22.6.10 Erythrocyte enzymopathies 5463 Alberto Zan

22.6.10 Erythrocyte enzymopathies 5463 Alberto Zanella and Paola Bianchi

22.6.10 Erythrocyte enzymopathies 5463 gene, with dominant inheritance). In both cases, abnormal cation flux results. Diagnosis of these rare conditions rests on the iden- tification of appropriate red cell morphology in the context of a family history of haemolytic anaemia; confirmatory genetic testing of candidate genes is possible, and red cell cation concen- trations may be measured in the research setting. Other conditions Other conditions associated with hereditary stomatocytosis in- clude the Rh deficiency syndromes, sitosterolaemia, and familial deficiency of high-density lipoproteins. The rare Rh deficiency syndromes are associated with mild to moderate haemolytic an- aemia and absent (Rhnull) to decreased (Rhmod) erythrocyte ex- pression of Rh antigens associated with mutation in the RHD and RHAG genes. Sitosterolaemia is associated with early-onset atherosclerosis, anaemia, and macrothrombocytopenia asso- ciated with mutation of the ABCG5/ABCG8 cotransporters, leading to increased intestinal absorption and decreased biliary elimination of sterols, particularly those derived from plants. Familial deficiency of high-density lipoproteins (Tangier disease, OMIM 205400) is due to mutation of ABCA1, a protein critical for cellular cholesterol export, leading to accumulation of tissue cholesterol esters manifest as enlarged orange-yellow tonsils, hepatosplenomegaly, cloudy corneas, neuropathy, and prema- ture atherosclerosis. Affected patients exhibit mild to moderate haemolytic anaemia. Acquired stomatocytosis has been observed in a large number of conditions, particularly hepatobiliary disease and acute alcoholism. Acquired stomatocytosis has also been seen in patients with various malignant neoplasms, cardiovascular disease, and after the adminis- tration of vinca alkaloids. Acanthocytosis Acanthocytes are dense, contracted erythrocytes with irregular ‘thorny’ projections. Acanthocytes may also been found on the peripheral smears of patients with

abetalipoproteinaemia, the McLeod red cell phenotype, or one of the neuroacanthocytosis syndromes. Abetalipoproteinaemia (OMIM 200100) is associated with hypolipidaemia, fat malabsorption, progressive ataxia, retin- itis pigmentosa, and poor growth in childhood due to the inability to produce or secrete the B apoproteins B100 and B48, or defects in the microsomal triglyceride transfer protein (MTTP), required for production of apoprotein B-containing β-lipoproteins. The X- linked McLeod phenotype (OMIM 314850) is due to mutation of XK, necessary for Kell antigen expression. Affected individuals experience compensated anaemia, susceptibility to Kell D antigen alloimmunization, and late-onset myopathy and nervous system abnormalities. The neuroacanthocytosis syndromes are a heterogeneous group of neurodegenerative disorders including the McLeod syndrome, chorea-acanthocytosis due to mutation of chorein or VPS13A, Huntington disease-like 2 due to mutation of junctophilin-3, and pantothenate kinase-associated neurodegeneration (formerly known as Hallervorden–Spatz syndrome and its allelic variant HARP syndrome—hypobetalipoproteinaemia, acanthocytosis, ret- initis pigmentosa, and pallidal degeneration) due to mutations of pantothenate kinase 2. The cause of the acanthocytosis in these dis- orders is unknown. Acquired acanthocytosis may be seen in patients with severe hepatic disease (commonly known as spur cell anaemia), hypothy- roidism, malnutrition, and after splenectomy. FURTHER READING Andolfo I, et al. (2016). New insights on hereditary erythrocyte mem- brane defects. Haematologica, 101, 1284–94. Bennett V, Healy J (2008). Organizing the fluid membrane bilayer:
diseases linked to spectrin and ankyrin. Trends Mol Med, 14, 28–36. Beris P, Picard V (2015). Non-immune hemolysis: diagnostic consid- erations. Semin Hematol, 52, 287–303. Da Costa L, et al. (2013). Hereditary spherocytosis, elliptocytosis, and other red cell membrane disorders. Blood Rev, 27, 167–78. De Franceschi L, Bosman GJ, Mohandas N (2014). Abnormal red cell features associated with hereditary neurodegenerative disorders: the neuroacanthocytosis syndromes. Curr Opin Hematol, 21, 201–9. Gallagher PG (2013). Abnormalities of the erythrocyte membrane. Pediatr Clin North Am, 60, 1349–62. King M-J, et al. (2015). The International Council for Standardization in Haematology guidelines for the laboratory diagnosis of nonimmune hereditary red cell membrane disorders. Int J Lab Hematol, 37, 304–25. Landry ML (2016). Parvovirus B19. Microbiol Spectr, 4, 3. Lusher JM, Barnhart MI (1980). The role of the spleen in the pathoophysiology of hereditary spherocytosis and hereditary elliptocytosis. Am J Pediatr Hematol Oncol, 2, 31. Lux SE 4th (2016). Anatomy of the red cell membrane skeleton: un- answered questions. Blood, 127, 187–99. Mohandas N (2018). Inherited hemolytic anemia: a possessive beginner’s guide. Hematology, 30, 377–81. Niss O, et al. (2016). Genotype-phenotype correlations in hereditary elliptocytosis and hereditary pyropoikilocytosis. Blood Cells Mol Dis, 61, 4–9. Pasini EM, et al. (2006). In-depth analysis of the membrane and cyto- solic proteome of red blood cells. Blood, 108, 791–801. Risinger M, Glogowska E, Chonat S, et al. (2018). Hereditary xero cytosis: Diagnostic considerations. Am J Hematol, 93, E67–E69. 22.6.10 Erythrocyte enzymopathies Alberto Zanella and Paola Bianchi ESSENTIALS Numerous enzymes, including those of the hexose monophosphate and glycolytic pathways, are active in the red cell. They are required for the generation of ATP (needed to supply energy for sodium ex- trusion) and the reductants NADH and NADPH (necessary to main- tain haemoglobin in its active ferrous atomic state, as well as for the integrity of sulphydryl groups present on essential proteins). 2,3- Diphosphoglycerate (2,3-DPG), an intermediate of glucose metab- olism, is a key regulator of the affinity of haemoglobin for oxygen, and accessory enzymes are also active for the synthesis of glutathione, dis- posal of oxygen free radicals, and for nucleotide metabolism.

section 22 Haematological disorders 5464 With the exception of heavy metal poisoning and rare cases of myelodysplasia, most red cell enzyme deficiency disorders are in- herited. They may cause haematological abnormalities, (most commonly nonspherocytic haemolytic anaemias, but also rarely polycythaemia or methaemoglobinaemia, manifest with autosomal recessive or sex-linked inheritance), and may also be associated with nonhaematological disease (e.g. neuromuscular symptoms) when the defective enzyme is expressed throughout the body. Some may mirror important metabolic disorders, without produ- cing haematological problems, making them of diagnostic value
(e.g. galactosaemia). Others are of no known clinical consequence. With rare exceptions, it is impossible to differentiate the enzymatic defects from one another by clinical or routine laboratory methods. Diagnosis depends on the combination of (1) accurate ascertain- ment of the family history; (2) morphological observations—these can determine whether haemolysis is present, rule out some causes of haemolysis (e.g. hereditary spherocytosis and other red blood cell membrane disorders), and diagnose pyrimidine 5′-nucleotidase de- ficiency (prominent red cell stippling); (3) estimation of red cell en- zyme activity; and (4) molecular analysis. The most common red cell enzyme defects are glucose-6-phos- phate dehydrogenase deficiency (see Chapter 22.6.11), pyruvate kinase deficiency, glucose-6-phosphate isomerase deficiency, pyr- imidine 5′-nucleotidase deficiency—which may also induced by exposure to environmental lead—and triosephosphate isomerase deficiency. Introduction Erythrocytes contain a large number of enzymes required to carry out a variety of metabolic processes. Some inherited deficiencies of these enzymes are designated red cell enzymopathies. They may cause haematological disorders, including nonspherocytic haemo- lytic anaemias, polycythaemia, and methaemoglobinaemia. Other deficiencies do not produce haematological disorders, but instead mirror important metabolic disorders such as galactosaemia that are therefore of diagnostic value. Moreover, when the defective enzyme is expressed throughout the body, haemolytic anaemia is associated with systemic nonhaematological manifestations such as neurological dysfunc- tion, intellectual disability, myopathy, and susceptibility to infec- tions. This section deals with those red cell enzyme defects that cause haemolytic anaemia. Many have been described; most are rare but some are sufficiently common that several hundred or even thousands of cases have been documented. Although the enzym- atic bases of these defects are very different, the clinical presenta- tion is similar and relatively nondescript. Except for pyrimidine 5′-nucleotidase deficiency, in which red cell stippling is a prominent feature, it is impossible reliably to differentiate the enzymatic defects from one another by clinical or routine laboratory methods. Red cell metabolism The two major pathways of red cell glucose metabolism are illus- trated in Fig. 22.6.10.1. Glucose is phosphorylated to glucose 6- phosphate in the hexokinase reaction. It is then either metabolized in the anaerobic Embden–Meyerhof pathway or is oxidized in the glucose 6-phosphate dehydrogenase (G6PD) reaction, entering the hexose monophosphate pathway. Anaerobic metabolism of glucose phosphorylates ADP to ATP, providing energy to maintain erythro- cyte shape and to transport molecules into and out of erythrocyte. It also reduces NAD to NADH, which serves to reduce meth-aemoglobin to haemoglobin. The hexose monophosphate pathway reduces NADP to the NADPH and thus serves to maintain gluta- thione and protein sulphydryl groups in the reduced state. These pathways are similar in red cells, other tissues, and in ‘lower’ organ- isms. However, the 2,3-diphosphoglycerate (2,3-DPG) shunt is a unique feature of the Embden–Meyerhof pathway in erythrocytes. This ‘energy clutch’ of erythrocyte metabolism not only allows flexi- bility in the amount of ATP that is generated in glycolysis, but also provides a source of 2,3-DPG, the key modulator of haemoglobin oxygen affinity. There are, in addition, many other metabolic functions

that the erythrocyte must carry out. Among these are the synthesis of gluta- thione, the synthesis and degradation of nucleotides and nucleosides, Glucose Glucose 6-phosphate Fructose 6-phosphate Fructose 1, 6-diphosphate ATP ADP 1, 3-Diphosphoglycerate 3-Phosphoglycerate 2-Phosphoglycerate Phosphoenolpyruvate 2 ADP 2 ATP Pyruvate Lactate Glyceraldehyde 3-phosphate Dihydroxyacetone- phosphate P e n t o s e Hexokinase Glucose phosphate isomerase Phosphofructokinase Glyceraldehyde-3-phosphate dehydrogenase Phosphoglycerate kinase Monophosphoglycerate mutase Enolase Pyruvate kinase Lactic dehydrogenase DPG mutase 2, 3 DPG phosphatase Aldolase Triose phosphate isomerase 2, 3- Diphosphoglycerate 3PG 2PG EM Pathway Hexose monophosphate shunt Rapoport- Luebering shunt ATP ADP 2 ADP 2 ATP 1, 3-Diphosphoglycerate NADH NAD+ Fig. 22.6.10.1 The relationship between the main red cell Embden– Meyerhof glycolytic pathway (EM) and the other metabolic pathways. The insert shows the production of 2,3-DPG in the Rapoport–Luebering shunt. DPG, diphosphoglycerate; PG, phosphoglycerate.

22.6.10 Erythrocyte enzymopathies 5465 the detoxification of active oxygen radicals, and the transport of small molecules into and out of the cell. The lack of protein synthesis in the mature red cell means that none of the enzymes in the metabolic pathways can be replaced during the red cell lifespan. Over the 120 days of normal red cell sur- vival, enzyme activities decline at variable but predictable rates. This decline probably contributes to the ageing process of the red cell. Many of the abnormalities that affect red cell metabolism pro- voke haemolytic anaemia. The type (acute or chronic) and degree of anaemia depends on the metabolic cycle involved, the relative importance of the affected enzyme, the functional properties of the mutant enzyme with regard to kinetic abnormalities and/or in- stability, and the ability to compensate for the enzyme deficiency by overexpressing isoenzymes or using alternative pathways. The degree of anaemia may therefore vary from mild or fully compen- sated haemolysis to life-threatening neonatal anaemia and jaun- dice necessitating exchange transfusion and subsequent continuous transfusion support. Typical clinical symptoms may also include splenomegaly, jaundice, gallstones, and iron overload (with the latter seen in both transfused and nontransfused patients due to the down-regulation of hepcidin). Genetics Hereditary red blood cell (RBC) enzymopathies are caused by mu- tations in genes coding for red cell enzymes. Half of the normal activity of red cell enzymes is generally sufficient for normal func- tion, thus most haemolytic anaemias due to red cell enzyme defi- ciencies are inherited as autosomal recessive conditions. The only exception is the haemolytic anaemia associated with elevated ad- enosine deaminase activity, which is inherited as an autosomal dominant disorder. Only two of the deficiencies, those of G6PD and phosphoglycerate kinase (PGK), are encoded by genes on the X chromosome. Extensive mutation analysis at the DNA level has been carried out on patients with most of red cell enzyme defects, and most mutations have been found to be missense mutations or nonsense mutations; a few deletions, insertions, and splicing mutations have also been described, as have occasional mutations of regulatory machinery. Many of the mutations that affect red cell metabolism and provoke haemolytic anaemia cause instability and premature inactivation of the enzyme; other mutations directly affect catalytic activity. The molecular characterization of the defective enzyme has assumed an increasingly valuable role in the diagnosis of these disorders, including prenatal diagnostics and genetic counselling, especially as our understanding of the genetic basis of these disorders expands thanks to the development of high-throughput technologies, such as next-generation sequencing. Epidemiology Red cell enzyme defects have a worldwide distribution. For most, there are no exact and verified figures regarding their prevalence, in part due to the lack of certified registries, and in part due to the diagnostic

difficulty resulting from their heterogeneous and over- lapping clinical pictures. Pyruvate kinase deficiency (PKD), which is the most common glycolytic defect, has an estimated preva- lence of 1:20 000 in the general Caucasian population as assessed by gene frequency studies. Reported cases, however, are fewer than predicted—likely because of under-reporting, misdiagnosis and prenatal death in severe cases. For some of the other rarer enzyme defects, only a few cases are reported in the literature (Table 22.6.10.1). Specific red cell abnormalities that may cause haemolytic anaemia Table 22.6.10.1 summarizes some of the clinical and genetic char- acteristics of red cell enzyme deficiencies. The more common red cell enzyme abnormalities G6PD deficiency This important enzymopathy is described in Chapter 22.6.11. Pyruvate kinase deficiency Pyruvate kinase (PK) catalyses the conversion of phos phoenolpyruvate to pyruvate, coupled with the synthesis of ATP. PKD, which is the most common glycolytic defect, is typical of con- genital nonspherocytic haemolytic anaemias (CNSHAs) caused by red cell enzymopathies. PKD has been shown to have a protective effect against rep- lication of the malaria parasite in human red cells, although it is not clear whether PKLR mutant alleles are more prevalent in malaria endemic areas. Clinical manifestations of PKD com- prise the usual hallmarks of chronic haemolysis of variable se- verity, that is, anaemia, jaundice, and splenomegaly. The degree of anaemia varies widely, from very mild or fully compensated to life-threatening neonatal anaemia and jaundice requiring ex- change transfusion and subsequent continuous transfusion sup- port. Hydrops fetalis has also been reported in rare cases. The anaemia tends to improve as infants grow, whereas it is constant in adults with exacerbations in the context of infections, stress, and pregnancy. Since 2,3-DPG is elevated in individuals with PKD, the anaemia may be better tolerated than in other conditions, be- cause the oxygen dissociation curve is shifted to favour unloading of oxygen in the tissues. Iron overload is common in PKD, in both chronically transfused and untransfused individuals. In nontransfused subjects, predis- posing factors for iron loading include splenectomy, a certain de- gree of ineffective erythropoiesis, and coinheritance of hereditary haemochromatosis mutations. Monitoring iron status and meas- uring tissue iron burden by R2 (Ferriscan) or T2* magnetic reson- ance imaging (MRI) methods is indicated. PKD is one of the most difficult red cell enzymopathies to diag- nose. As well as its clinical heterogeneity, the enzyme itself is com- plex with allosteric properties. In PKD, the residual enzyme activity is not always greatly reduced and may even be normal in some cases (Fig. 22.6.10.2). Meaningful enzyme levels can only be achieved after total removal of leucocytes, which have up to 300 times the PK activity of red cells. No correlation has been observed between the residual PK activity, the degree of haemolysis, and the overall clinical severity. The block of glycolysis at the level of PK is associ- ated with an elevated 2,3-DPG concentration (two to three times normal).

section 22 Haematological disorders 5466 Table 22.6.10.1 Main features of red cell enzyme deficiencies OMIM Transmission Gene Chromosome No. of cases No. of mutations Haematological symptoms Other symptoms Response to splenectomy Embden–Meyerof pathway Hexokinase 235700 AR HK1 10q22.1 20 cases 5 CNSHA ++ Glucose-6-phosphate isomerase 172400 AR GPI 19q13.11

> “ 50 fam 31 CNSHA Intellectual disability? ++ Phosphofructokinase 232800 AR PFK-M PFK-L 12q13.11 21q22.3 50–100 cases 23 Erythrocytosis Minimal haemolysis Muscle disease, Tarui’s disase (glycogenosis type VII) 0 Aldolase

> 103850 AR ALDOA 16p11.2 6 cases 4 CNSHA Intellectual disability Dysmorphism ? Triosephosphate isomerise 190450 AR TPI1 12p13 50–100 cases 18 CNSHA Neuromuscular disease Infections 0 Phosphoglycerate kinase 300653 X-linked PGK1 X13.3 40 cases 20 CNSHA Neuromuscular disease ++ Pyruvate kinase 266200 AR PKLR 1q22 500 fam 200 CNSHA ++ Rapoport–Luebering shunt Bisphosphoglycerate mutase 222800 AR BPGM 7q33 6 fam 3 Erythrocytosis, CNSHA Hexose-monophosphate shunt Glucose-6-phosphate dehydrogenase 305900 X-linked G6PD Xq28 400 × 106 cases 180 CNSHA—acute Favism

- Glucose-6-phosphate dehydrogenase (class I) 305900 X-linked G6PD Xq28

> 50 fam 60 CNSHA—chronic

- Glutathione metabolism Glutathione synthetase AR GSS 20q11.22

> 50 fam 32 CNSHA Metabolic acidosis (5-oxoprolinuria) Neurological symptoms Drug/infections-induced haemolysis 0 Glutathione reductase 138300 AR GSR 8p21.1 2 fam 3 Induced oxidative HA, favism, neonatal jaundice Cataract ? γ-Glutamylcysteine synthetase (glutamate cysteine ligase) 230450 AR GCLC GCLM 6p12.1 1p21 12 fam 6 CNSHA, oxidative HA Metabolic acidosis (5-oxoprolinuria) Neurological symptoms ? Glutathione peroxidase AR GPX1 3p21.3 1 0 Acute intravascular haemolysis? ? Nucleotide metabolism Adenosine deaminase (hyperactivity) 102730 AD ADA 20q13.12 3 fam 0 CNSHA Adenylate kinase AR AK1 9q34.11 12 fam 7 CNSHA Motor impairment Intellectual disability ? Pyrimidine-5’-nucleotidase 606224 AR NT5C3A 7p14.3 60 fam 26 CNSHA ++ NADH-cytochrome b5 reductase 230800 AR CYB5R3 22q13.2 50 cases 45 Methaemoglobinaemia Neuromuscular disease Intellectual disability AD, autosomal dominant; AR, autosomal recessive; CNSHA, congenital nonspherocytic haemolytic anaemia; fam, families; HA, haemolytic anaemia. On a scale of 0 to 4+ where 4+ is a complete response, not usually required. In many cases data are meagre.

22.6.10 Erythrocyte enzymopathies 5467 Red cell morphology is commonly unremarkable, displaying anisocytosis and a variable portion of spur cells or acanthocytes, particularly after splenectomy. None of these findings is specific for PKD. Since the younger PK-deficient erythrocytes are selectively se- questered by the spleen, splenectomy usually results in a marked in- crease of reticulocytes. More than 220 different mutations have been described in PKLR gene, causing PKD; many individuals are compound heterozygotes. Not surprisingly, there is enormous genetic heterogeneity between affected subjects, reflected in the multiplicity of quantitative and

kin- etic defects. In European populations, the most common mutations are c.1529 G>A (Arg510Gln) and c.1456 C>T (Arg486Trp). These mutations have not been detected among Asians with PKD; among Roma peoples, the characteristic mutation results in a deletion of exon 11, while a mutation at c.1436G>A (Arg479His) is commonly found in Amish populations. No curative therapy for PKD is available to date and the treatment is therefore based on supportive measures. Red cell transfusions may be required in severely anaemic patients, particularly in the first years of life; the haemoglobin then tends to stabilize in many cases at about 80 g/litre. Splenectomy does not arrest haemolysis, and should be reserved for severely affected, young patients who need regular blood transfusions, and to patients who do not tolerate anaemia. It usually results in an increase of 10 to 30 g/litre in haemoglobin, re- ducing or even eliminating in most cases transfusion requirements (Fig. 22.6.10.3). Bone marrow transplantation has been performed in a few very severely affected children. Other red cell enzyme abnormalities These are summarized in Box 22.6.10.1. Glucose-6-phosphate isomerase deficiency Glucose-6-phosphate isomerase deficiency is a further cause of CNSHA along with G6PD deficiency and PKD. This enzyme cata- lyses the second step of glycolysis, the interconversion of glucose 6-phosphate to fructose 6-phosphate. It is also known as phos phohexose isomerase, phosphoglucose isomerase, autocrine motility factor, and neuroleukin, indicating that the protein has other actions in other cells. Most reported cases of glucose-6-phosphate isomerase deficiency present with mild to moderate haemolytic anaemia, but hydrops fetalis has also been described. In some rare deficient patients, neurological impairment or intellectual disability has been reported. 16 16 12 12 10 8 8 6 4 4 0 1600 1200 800 400 0 0 2 4 6 8 10 12 14 0 2 4 6 PK activity (IU/gHb) PK activity (IU/gHb) PK activity (IU/gHb) (a) (b) (c) Hb (g/dl) Reticulocytes (109/litre) 8 10 12 14 2 Fig. 22.6.10.2 Enzyme activity in PK deficient patients. ●, not splenectomized patients, ●, splenectomized patients. TX/yr

> 2 0 18 16 14 12 10 8 6 4 2 0 Hb (g/dl) 16 12 Children (a) (b) Adults 11 10 9 8 7 6 5 4 3 Fig. 22.6.10.3 Effect of splenectomy on (a) Hb levels and on (b) transfusion needs in PKD patients. ●/■, before splenectomy, ○/□, after splenectomy.

section 22 Haematological disorders 5468 About 30 different mutations have been identified, underlining the molecular heterogeneity and consequently the clinical hetero- geneity of this disorder. The response to splenectomy is usually sat- isfactory although it does not fully correct the haemolysis. Pyrimidine 5′-nucleotidase deficiency Pyrimidine 5′-nucleotidase catalyses the dephosphorylation of the pyrimidine nucleotides uridine monophosphate (UMP) and cytidine monophosphate (CMP) in the corresponding nucleosides uridine and cytidine. The defect of pyrimidine 5′-nucleotidase leads to a marked accumulation of pyrimidine nucleotides that interferes with the adenine nucleotide pool, and results in a haemolytic anaemia characterized by pronounced basophilic stip- pling in the red cells (Fig. 22.6.10.4). The appearance of the blood film is similar to that seen in lead poisoning and the haemolytic anaemia associated with this condition is likely to be due to the inhibition of pyrimidine 5′-nucleotidase. In congenital forms, the haemolysis is usually mild to moderate, although severe cases have also been reported. The accumulation of pyrimidine nucleotides can be easily documented by measuring the ultraviolet absorp- tion spectrum. Splenectomy commonly results in stabilization of the haemoglobin to higher levels. Pyrimidine 5′-nucleotidase activity may also be decreased in aplastic anaemia and the tran- sient erythroblastopenia of childhood, which can potentially lead to misdiagnosis. Triose-phosphate

isomerase deficiency Triose-phosphate isomerase (TPI) catalyses the interconversion of dihydroxyacetone-phosphate and glyceraldehyde 3-phosphate, which is then metabolized along the glycolytic pathway. The en- zyme is ubiquitously expressed. TPI deficiency, reported in more than 50 cases, results in a multisystem disorder consisting of chronic haemolytic anaemia, increased propensity to infections, and severe progressive neuromuscular degeneration (the latter likely due to the formation of toxic protein aggregates induced by misfolded TPI). With few exceptions, death occurs usually at about the age of 5 years, and may occur as a consequence of cardiac dys- rhythmia. Several point mutations have been identified that lead to this disease; the most predominant is Glu104Asp, linked by common haplotypes suggesting descent from a common ancestor. There is no specific treatment, but supportive care, including as- sisted ventilation, may prolong life in some instances. The rare red cell enzyme deficiencies Hexokinase deficiency Hexokinase catalyses the phosphorylation of glucose to glucose 6-phosphate, the first step in the glycolytic pathway. The defect of this enzyme has been recorded in about 20 cases characterized by molecular and phenotypic heterogeneity. Most patients have mod- erately reduced activity; complete hexokinase deficiency is prob- ably lethal. The enzyme deficiency results in moderate to mild anaemia, but some cases present with severe anaemia and death in the neonatal period. Typically, reduced hexokinase activity is as- sociated with a low concentration of 2,3-DPG within the red cells. Patients have lower exercise tolerance for a given level of haemo- globin than would be expected, because of the left shift in the oxygen dissociation curve. Hexokinase deficiency is often difficult to diagnose because the activity of this enzyme is much higher in young red cells than in older erythrocytes. As a result, hexokinase activity is usually in- creased in patients with haemolytic anaemia of any type. In patients with hexokinase deficiency, this often gives rise to the anomalous finding that the red cell enzyme activity in the affected patient is normal, and indeed is usually higher than that found in the hetero- zygous parents. A careful examination of erythrocyte enzyme activ- ities is therefore recommended, using other age-dependent enzymes such as PK or G6PD as an internal control. Phosphofructokinase deficiency Phosphofructokinase catalyses a reaction in which fructose 6- phosphate is phosphorylated to fructose 1,6-diphosphate, ATP being the donor of the phosphate group. Under normal physio- logical conditions, this may be the major rate-limiting step in gly- colysis in the red cell. Erythrocytes contain two types of genetically distinct phosphofructokinase subunits, L (liver) and M (muscle), organized in tetramers composed of M and L subunits; there may be five isoenzymes composed of different numbers of L and M sub- units. Phosphofructokinase is a homotetramer of M subunits (M4) in muscle and of L subunits (L4) in liver. A third subunit is found in platelets. Deficiency of the M subunit causes haemolysis, but the haemoglobin level in the blood is often normal or even higher than normal because of the diminished 2,3-DPG levels that are Box 22.6.10.1 Other red cell enzyme abnormalities • Glucose-6-phosphate isomerase deficiency • Pyrimidine 5′-nucleotidase deficiency • Triosephosphate isomerase deficiency Very rare enzyme defects • Hexokinase deficiency • Phosphofructokinase deficiency • Phosphoglycerate kinase • Aldolase deficiency • Deficiency of enzymes of glutathione cycle • Adenylate kinase deficiency • Red cell adenosine deaminase hyperactivity Fig. 22.6.10.4 Peripheral blood film in pyrimidine 5′-nucleotidase deficiency showing basophilic stippling (arrows).

22.6.10 Erythrocyte enzymopathies 5469 characteristic of this disorder. Muscle enzyme activity is also com- promised, and a myopathy results. It is characterized by muscle cramps and myoglobinuria on exertion. This disorder is sometimes designated Tarui’s disease or type VII glycogenosis. Shortened red cell viability may be a minor component of this disease. Deficiency of

the L subunit of phosphofructokinase has also been reported, but without any clinical consequence. Aldolase deficiency Aldolase catalyses the conversion of fructose 1,6-diphosphate to glyceraldehyde 3-phosphate and dihydroxyacetone phosphate. There are three aldolase isoenzymes in human tissues, A, B, and C; A isoenzyme is expressed in red cells. The deficiency of aldolase A is extremely rare. The defect results in haemolytic anaemia which may be associated with intellectual disability, dysmorphic features, or myopathy. Phosphoglycerate kinase PGK catalyses the reversible phosphotransfer reaction from 1,3- bisphosphoglycerate to 3-phosphoglycerate with production of ATP. PGK deficiency is an uncommon X-linked inherited disorder. The enzyme is monomeric and is expressed in all tissues. PGK defi- ciency shows a wide clinical phenotype: it may present with chronic nonspherocytic haemolysis, behavioural disturbances, neurological impairment (intellectual disability, and ataxia), and myopathy (ex-ercise intolerance or muscle weakness). A few affected individuals suffer the full spectrum of symptoms, whereas some cases are de- scribed with myopathy and no haemolysis. Diphosphoglycerate mutase deficiency The DPG mutase is a multifunctional enzyme catalysing the reaction from 1,3-DPG to 2,3-DPG. DPG mutase deficiency more frequently leads to erythrocytosis than haemolytic anaemia, because a lack of this enzyme prevents the formation of 2,3-DPG. Consequently, the oxygen affinity of the red cells is increased, stimulating erythropoi- esis and resulting in secondary erythrocytosis. Enzymes of glutathione synthesis Glutathione is the major intracellular thiol in aerobic cells, and is equally important in the erythrocytes. It has a number of critical functions: protecting cells against oxidative damage, participa- tion in detoxification of foreign compounds, maintenance of pro- tein sulphydryl groups in a reduced state, and possibly transport of amino acids. In red cells, its main function is as an antioxidant. Glutathione is synthesized from glutamate, cysteine, and glycine by two ATP-dependent reactions catalysed by γ-glutamylcysteine synthetase and glutathione synthetase (Fig. 22.6.10.5). Defects of both enzymes, occurring with a recessive mode of transmis- sion, are rare and result in chronic nonspherocytic haemolytic anaemia with increased susceptibility to oxidative stress. Severe deficiency leads to 5-oxoprolinuria, metabolic acidosis, and in- tellectual disability. Complete loss of glutathione synthesis is probably lethal. Oxidized glutathione is reduced to glutathione by the action of glutathione reductase, the hydrogen donor being NADPH. Only a single family with severe, hereditary deficiency of gluta- thione reductase has been described. No haemolysis was present, ex- cept after ingestion of fava beans. Low activity of red cell glutathione reductase, a flavin enzyme, is found when the intake of riboflavin is suboptimal, but this mild or moderate enzyme deficiency has no clinical consequences. Adenylate kinase deficiency Adenylate kinase modulates the interconversion of adenine nu- cleotides catalysing the reversible phosphoryl transfer reac- tion from ATP to AMP and ADP. Adenylate kinase deficiency is a rare genetic disorder, with only 12 affected families reported in the literature. From a clinical point of view, erythrocyte adenylate Glutamic acid + Cysteine Glycine + y-Glutamylcysteine y-Glutamylcysteine synthetase Glutathione synthetase Glutathione S-transferase Glutathione peroxidase Glutathione reductase NADPH MetHb reductase Hb Fe2+ MetHb Reduced glutathione Oxidized glutathione H2O H2O2 Catalase NADP+ NADPH G6PD G6P 6PG ATP ATP RSSR RSH ADP ADP Fig. 22.6.10.5 The glutathione cycle and synthetic pathway. Redox control is exercised by the glutathione cycle linked to the NADPH of the pentose phosphate pathway by glutathione reductase.

 kinase deficiency is associated with moderate to severe chronic nonspherocytic haemolytic anaemia in all cases but one. In add- ition, psychomotor impairment has also been observed in a few patients. The relationship between this enzyme

deficiency and haemolytic anaemia remains unclear. Red cell adenosine deaminase hyperactivity Adenosine deaminase (ADA) catalyses the deamination of both adenosine to inosine and 2′-deoxyadenosine to 2′-deoxyinosine. Mutations causing a decreased ADA activity do not affect RBC metabolism, but induce a severe immunodeficiency with an auto- somal recessive inheritance. Only the overexpression of ADA, with an autosomal mode of inheritance, produces haemolytic anaemia. The ADA that is formed appears to be normal and the abnormality that causes this tissue-specific increase in enzyme activity has not yet been discovered. Specific red cell abnormalities that do not cause haemolytic anaemia Severe deficiencies of many red cell enzymes do not produce haem- atological abnormality or, indeed, in many cases, any clinical ab- normality at all. Included are deficiencies of 6-phosphogluconate dehydrogenase, δ-aminolaevulinic acid dehydrase, acetyl-cholinesterase, AMP deaminase, carbonic anhydrase, catalase, galactokinase, galactose-1-phosphate uridyltransferase, glutathione peroxidase, hypoxanthine-guanine phosphoribosyltransferase, ITPase, and phosphoglucomutase. Discussion of these enzyme defi- ciencies is beyond the scope of this chapter. General approach in diagnosis of red cell enzymopathies, differential diagnosis This is summarized in Box 22.6.10.2. The clinical and haematological features of most enzyme defects are common to other haemolytic diseases, so that the diagnostic process in these rare disorders is the final step of a diagnostic workup based not only on laboratory tests but also on clinical examination, personal and family history, and the exclusion of the most common causes of acquired haemolytic anaemia. The diag- nosis ultimately depends upon the demonstration of low enzyme activity and molecular analysis. However, given the rarity and the wide clinical heterogeneity, the diagnosis of these defects can be difficult. The main laboratory features include the following: • Increased unconjugated bilirubin and lactate dehydrogenase levels, reduced haptoglobin, increased absolute reticulocyte number, and negative direct antiglobulin test. • Normochromic anaemia, sometimes producing a slight macrocytosis. An increased mean cell haemoglobin concentration is occasionally seen in severe cases due to dehydration brought about by ATP deficiency. • Usually unremarkable RBC morphology with anisocytosis and a variable portion of spur cells (which are not specific), particularly after splenectomy. The presence of prominent red cell stippling suggests a diagnosis of pyrimidine 5′-nucleotidase deficiency. • Normal osmotic fragility, normal or sometime increased fluor- escence intensity of RBC labelled with eosin-5-maleimide (flow- cytometry EMA-binding test). • Noninformative osmotic gradient ektacytometry or Laser-assisted Optical Rotational Cell Analyzer (LoRRca). The coexistence of CNSHAs and neuromuscular symptoms is suggestive of triose-phosphate isomerase deficiency, PGK, or other rare enzyme defects. Specific laboratory tests include red cell enzyme activity assays and molecular testing. Red cell enzyme assays The diagnosis of the causative enzyme disorder underlying CNSHAs is best achieved by measuring red cell enzyme activity by a quan- titative assay using spectrophotometric methods. Quantification of rarer RBC enzyme activities is a more specialized task that can be ac- complished by the use of standardized techniques in an experienced laboratory. There are a number of caveats that must be taken into account, both with respect to the performance of red cell enzyme assay and the interpretation of the results: • Leucocyte contamination: in some cases (i.e. PKD), the leucocyte isoenzyme displays much higher activity than that of red cells. Thus, contamination of a red cell suspension with a relatively small number of white cells may obscure the diagnosis. • Reticulocytosis: the interpretation of the results of a red cell en- zyme assay may also be confounded by the fact that the blood of patients with haemolytic anaemia is enriched with reticulocytes and young erythrocytes. Since many of the mutations that cause red cell enzymopathies result in the production of unstable en- zymes, the young circulating erythrocytes

may actually contain normal or near-normal levels of enzyme. It is therefore essential to take into account the age of the circulating cells. Family studies may help the diagnosis in these cases. • Recent transfusions: it is clearly best to wait until just before a transfusion to draw blood for testing. Moreover, problems in interpretation may also arise when the activity of an enzyme as measured in vitro does not accurately re- flect its intracellular in vivo activity. This arises because of the ne- cessity of using exceedingly high substrate concentrations for the in vitro assay. This can be particularly problematic in the case of PKD, because this complex allosteric enzyme has binding sites for two Box 22.6.10.2 General approach in diagnosis of red cell enzymopathies • The diagnostic process is the final step of a diagnostic workup based not only on laboratory tests but also on clinical examination, and per- sonal and family history. • Due to the rarity and the wide clinical heterogeneity, the diagnosis of these defects can be difficult. • The diagnosis of red cell enzymopathies ultimately depends on the exclusion of other causes of haemolytic anaemia and upon the dem- onstration of defective enzyme activity. • Molecular testing is strongly recommended to confirm the diagnosis, and for genetic counselling.

22.6.10 Erythrocyte enzymopathies 5471 substrates, ADP and phosphoenolpyruvate, and also for fructose di- phosphate, an allosteric effector. Molecular characterization Molecular testing now plays an increasing role in the diagnosis of most red cell enzyme defects. Molecular characterization confirms the diagnosis and is necessary for genetic counselling. Genotype–phenotype correlations have been performed in some enzyme defects such as PK, glucose-6-phosphate isomerase, PGK, and pyr- imidine 5′-nucleotidase deficiencies. Generation and characteriza- tion of recombinant variants helped to define the effects of amino acid replacement on the enzyme's functional properties, and to cor- relate genotype to clinical phenotype. However, different putative modifiers may alter the expression of the mutant enzyme, such as differences in splenic function, differences in genetic background (such as some functional polymorphisms of other glycolytic en- zymes, e.g. HFE and UGT1A1 genotypes), compensatory expression of other isozymes, a variable degree of ineffective erythropoiesis, or other abnormalities in unknown regulatory regions/unknown genes. In case of incomplete molecular characterization (mutations identified at heterozygous level or no mutation at all identified in the suspected gene), other causes of haemolysis should be excluded wherever possible. Molecular analysis has some advantages over enzyme assay-based diagnosis. First, DNA is very stable, even before it is purified, so transporting samples of blood to reference laboratories is less of a logistical problem. Transfused red cells do not pose a problem in performing DNA-based diagnosis, since transfused leucocytes do not persist in the circulation. Complications Iron overload may be a complication in RBC enzyme defects, not only in chronically transfused patients but also in untransfused subjects, particularly in PKD. Predisposing factors for iron loading include splenectomy, a certain degree of ineffective erythropoiesis, and coinheritance of hereditary haemochromatosis (HFE gene) mu- tations. Ferritin levels should be monitored and R2/T2* MRI moni- toring may be required. Gallstone formation occurs frequently in haemolytic anaemias and may requires periodic abdominal ultrasound monitoring. Extramedullary haematopoiesis is a potential complication, and paraspinal lesions causing cord compression may arise. Leg ulcers, similar to those reported in patients with sickle cell disease and her- editary spherocytosis, have been reported in patients with severe anaemia. Management of red cell enzyme defects The treatment of red cell enzyme defects is based on supportive measures: folate therapy is recommended in severe and moderate forms of haemolytic anaemia; red cell transfusions may be re- quired in severely anaemic patients, particularly in the first years of life, during aplastic crises, infections, and pregnancy. Some pa- tients may require splenectomy. Considering the heterogeneous aetiology it is not surprising that

the response may be difficult to predict. In general, splenectomy is beneficial in some patients suffering from deficiencies of PK, hexokinase, glucose-6-phosphate isomerase, PGK, and pyrimidine 5′-nucleotidase. Prior to splen- ectomy, all patients should receive immunizations according to the Centers for Disease Control and Prevention guidelines for patients with asplenia, including the pneumococcal, meningococcal, and haemophilus influenzae vaccines. Postsplenectomy, these vaccines should be boosted at regular intervals according to the same guide- lines and any newly recommended vaccines should be administered in children and adults. Penicillin prophylaxis generally is recom- mend for 1 to 2 years postsplenectomy; some clinicians recommend lifelong prophylaxis, though this is more controversial. PKD has rarely been treated by stem cell transplantation. Future directions for therapy No specific drug therapies are available for the routine clinical treat- ment of enzyme defects. A pharmacological activator of PKLR is presently in clinical trials. A gene therapy strategy has not been so far attempted. Studies in mice have shown prolonged expression of PK in the blood and haematopoietic organs of mice after intro- duction of a viral vector expressing human liver-type PK cDNA. Additionally, other investigators have shown increased expression of PK in transgenic mice using a gene-addition strategy. More recently, healthy erythroid cells has been obtained from gene-edited PKD- induced pluripotent stem cells by nonintegrative lentiviral vectors. FURTHER READING Ayi K, et al. (2008). Pyruvate kinase deficiency and malaria. N Engl J Med, 358, 1805–10. Beutler E, et al. (1977). International committee for standardization in haematology: recommended methods for red cell enzyme analysis. Br J Haematol, 35, 331–40. Beutler E (1984). Red cell metabolism: a manual of biochemical meth- ods, 3rd edition. Grune & Stratton, New York. Beutler E (2007). PGK deficiency. Br J Haematol, 136, 3–11. Bianchi P, et al. (2003). Molecular characterization of six unrelated Italian patients affected by pyrimidine 5’-nucleotidase deficiency. Br J Haematol, 122, 847–51. Chiarelli LR, et al. (2012). Molecular insights on pathogenic effects of mutations causing phosphoglycerate kinase deficiency. PLoS One, 7, e32065. Fermo E, et al. (2012). A new variant of phosphoglycerate kinase de- ficiency (p.I371K) with multiple tissue involvement: molecular and functional characterization. Mol Genet Metab, 106, 455–61. Garate Z, et al. (2015). Generation of a high number of healthy eryth- roid cells from gene-edited pyruvate kinase deficiency patient- specific induced pluripotent stem cells. Stem Cell Reports, 5, 1053–66. Grace RF, et al. (2015). Erythrocyte pyruvate kinase deficiency: 2015 status report. Am J Hematol, 90, 825–30. Hipkins R, et al. (2009). Images in haematology. Paravertebral extramedullary haemopoiesis associated with pyruvate kinase defi- ciency. Br J Haematol, 147, 275. Koralkova P, van Solinge WW, van Wijk R (2014). Rare hereditary red blood cell enzymopathies associated with hemolytic anemia: patho- physiology, clinical aspects, and laboratory diagnosis. Int Jnl Lab Hem, 36, 388–97.

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