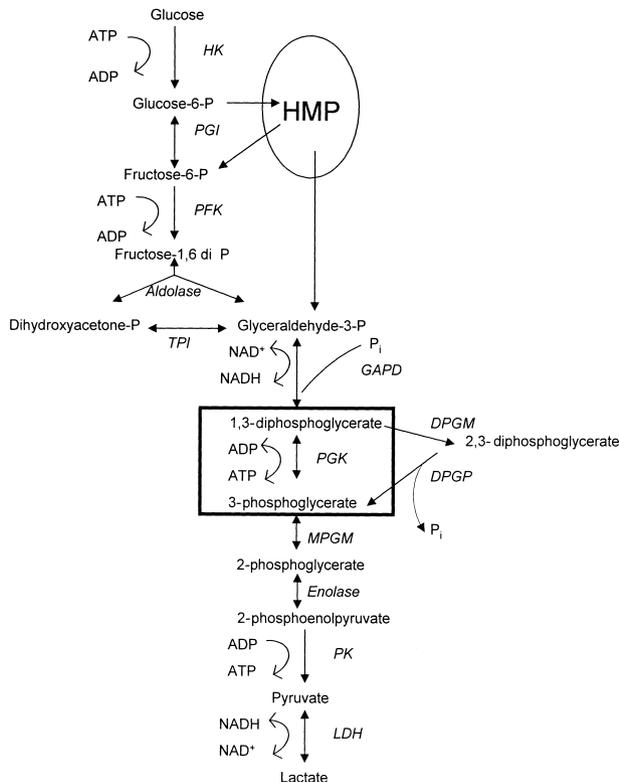E-mail: beutler@scripps.edu

Fig 1. The glycolytic pathway of erythrocytes. The enzymes catalyzing the reactions of the pathway or their abbreviations are shown in italics. The phosphoglycerate kinase *(PGK)* reaction is boxed. The following abbreviations are used: HK, hexokinase; PGI, phosphoglucose isomerase; HMP, hexomonophosphate shunt; PFK, phosphofructokinase; TPI, triosephosphate isomerase; GAPD, glyceraldehyde phosphate dehydrogenase; DPGM, diphosphoglyceromutase; DPGP, diphosphoglycerophosphatase *(DPGM* and *DPGP* are different activities of the same enzyme); PGK, phosphoglycerate kinase; MPGM, monophospheroglycerate mutase; PK, pyruvate kinase; LDH, lactate dehydrogenase.

transmission suggested X-linkage. Based on these findings, we attempted to prove X-linkage of PGK by developing a method for enzyme electrophoresis on starch gel (Beutler, 1969), but found no variants among 92 White males, 121 White females, 114 Black males, 10 Black females, four Asian males, and four Asian females. However, Chen *et al* (1971) found three electrophoretic variants among 161 blood specimens from New Guinea natives, and established that transmission was, indeed, X-linked. As early as 1972, the mutation in this PGK variant, found in Samoa and New Guinea, was established at the amino acid level by protein fingerprinting (Yoshida *et al*, 1972).

Clinical manifestations

We have found reports of 26 families in which clinically significant PGK deficiency has been documented. These are summarised in Table I. Various combinations of haemolytic anaemia, mental retardation, and myopathies were encountered. In the few instances when more than one family member

was affected, the clinical phenotype in the affected individuals, usually male siblings, were similar. Haemolytic anaemia was present in most of the reported patients, being documented in 18 of the families. Splenectomy was performed on a few occasions and appeared to have been beneficial. Myopathies were present in patients in 10 families, without anaemia in 7 of these. Thus, there seemed to be a tendency toward dichotomisation into a myopathic form, on the one hand, and a haemolytic form, on the other. Varying degrees of mental retardation were common and other neurological symptoms were present in some patients, as indicated in the table.

Genetics

The gene encoding the form of PGK found in most tissues, including the erythrocyte, was cloned in 1983 (Michelson *et al*, 1983). It was localised to Xq13 (Willard *et al*, 1985) and is designated *PGK1*. A pseudogene (*PGK1P1*) has also been found about 10 megabases centromeric to the *PGK1* gene. A related sequence, immunologically distinct, was identified by Chen *et al* (1976), and is the intronless form of the gene that is expressed in the testis (Michelson *et al*, 1985; McCarrey & Thomas, 1987). This gene, localised to chromosome 6 (Michelson *et al*, 1985), is designated *PGK2*.

There is one known polymorphic mutation of the *PGK1* gene, which is the polymorphism that was used to establish X linkage of the gene. The amino acid substitution was found on peptide mapping of a tryptic digest of the purified enzyme (Yoshida *et al*, 1972), and subsequently the site of the mutation was identified by DNA sequencing (Fujii *et al*, 1981). The frequency of this polymorphism was estimated to be approximately 0·014 in a New Guinea population (Chen *et al*, 1971). While most of the remaining known mutations of the *PGK1* gene were identified by investigating patients with clinical disease, there is one exception. PGK München was found by screening 3000 normal blood samples for decreased PGK activity (Krietsch *et al*, 1977). Presumably because the residual activity of this variant is 21% of normal, no clinical or biochemical abnormalities seem to result. Among the families with disease manifestations due to severe PGK deficiency, 19 different mutations have been reported. The available data are summarised in Table II.

There is considerable confusion in the literature about the actual position of some of the described mutations within the *PGK1* gene. This is partly due to the fact that PGK is one of the few enzymes in which mutations were detected at the amino acid level before DNA sequencing was possible; in part, it is a consequence of the unfortunate tendency of many investigators to use amino acid nomenclature as a primary nomenclature for mutations (Beutler *et al*, 1996). Yoshida, who characterised many of the mutations with his collaborators, depended initially on protein sequence and therefore designated the acetylserine at the N-terminus of the mature enzyme as amino acid 1. This non-standard nomenclature has been followed in describing many of the subsequently

Table I. A summary of clinical data obtained in 26 families with clinically significant PGK deficiency and in mutants observed as a result of population screening.

Family	Designation	Ethnic background	Reference	Patients	Anaemia	Myopathy	Splenectomy	Mental retardation	Other
1			Kraus et al (1968)	1	+	0		0	
2	New York	Asian and European	Valentine et al (1969)	7M; 2F	+	0	Beneficial	+	
3		French (White)	Cartier et al (1971)	1	+	0	Beneficial	+	Severe Neonatal Jaundice
4	Matsue	Japanese	Miwa et al (1972)	1	+	0	Some benefit	+	'Cerebral palsy'
5		Italian	Arese et al (1973)	1	+	0			
6		US White	Konrad et al (1973)	2	+	0		+	Neurological abnormalities
7	Amiens	French	Boivin et al (1974)	1	+	0		+	
8	Uppsala	Swedish	Hjelm et al (1980)	1	+	0		+	
9	Tokyo	Japanese	Akatsuka et al (1973)	2	+	0		+	Convulsions
10	Creteil	French	Rosa et al (1979, 1982)	1	0	+		0	Rhabdomyolysis without any anaemia. RBC activity 27% of normal RBC activity 20% of normal
11		Japanese	DiMauro et al (1983)	1	0	+			
12	Hamamatsu	Japanese	Sugie et al (1989)	1	0	+		+	No anaemia. RBC activity 8% of normal
13	Shizuoka	Japanese	Fujii et al (1992)	1	+	+			
14	Michigan	White	Maeda et al (1992)	1	+	0		+	Abnormal EEG
15	Alberta	East Indian	DiMauro et al (1983)	1	0	+		0	
16	North Carolina	USA	Tsujino et al (1994)	1	+	0	+	+	
17	Alabama		Yoshida et al (1995)	1	+	0		0	Haemolysis well compensated
18	Herlev	Danish	Valentin et al (1998)	1	+	0		0	
19	Antwerp	Belgian	Ookawara et al (1996)	2	0	+		0	
20	Fukui	Japanese	Hamano et al (2000)	1	0	+			
21	Kyoto	Japanese	Morimoto et al (2003)	1	+	+		+	
22		Romanian	Spanu and Oltean (2003)	2	No data	+			
23	Barcelona	Spanish	Noel et al (2005)	1	+	0		+	
24	Murcia	Spanish	Noel et al (2005)	1	+	?		+	Died age 7 years of severe encephelopathy
25		Japanese	Shirakawa et al (2006)	1	0	+		+	
26	New York/Amiens	English/Irish/Native American	Flanagan et al (2006)	2	+	0		+	Hemiplegic migraines and seizures
Population Survey	München	German	Krietsch et al (1977)	7 M; 12 F	0	0		0	Discovered as a result of population screening
Population Survey	PGKII/'Samoa'	Samoan	Chen et al (1971)		0	0		0	A polymorphism

RBC, red blood cell; EEG, electroencephalography

Table II. Mutations found in the variants summarised in Table I. Only families in which the mutation has been identified are included.

Family number	Designation	Mutation reported	Mutation (corrected/standard) nomenclature	Mutation reference	Comment
2	New York	491 A → T (D163V)	491A → T (D164V)	Turner et al (1995)	Also found in a US White family, but in the context of a different haplotype (Flanagan et al, 2006)
4	Matsue	266T → C (L88P)	267T → C (L89P)	Maeda and Yoshida (1991)	
7	Amiens	571 A → T (D163V)	491 A → T (D164V)	Cohen-Solal et al (1994)	
8	Uppsala	R206P	617G → C(R206P)	Fujii and Yoshida (1980)	
9	Tokyo	V266M	*	Fujii et al (1981)	
10	Creteil	1022G → A (D314N)	943G → A (D315N)	Cohen-Solal et al (1994)	
12	Hamamatsu	837T → C (I252T)	758T → C (I253T)	Sugie et al (1998)	Mutation shown in incorrect location in figure
13	Shizuoka	(G157V)	473G → T (G158V)	Fujii et al (1992)	
14	Michigan	(C315R)	947T → C (C316R)	Maeda et al (1992)	
17	Alabama	Del 571–573 or 574–576 AAG (del L190 or L191)	del 571–573 AAG (del L191 or 192)	Yoshida et al (1995)	Deletion is one of two tandem lysines
18	Herlev	D285V	855A → T(D285V)	Valentin et al (1998)	A somatic, not germline, mutation in a chimaeric patient
19	Antwerp	755A → C (E251A)	755A → C(E252A)	Ookawara et al (1996)	
20	Fukui	del 716–719 GGCG	del 706–709 GGCG (fs)	Hamano et al (2000)	Also produces splicing abnormality
21	Kyoto	1060G → C (A353P)	1060G → C (A354P)	Morimoto et al (2003)	
23	Barcelona	140T → A (I46N)	140T → A (I47N)	Noel et al (2005)	
24	Murcia	958G → A (S319N)	959G → A (S320N)	Noel et al (2005)	
25		IVS7 + 5 G → A (fs)	IVS7 + 5 G → A (fs)	Shirakawa et al (2006)	
26	New York/Amiens	491A → T (D164V)	491A → T (D164V)	Flanagan et al (2006)	
Population Survey	München	(D268N)	802G → A (D268N)	Fujii et al (1980)	
Population Survey	PGKII/'Samoa'	1055C → A (T352N)	1055C → A (T352N)	Yoshida et al (1972); Fujii et al (1981)	

*The valine 266 that was reported to have been changed to a methionine is encoded by a codon (GTC) that cannot be changed to the methionine codon, ATG, by a single nucleotide change.

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                    ↓
482   ATGTCAATGATGCTTTTGGCACTGCTCACAGAGCCCACAGCTCCATGGTAGGAGTCA   538
      ||||||||| |||||||||||||||||||||||||||||||||||||||||||||||
      ATGTCAATGGTGCTTTTGGCACTGCTCACAGAGCCCACAGCTCCATGGTAGGAGTCA
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Fig 2. The portion of the *PGK1* pseudogene sequence (GenBank accession number K03201.1) aligned with the homologous portion of the X-linked PGK sequence. The X-linked functional gene is shown above. The nucleotide numbering is that of the functional gene. The lower sequence is that of the pseudogene. The arrow indicates the nucleotide that has been subject to recurrent mutation from the normal A to a T. Although the pseudogene has a G in this position it is tempting to consider that this nucleotide is particularly vulnerable to mutation, in the case of PGK New York to a T.

identified variants. There were also some errors in counting amino acids. For example, in describing both the mutation of PGKII and PGK Uppsala (Fujii & Yoshida, 1980), there are errors in the published sequence; the insertion of a lysine in position 39. This results in reporting codon numbers that correspond with the standard numbering in which the N-terminal methionine is counted as the first amino acid. In the case of another mutation, PGK Tokyo (Fujii *et al*, 1981), the valine 266 that was reported to have been changed to a methionine is encoded by a codon (GTC) that cannot be changed to the methionine codon, ATG, by a single nucleotide change. The mutation of PGK Murcia was mistakenly reported as being at nt 958 (Noel *et al*, 2005), when it reality it is at nt 959.

With one exception, each reported family manifested a unique mutation. However, the 491 A → T (D164V) mutation has been encountered three times: once in the original Chinese family, described by Valentine *et al* (1969) and later designated PGK New York; then in a French family, a variant designated PGK Amiens (Boivin *et al*, 1974); and most recently in a North American family with English/Irish/Native American ancestry (Flanagan *et al*, 2006). The mutation in the American family was found to be in the context of different haplotype than that in the original Chinese kindred. Accordingly, it seems likely that these mutations arose independently. It is of interest in this regard, as shown in Figure 2, that this mutation occurs in cDNA nucleotide 491, the only nucleotide mismatching the X-linked PGK pseudogene in a long homologous sequence. Although the pseudogene sequence has a G in this position and a T exists in the New York/Amiens mutation, it is attractive to suppose that this is a recurrent mutation resulting from gene conversion involving the closely homologous pseudogene, as in some other recurrent mutations. A computer-based search of Reference Sequence Release 9 (Bischof *et al*, 2006) identified six gene-pseudogene pairs in which gene conversion had occurred. The *c.* 837T > C mutation (Sugie *et al*, 1998) of *PGK* was one of these. A review of the 13 point mutations known in the PGK coding region indicates that among these the only other mutation in which the sequence of the pseudogene differs from that of the functional gene is the recurrent c.491A → T mutation.

PGK is a bilobed enzyme with a C and an N domain. The phosphoglycerate binds to the N domain and the Mg-complexed nucleotide to the C domain (Varga *et al*, 2005). Valentin *et al* (1998) suggested that, in PGK Matsue, the mutant proline may change the position of the short beta strand that follows it. They proposed that in PGK Shizooka,

the larger valine residue substituted for a glycine and in the case of PGK Tokyo, the substitution methionine for valine may be introducing an amino acid too large to fit into the available space. The new hydrophobic side chain of the valine introduced into the New York/Amiens variant and into PGK Herlev introduces a hydrophobic valine into a region accessible to solvent. Valentin *et al* (1998) also proposed that the mutation in PGK Upsala may perturb motion at the hinge that connects the two domains of PGK and that in PGK Creteil, the substitution is adjacent to a leucine involved in nucleotide binding.

Mutations that produce clinical disease are found in both the C and the N domains. Figure 3 depicts the structure of the PGK molecule based on the crystal structure of the pig enzyme (Flachner *et al*, 2004). Since only 23 of the 417 amino acids of the pig *PGK1* differ from the human enzyme, it is to be presumed that the structure is almost identical. More recently, Noel *et al* (2005), using the pig muscle structure as a model, suggested that the two mutations that they had described, PGK Barcelona and PGK Murcia both tended to disrupt the tertiary

Fig 3. Three-dimensional model of human phosphoglycerate kinase (PGK) shown in cartoon representation using the VMD program. ILE47 at the N-domain and SER320 at the C-domain of the molecule are shown as yellow spheres, whereas other relevant amino acids corresponding to previously reported pathological amino acid substitutions mutations are shown as green rods. PGK mutants referred to Murcia S320N, Matsue L89P, Shizuoka G158V, Amiens D164V, Uppsala R206P, Antwerp E252A, Hamamatsu I253T, München D268N, Herlev D285V, Creteil D315N, Michigan C316R, Barcelona I47N and Kyoto A354P. Reproduced from Noel *et al* (2005).

structure of the molecule, affecting its stability. They also pointed out that there is a tendency for the single amino acid substitutions to occur in three regions of the enzyme molecule. However, they noted that no correlation could be found between these different locations and the clinical expression of the enzyme deficiency.

Biochemical characteristics

The biochemical characteristics of the residual enzyme have been studied in many of the variants. No pattern has emerged that would explain the different clinical manifestations of the various mutations. In PGK Creteil, found in a patient with rhabdomyolysis without haemolytic anaemia (Rosa et al, 1982), there was nonetheless striking decrease of red cell enzyme activity, as well as muscle enzyme activity. Leucocyte and platelet activity was also decreased. Similarly, the patient with PGK North Carolina only had 2·7% of normal red cell enzyme activity but no anaemia (Tsujino et al, 1994). Sugie et al (1989) found kinetically normal enzyme in a child with mental retardation and myoglobinuria, but in another patient with myoglobinuria, Rosa et al (1982) found a marked increase in the K_m for ATP and ADP. In the case of PGK Uppsala (Fujii & Yoshida, 1980), a variant associated with haemolytic anaemia and mild mental retardation, there was a considerable increase of the K_m not only for ADP and ATP, but also for 1,3-DPG and 3-PGA. As is usually the case in partial defects of glycolytic enzymes, there was relatively little decrease in glucose consumption and lactate production. This is presumably because the increased 3-diphosphoglycerate level that results from the PGK deficiency, compensates, at least in part, for the deficient enzyme.

Discussion

Glycolysis, the metabolism of glucose to pyruvate and lactate, is an ancient metabolic pathway that is found in most living organisms. There is little redundancy in the reactions that comprise glycolysis, and one might conclude that loss or marked impairment of this pathway would be lethal, and therefore never encountered in medicine. However, mutations of enzymes catalyzing this pathway are encountered in patients with hereditary non-spherocytic haemolytic anaemia (HNSHA), a relatively rare disorder that is caused by a variety of inherited red blood cell defects. These mutations are compatible with life because the mutant gene is a hypomorph, i.e. some protein, albeit abnormal, is expressed. Phosphoglycerate kinase is one of these enzymes.

Most of the 18 mutations associated with clinical disease are missense mutations. Exceptions are PGK Alabama, in which there is a single amino acid deletion, PGK Antwerp, in which there is missplicing in addition to an amino acid substitution, PGK North Carolina, which has a splicing error that results in the insertion of 10 additional amino acids as a result of the utilisation of an alternative splicing site, and PGK Fukui, in which a four base pair deletion in exon 6 predicts the formation of a truncated protein. It is somewhat surprising that the latter mutation is compatible with life; more than one-third of the enzyme molecule would be missing.

In the case of some glycolytic enzyme deficiencies, e.g. pyruvate kinase deficiency, the clinical phenotype is limited almost entirely to the red blood cell. Other tissues may be spared in such genetically determined enzyme deficiencies for several reasons. The stability of the mutant enzyme is less in the red cell than in other tissues, the red cell lacks the capacity to synthesise enzyme to replace mutant enzyme that has been lost as a result of instability, and the metabolic compensation provided by mitochondria in most tissues is not available in red cells. There may also be other redundant metabolic pathways, regulated by other genes, in tissues other than the erythrocyte. In contrast to patients with pyruvate kinase deficiency, a deficiency of triosephosphate isomerase, the anaemia is almost always associated with severe neuromuscular disease. PGK deficiency is intermediate between these two extremes. It sometimes manifests only chronic haemolytic anaemia, but in many cases other clinical findings are present, particularly mental retardation and muscle disease, and these may occur with or without the anaemia.

It is not altogether unusual for different mutations of the same gene to cause very different disease phenotypes. Some examples from haematology include hereditary methemoglobinemia due to NADH diaphorase deficiency, which may occur with or without mental retardation (Beutler, 2006); mutations of the gene encoding glucocerebrosidase which may produce visceral disease either without any neurological impairment (Beutler, 1992) or with a very severe neurological impairment; and glutathione synthase deficiency, which may produce haemolytic anaemia with or without 5-oxoprolinuria and mental retardation (Beutler, 1976). In these examples, the pleomorphic clinical presentation is probably the result of different tissue distributions of the deficiency: In the mild forms of NADPH diaphorase and glutathione synthase deficiencies only the red cells seem to manifest a severe lack of enzyme.

We have proposed that one mechanism by which there may be selective loss of enzyme might be based upon the different types of proteases that are active within various tissues. Some mutant enzymes may bear a lesion that marks them for destruction only in erythroid precursors and erythrocytes. Others may bear lesions that also result in their destruction in cells of the central nervous system or other tissues (Beutler, 1983). But selection from loss of enzyme activity does not seem to explain the pleomorphic clinical presentation in PGK deficiency. In the case of PGKs Creteil (Rosa et al, 1982) and North Carolina (Tsujino et al, 1994), no haemolysis was present, although the patients' red cell activity was recorded at only 2·75% of the mean normal; some patients with considerably higher levels of erythrocyte enzyme show severe haemolysis. Perhaps the pleomorphic disease manifestations may result from lesions in one portion of the molecule

producing one phenotype while those in another have a different clinical effect. One such example is Chuvash polycythaemia, where the gene encoding the Von Hippel–Lindau protein is mutated. One mutation of this protein produces a disturbance in the regulation of erythropoiesis so that polycythaemia ensues. The site of this mutation in the protein prevents a necessary step in the degradation of HIF1α in the presence of oxygen. Other mutations of the same protein produce an entirely different clinical syndrome, namely, an increased incidence of cancer (Ang *et al*, 2002a,b). One may speculate that some mutations of PGK impair its functions more in muscle cells than in erythrocytes and that in others, the reverse is true. It may be, for example, that if tethering to the erythrocyte membrane is important for the function of PGK, as has been implied, that some mutations interfere with this effect in erythrocytes while others do not. The location of the various mutations within the PGK molecule tell us relatively little in this regard, although it is notable that the three mutations that are known to produce rhabdomyolysis without producing anaemia are all localised in a circumscribed region of the C domain of the enzyme. Finally, one may consider the possibility that PGK is a 'moonlighting' enzyme. Such enzymes have two totally disparate functions. In other glycolytic enzymes, glucose phosphate isomerase is known to have this property. In addition to isomerising glucose-6-phosphate di-fructose-6-phosphate, the same protein is identical to neuroleukin, an enzyme with lymphokine properties (Kugler *et al*, 1998). Perhaps PGK has another job to do, one that we do not yet know about.

PGK deficiency is a rare disease, and rare diseases sometimes teach us more than common disorders. This deficiency presents us with some interesting puzzles that challenge us to solve them. Prominent among these is the question of why the presentation of disease manifestations tends to be dimorphic, with relatively little overlap: Why do some patients develop myopathies, others haemolytic anaemia? Another unanswered question is how patients with major disruptions of PGK can survive? Is there activation of other forms of PGK in the absence of PGK1? New discoveries and the development of new insights may solve some of these questions.

Acknowledgements

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