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Point Mutations That Reduce Erythrocyte Resistance to Oxidative Stress

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1. Introduction

Oxygen transport is a primary goal of erythrocytes. The high maintenance of oxygen in erythrocytes defines high speed of formation of active forms of oxygen – superoxide ($O2^-$), a hydrogen peroxide (H2O2) and a hydroxyl radical (·OH). A constant source of active forms of oxygen in erythrocytes is hemoglobin oxidation in a methemoglobin with formation of superoxide ($O2^-$). Therefore erythrocytes should have a powerful antioxidant system, which prevents the toxic action of active forms of oxygen on hemoglobin and erythrocyte membrane. Mature erythrocytes have neither cytoplasmic organelles nor a nucleus and consequently are not capable to synthesize proteins and lipids, to carry out oxidative phosphorylation or to maintain tricarboxylic acid cycle reactions. The energy of erythrocytes comes for the most part from anaerobic glycolysis – via the Embden-Meyerhof-Parnas pathway (EMP pathway). Thus, glucose catabolism provides preservation of structure and function of hemoglobin, integrity of an erythrocyte membrane and formation of energy for the work of ionic pumps. Anaerobic glycolysis in itself is a power-consuming process. Glucose arrives in erythrocytes by the facilitated diffusion by glucose transporter type 1. Hexokinase is the first enzyme of EMP pathway, it provides glucose phosphorylation. Further during consecutive reactions with participation of glucose-6-phosphate isomerase, phosphofructokinase, aldolase, glyceraldehydes 3-phosphate dehydrogenase, phosphoglycerate kinase, phosphoglycerate mutase, enolase, pyruvate kinase one molecule of glucose gives 4 molecules of adenosine triphosphate (ATP) and 2 molecules of restored nicotinamide adenine dinucleotide (NADH), and at the same time, 2 ATP molecules are spent at the initial stage of EMP pathway. A certain quantity of glucose with formation of restored compounds – glutathione (GSH) and nicotinamide adenine dinucleotide phosphate (NADPH) is taken away through pentose phosphate pathway (aerobic glycolysis). Glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase provide the stages of pentose phosphate pathway. The hydroxyl radical, the most active component of oxidative stress, is neutralized by GSH. Methemoglobin reductase restores a methemoglobin into hemoglobin, NADPH being the donor of hydrogen, which is formed in EMP pathway and NADPH is in its turn formed in pentose phosphate pathway. Superoxide dismutase 1 enzyme contributes superoxide ($O2^-$) to turn into hydrogen peroxide. The hydrogen peroxide is destroyed by catalase and glutathione peroxidase, GSH being the donor of hydrogen. Peroxiredoxin 2 is an antioxidant enzyme that uses cystein residues to

decompose peroxides. Peroxiredoxin 2 is the third most abundant protein in erythrocytes, and competes effectively with catalase and glutathione peroxidase to scavenge low levels of hydrogen peroxide, including that derived from hemoglobin autoxidation. GSH reductase restores oxidized GSH at the expense of NADPH energy. The final step in GSH synthesis is catalysed by the glutathione synthetase. Thus, resistance of erythrocytes to oxidative stress will depend on the activity of glucose transporter type 1, glycolysis enzymes, glutathione synthetase, glutathione reductase, glutathione peroxidase, peroxiredoxin 2, superoxide dismutase 1, catalase and nucleotide metabolism. Activation of oxidative stress occurs in case of infection, hypoxic ischemia, acidosis, effect of some medications and toxins. Low resistance of erythrocytes to oxidative stress leads to hemoglobin precipitation and erythrocytes hemolysis. Thus, erythrocytes become sources of active forms of oxygen. Oxidative stress has been implicated in many human diseases. Activity of erythrocyte antioxidant enzymes is closely studied to reveal oxidative stress status in various pathological conditions.

Herein we describe the recent updates regarding point mutations, which contribute to the decrease of antioxidant protection of erythrocytes.

2. Point mutations in proteins and enzymes providing a metabolism of erythrocytes

2.1 Point mutation in glucose transporter GLUT 1

GLUT1 was the first glucose transporter isoform to be identified, and is one of 13 proteins that comprise the human equilibrative glucose transporter family. GLUT1 is a membrane-spanning glycoprotein of 492 amino acids, containing 12 transmembrane domains with both *N*- and *C*-termini located in cytosol, and its gene being located on chromosome 1 (1p35-31.3) is composed of ten exons and nine introns. GLUT1 is expressed at the highest levels in the plasma membranes of proliferating cells forming the early developing embryo, in cells forming the blood-tissue barriers, in human erythrocytes and astrocytes, and in cardiac muscle (Carruthers et al., 2009). Heterozygous mutations in the GLUT1 gene have been reported in sporadic patients and results in autosomal dominant pedigrees. Expression of mutant transporters resulted in a significant decrease in transport activity of GLUT1. Impaired glucose transport across brain tissue barriers is reflected by hypoglycorrhachia and results in epilepsy, mental retardation and motor disorders. The first autosomal dominant missense mutation (G272A) has been reported within the human GLUT1 gene and wos shared by three affected family members. Substitution of glycine-91 by site-directed mutagenesis with either aspartate or alanine was studied in oocytes. The data agree with 3-O-methyl-glucose uptake into patient erythrocytes and indicate that the loss of glycine rather than a hydrophilic side chain (Gly91→Asp) defines the functional consequences of this mutation. (Klepper et al., 2001). Recently, mutations in GLUT1 gene have been identified as a cause in some patients with autosomal dominant paroxysmal exercise-induced dyskinesias (PED). PED are involuntary intermittent movements triggered by prolonged physical exertion. Some patients had a predating history of childhood absence epilepsy and a current history of hemiplegic migraine as well as a family history of migraine (Schneider et al., 2009). In certain cases PED was accompanied by hemolytic anemia with echinocytosis, and altered erythrocyte ion concentrations. Using a candidate gene approach,

a causative deletion of 4 highly conserved amino acids (Q282_S285del) in the pore region of GLUT1 was identified. Functional studies in Xenopus oocytes and human erythrocytes revealed that this mutation decreased glucose transport and caused a cation leak that alters intracellular concentrations of sodium, potassium, and calcium. In families where PED is combined with epilepsy, developmental delay, or migraine, but not with hemolysis or echinocytosis 2 GLUT1 mutations were identified (A275T, G314S) that decreased glucose transport but did not affect cation permeability (Weber et al., 2008). The causative mutations for some forms of hereditary stomatocytosis have been found to result from mutations in SLC2A1, encoding GLUT1. Stomatocytosis was associated with a cold-induced cation leak, hemolytic anemia and hepatosplenomegaly but also with cataracts, seizures, mental retardation and movement disorder (Flatt et al., 2011).

2.2 Point mutation in glycolysis enzymes

2.2.1 Point mutation in hexokinase

Hexokinase (HK) catalyses the phosphorylation of glucose to glucose-6-phosphate using adenosine triphosphate as a phosphoryl donor. The four isozymes of the HK family (HK1, HK2, HK3, and glucokinase) contribute to commit glucose to the glycolytic pathway, each of which is encoded by a separate gene. The predominant HK1 isozyme is expressed in the vast majority of cells and tissues, including cells that are strictly dependent on glucose uptake for their metabolic needs. While most tissues express more than one HK isozyme, erythrocytes glucose metabolism only depends on HK1 activity. HK1 is one of the rate-limiting enzymes in erythrocytes glycolysis. Gene structure and exon-intron organization of the HK1 gene have been elucidated from a sequence of three contiguous genomic clones localized at human chromosome 10. The sequence spans about 131 kb, and consists of 25 exons, which include 6 testis- and 1 erythroid-specific exons. The HK1 and erythroid-specific HK-R transcripts being produced by using two distinct promoters. Thus, the first and second exons are specifically utilized for the erythroid-specific HK-R and ubiquitously expressed HK1 isozymes respectively (Kanno 2000; Murakami et al., 2002; van Wijk at al., 2003; Bonnefond et al., 2009). In humans, mutations including nonsynonymous substitutions in the active site of HK1 and intragenic deletions have been shown to cause HK1 enzymatic deficiency associated with autosomal recessive severe nonspherocytic hemolytic anemia (Bonnefond et al., 2009). Mutation affecting the substrate affinites of the enzyme, regulatory properties, heat stability have been described (Rijksen et al., 1983; Magnani et al., 1985). HK deficiency is a very rare disease with a clinical phenotype of hemolysis. PCR amplification and sequence of the cDNA in patients with HK deficiency revealed the presence of a deletion and of a single nucleotide substitution, both in heterozygous form. In particular, the deletion, 96 bp long, concerns nucleotides 577 to 672 in the HK cDNA sequence and was not found in the cDNAs of 14 unrelated normal subjects. The sequence of the HK allele without deletion showed a single nucleotide substitution from T to C at position 1667 which causes the amino acid change from Leu529 to Ser (Bianchi et al., 1995). The T1667→C substitution, causing the amino acid change Leu529→Ser, is responsible for the complete loss of the hexokinase catalytic activity, while the 96 bp deletion causes a drastic reduction of the hexokinase activity (Bianchi et al., 1997). A homozygous missense mutation in exon 15 (2039C→G, HK Utrecht) of HK1, the gene that encodes red blood cell-specific hexokinase-R,

in a patient previously diagnosed with hexokinase deficiency has been reported. The Thr680→Ser substitution predicted by this mutation affects a highly conserved residue in the enzyme's active site that interacts with phosphate moieties of adenosine diphosphate, adenosine triphosphate, and glucose-6-phosphate inhibitor (van Wijk et al. 2003). On the paternal allele in a patient with chronic hemolysis two mutations in the erythroid-specific promoter of HKI: 373A→C and 193A→G were identified. Transfection of promoter reporter constructs showed that the 193A→G mutation reduced promoter activity to 8%. Hence, 193A→G is the first mutation reported to affect red blood cell-specific hexokinase specific transcription. On the maternal allele there was a missense mutation in exon 3: 278G→A, encoding an arginine to glutamine substitution at residue 93 (Arg93→Glu), affecting both hexokinase-1 and erytrocytes specific-hexokinase. This missense mutation was shown to compromise normal pre-mRNA processing. Reduced erythroid transcription of HK1 together with aberrant splicing of both hexokinase-1 and erytrocytes specific-hexokinase results in HK deficiency and mild chronic hemolysis (de Vooght et al. 2009).

2.2.2 Point mutation in glucose-6-phosphate isomerase

Glucose-6-phosphate isomerase (GPI) catalyzes interconversion of glucose-6-phosphate and fructose 6-phosphate in the Embden-Meyerhof glycolytic pathway. GPI is an essential enzyme for carbohydrate metabolism in all tissues. In humans, the GPI gene locus is located on chromosome 19, and the gene spans more than 40 kb, including 18 exons and 17 introns. The cDNA sequence encodes 558 amino acid residues. The enzyme consists of two identical subunits. In mammals, GPI can also act as an autocrine motility factor, neuroleukin, and maturation factor. GPI deficiency is a well-known congenital autosomal recessive disorder with the typical manifestation of nonspherocytic hemolytic anemia of variable severity in humans. GPI deficiency is one of the most common cause of congenital nonspherocytic hemolytic anemia caused by deficiency of glycolytic enzymes, the commonest being deficiency of glucose-6-phosphate dehydrogenase and pyruvate kinase. Patients with inherited GPI deficiency present with nonspherocytic anemia of variable severity and with neuromuscular dysfunction. Mutations in the GPI gene usually have negative influences on catalytic parameters, particularly k(cat), as well as structure stability. Mutations at or close to the active site, including R273H, H389R, and S278L, cause great damage to the catalytic function, yet those at distance can still reduce the magnitude of k(cat). At the nucleotide level, 29 mutations have been reported. Mutations decrease the enzyme tolerance to heat by mechanisms of decreasing packing efficiency (V101M, T195I, S278L, L487F, L339P, T375R, I525T), weakening network bonding (R75G, R347C, R347H, R472H, E495K), increasing water-accessible hydrophobic surface (R83W), and destabilizing the ternary structure (T195I, R347C, R347H, and I525T). A300P, L339P, and E495K mutations may also negatively affect the protein folding efficiency (Merkle S. at al., 1993; Kanno H. at al., , 1996; Kugler W. at al., 2000; Haller J.F. et al., 2009; Haller J.F., et al., 2010). The neurologically affected patient (GPI Homburg) is compound heterozygous for a 59 A→C (H20P) and a 1016 T→C (L339P) exchange. Owing to the insertion of proline, the H20P and L339P mutations are likely to affect the folding and activity of the enzyme. Point mutations identified at 1166 A→G (H389R) and 1549 C→G (L517V), which are located at the subunit interface showed no neurological symptoms. Thus mutations that lead to incorrect folding destroy both catalytic

(GPI) and neurotrophic activities, thereby leading to the observed clinical symptoms (GPI Homburg). Those alterations at the active site, however, that allow correct folding retain the neurotrophic properties of the molecule (GPI Calden) (Kugler, 1998). The similarity of the mutant enzymes to the allozymes found in human GPI deficiencies indicates the GPI deficient mouse mutants to be excellent models for the human disease (Padua et al., 1978; Pretsch et al., 1990). A heterozygous mouse mutant exhibiting approximately 50% of wild-type GPI activity. Biochemical and immunological studies revealed no differences in physicochemical, kinetic and immunological properties between the erythrocytic enzyme of heterozygous and wild-type mouse. The genetic and physiological analyses provided no indications for further altered traits in heterozygous animals including fertility, viability and several other traits. Homozygous null mutants died at an early post-implantation stage of embryogenesis (West et al., 1990; Merkle et al., 1992). Homozygous GPI deficiency in humans are responsible for chronic nonspherocytic hemolytic anemia. The homozygous missense A346H mutation replacement in cDNA position 1040G→A, which causes a loss of GPI capacity to dimerize, which renders the enzyme more susceptible to thermolability and produces significant changes in erythrocyte metabolism was described in patient with chronic nonspherocytic hemolytic anemia (Repiso et al., 2005). Biochemical and molecular genetic studies performed with the enzyme variants of GPI Zwickau and GPI Nordhorn showed that in both cases the simultaneous occurrence of a single amino acid substitution affecting the active site, together with a nonsense mutation leading to the loss of major parts of the enzyme probably explains the severe clinical course of the disease (Huppke et al., 1997). Molecular characteristics of erythrocytes GPI deficiency were described in Spanish patients with chronic nonspherocytic hemolytic anemia. Residual GPI activity in erythrocytes of around 7% (GPI-Catalonia), in an individual is homozygous for the missense mutation 1648A→G (Lys550→Glu) in exon 18 was described and residual activity in erythrocytes of around 20% (GPI-Barcelona), was found in a compound heterozygote for two different missense mutations: 341A→T (Asp113→Val) in exon 4 and 663T→G (Asn220→Lys) in exon 7. Molecular modeling using the human crystal structure of GPI as a model was performed to determine how these mutations could affect enzyme structure and function (Repiso et al., 2006). Chinese hamster (CHO) cell lines with ethylmethane sulfonate induced mutations in GPI and consequent loss of GPI activity have been reported. GPI activity was reduced by 87% in GroD1 isolated from this population. Expression cloning and sequencing of the cDNA obtained from GroD1 revealed a point mutation Gly189→ Glu. This resulted in a temperature sensitivity and severe reduction in the synthesis of glycerolipids due to a reduction in phosphatidate phosphatase (PAP). Overexpression of lipin 1 in the GPI-deficient cell line, GroD1 resulted in increased PAP activity, however it failed to restore glycerolipid biosynthesis. Fluorescent microscopy showed a failure of GPI-deficient cells to localize lipin 1α to the nucleus. Glucose-6-phosphate levels in GroD1 cells were 10-fold over normal. Lowering glucose levels in the growth medium partially restored glycerolipid biosynthesis and nuclear localization of lipin 1α. Thus, GPI deficiency results in an accumulation of glucose-6-phosphate, and possibly other glucose-derived metabolites, leading to activation of mTOR and sequestration of lipin 1 to the cytosol, preventing its proper functioning. These results may also help to explain neuromuscular symptoms associated with inherited GPI deficiency (Haller et al., 2010; 2011). GPI deficiency was found to be the cause of recurrent haemolytic crises that has required frequent blood transfusion.

Hemolysis is often ameliorated by splenectomy (Neubauer et al., 1990; Shalev et al., 1994; Alfinito et al., 1994). GPI deficiency can become a clinically relevant consequence of the administration of drugs. GPI deficiency can lead to impairment of the system that removes free radicals generated by amoxicillin, thereby resulting in oxidation of hemoglobin and destabilization of erythrocytes membranes, with acute hemolysis and severe hemoglobinuria (Rossi et al., 2010).

2.2.3 Point mutation in phosphofructokinase

Phosphofructo-1-kinase (PFK) is a tetrameric enzyme that phosphorylates fructose-6-phosphate to fructose-1,6-bisphosphate, committing glucose to glycolysis. Three PFK isoenzymes, encoded by separate genes, have been identified in mammals: muscle-type (PFKM), liver-type (PFKL), and platelet-type (PFKP), all of which are expressed in a tissue specific manner. Skeletal muscle expresses only PFKM homotetramers, liver mainly PFKL homotetramers, while erythrocytes contain PFKM and PFKL heterotetramers (Vora et al., 1983). Inherited deficiency of muscle PFK is known to occur in man and dog (Vora et al., 1983; Skibild et al., 2001). PFK deficiency was the first recognized disorder that directly affects glycolysis. Ever since the discovery of the disease in 1965, a wide range of biochemical, physiological and molecular studies of the disorder have been carried out (Nakajima et al., 2002). Several mutations in PFKM cause type VII glycogen storage disease (GSDVII), which is a rare disease described by Tarui (Tarui's disease). GSDVII is characterized by the coexistence of a muscle disease and a hemolytic process. Clinical manifestations of the disease range from the severe infantile form, leading to death during childhood, to the classical form, which presents mainly with exercise intolerance. Typically, the disease begins in early childhood and consists of easy fatigability, transient weakness and muscle cramps and myoglobinuria after vigorous exercises (Vora et al., 1987; García et al., 2009). A G-to-A transition at codon 209-in exon 8 of the PFK-M gene, changing an encoded Gly to Asp, is responsible for the GSDVII in a homozygous French Canadian patient. The Swiss patient is a genetic compound, carrying a G-to-A transition at codon 100 in exon 6 (Arg to Gln) and a G-to-A transition at codon 696 in exon 22 (Arg to His) (Raben et al., 1995). PFK deficiency include isolated hemolytic anemia, compensated hemolysis or asymptomatic state (Etiemble et al., 1983; Fogelfeld et al., 1990). The concomitant haemolysis in patients with inherited PFK deficiency of the muscle isoenzyme may be explained by a diminished erythrocyte deformability due to Ca2+ overload (Ronquist et al., 2001). PFK deficiency include early-onset neonatal seizures (Al-Hassnan et al., 2007). Portal and mesenteric vein thrombosis in patient with a known case of PFK deficiency has been described (Madhoun et al., 2011).

2.2.4 Point mutation in aldolase

Aldolase, a homotetrameric protein encoded by the ALDOA gene, converts fructose-1,6-bisphosphate to dihydroxyacetone phosphate and glyceraldehyde-3-phosphate. Three isozymes are encoded by distinct genes. The sole aldolase present in erythrocytes and skeletal muscle is the A isozyme. Aldolase B is mainly expressed in the liver, kidney and small intestine, where it plays a role in exogenous fructose utilization. Aldolase C is expressed predominantly in the brain. Aldolase B deficiency has been widely described in humans, because it causes hereditary fructose intolerance, which is an autosomal recessive

disease that may induce severe liver damage, leading, in extreme cases, to death if fructose is not eliminated from the diet. To date, nearly 25 HFI-related aldolase B mutants have been identified. In contrast, cases of aldolase A deficiency, which has been associated with nonspherocytic haemolytic anemia, are much rarer (Esposito et al., 2004). Human aldolase A is composed of four identical subunits encoded by a single gene located on chromosome 16 (16q22–q24). Aldolase A deficiency has been reported as a rare, autosomal recessive disorder (Kreuder et al., 1996; Yao et al., 2004). Alterations in the aldolase A gene leading to amino acid substitutions: Asp128→Gly (Kishi et al., 1987), Glu206→Lys (Kreuder et al., 1996), Gly346→Ser (Esposito et al., 2004) have been described. The Glu206→Lys mutation destabilizes the aldolase A tetramer at the subunit interface, the Gly346→Ser mutation limits the flexibility of the C-terminal region. Biochemical and thermodynamic data are available for the Asn128→Gly mutant have never been characterized. Yao D.C. et al. described the case of a girl of Sicilian descent with aldolase A deficiency. Clinical manifestations included transfusion-dependent anemia until splenectomy at age 3 and increasing muscle weakness, with death at age 4 associated with rhabdomyolysis and hyperkalemia. Sequence analysis of the ALDOA coding regions revealed 2 novel heterozygous ALDOA mutations in conserved regions of the protein. The paternal allele encoded a nonsense mutation, Arg303X, in the enzyme-active site. The maternal allele encoded a missense mutation, Cys338→Tyr, predicted to cause enzyme instability as reported (Yao et al., 2004). Hemolytic crisis in patients with aldolase A deficiency can be provoked by fever (Kiriyama et al., 1993) and upper respiratory infections (Miwa et al., 1981).

2.2.5 Housekeeping genes in glyceraldehyde 3-phosphate dehydrogenase

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) specifically catalyzes the simultaneous phosphorylation and oxidation of glyceraldehyde-3-phosphate to 1,3-biphosphoglycerate. GAPDH comprises a polypeptide chain of 335 amino acids. Structural studies identified two regions, namely the glyceraldehyde-3-phosphate catalytic site and the nicotinamide adenine dinucleotide binding site. The glycolytic function mainly relies on critical amino acids that include Cys152 and His179, and on its tetrameric structure composed of four identical 37-kDa subunits (Colell et al., 2009). GAPDH was considered a classical glycolytic protein involved exclusively in cytosolic energy production. However, recent evidence suggests that it is a multifunctional protein displaying diverse activities distinct from its conventional metabolic role. New investigations establish a primary role for GAPDH in a variety of critical nuclear pathways apart from its already recognized role in apoptosis. These new roles include its requirement for transcriptional control of histone gene expression, its essential function in nuclear membrane fusion, its necessity for the recognition of fraudulently incorporated nucleotides in DNA, and its mandatory participation in the maintenance of telomere structure. Other investigations relate a substantial role for nuclear GAPDH in hyperglycemic stress and the development of metabolic syndrome. GAPDH is a highly conserved gene and protein, with a single mRNA transcribed from a unique gene (Sirover 1997, 2005, 2011). GAPDH has been referred to as a "housekeeping" protein and based on the view that GAPDH gene expression remains constant under changing cellular conditions, the levels of GAPDH mRNA have frequently been used to normalize northern blots (Tatton, 2000). Evidence of an impairment of GAPDH glycolytic function in Alzheimer's and Huntington's disease subcellular fractions despite unchanged gene expression has been reported (Mazzola & Sirover, 2001).

2.2.6 Point mutation in phosphoglycerate kinase

Phosphoglycerate kinase (PGK) plays a key role for ATP generation in the glycolytic pathway. The PGK, which exists universally in various tissues of various organisms, is encoded by a single structural gene on the X-chromosome q13 in humans. The PGK consists of 417 amino acid residues with acetylserine at the NH2-terminal and isoleucine at the COOH-terminal and is a monomeric enzyme that is expressed in all tissues (Huang et al. 1980; Maeda et al. 1991). PGK deficiency is generally associated with chronic hemolytic anemia, although it can be accompanied by either mental retardation or muscular disease (Cohen-Solal et al. 1994). The structure of some PGK mutants has been described. PGK Matsue variant is a point mutation, a T/A→C/G transition in exon 3, that cause Leu88→Pro substitution associated with severe enzyme deficiency, congenital nonspherocytic hemolytic anemia, and mental disorders (Maeda et al. 1991). PGK Shizuoka variant is a single nucleotide substitution from guanine to thymine at position 473 of PGK messenger RNA, associated with chronic hemolysis and myoglobinuria. This nucleotide change causes a single amino acid substitution from Gly157→Val (Fujii et al. 1992). PGK Créteil variant arises from a G→A nucleotide interchange at position 1022 in cDNA (exon 9), resulting in amino acid substitution Asp314→Asn associated with rhabdomyolysis crises but not with hemolysis or mental retardation. PGK Amiens/New York variant, which is associated with chronic hemolytic anemia and mental retardation is a point mutation, an A→T nucleotide interchange at position 571 in cDNA (exon 5); this leads to amino acid substitution Asp163→Val (Cohen-Solal et al., 1994; Flanagan et al., 2006). Variants of PGK Barcelona and PGK Murcia are described in Spain. PGK Barcelona variant, which causes chronic hemolytic anemia associated with progressive neurological impairment is a point mutation, 140 T→A substitution that produces an Ile46→Asn change. The increase of 2,3-bisphosphoglycerate and the decrease of adenosine triphosphate levels in erythrocytes are the detected metabolic changes that could cause hemolytic anemia. PGK Murcia variant is a point mutation, 958 G→A transition that cause a Ser319→Asn substitution. The crystal structure of porcine PGK was used as a molecular model to investigate how these mutations may affect enzyme structure and function. In both cases – the mutations did not modify any of the PGK binding sites for ATP or 3PG, so their effect is probably related to a loss of enzyme stability rather than a decrease of enzyme catalytic function (Noel N. et al., 2006; Ramírez-Bajo M.J. et al., 2011). Mutants PGK München (Krietsch et al., 1980), PGK Herlev (Valentin et al., 1998), PGK Uppsala (Hjelm et al., 1980), PGK San Francisco (Guis et al., 1987), PGK II (Huang et al., 1980), PGK Michigan, PGK Tokyo (Cohen-Solal et al., 1994) are also described.

2.2.7 Point mutation in phosphoglycerate mutase

Phosphoglycerate mutase (PGAM) is a glycolytic enzyme that catalyses the interconversion of 2-phosphoglycerate and 3-phosphoglycerate, with 2,3-bisphosphoglycerate being required, in mammals, as a co-factor. In mammals, PGAM is present in three isozymes which result from the homodimeric and heterodimeric combinations of two different subunits, M and B, coded by two different genes, although the gene coding subunit B is unknown. Only the homodimer BB is present in erythrocytes. Only one PGAM BB deficiency has been reported. In a patient with clinical diagnosis of Hereditary Spherocytosis and partial deficiency (50%) of erythrocytes PGAM activity, a homozygous point mutation with cDNA 690G→A substitution that produces a Met230→Ile change has

recently been reported. The mutated PGAM shows an abnormal behaviour on ion-exchange chromatography and is more thermo-labile that the native enzyme. The increased instability of the mutated enzyme can account for the decreased erythrocytes PGAM activity (Repiso et al., 2005; de Atauri et al., 2005).

2.2.8 Point mutation in enolase

Enolase, an essential enzyme of glycolysis and gluconeogenesis, catalyses the interconversion of 2-hosphoglyceric acid and phosphoenolpyruvate. Enolases from most species are dimeric, with subunit molecular masses of 40000–50000 Da. Mammals have three genes for enolase, coding for the α, β and γ subunits; the subunits associate to form both homo- and heterodimers. The α gene is expressed in many tissues, γ primarily in neurones and β in muscle (Zhao et al., 2008). Erytrocytes enolase deficiency is rare, and its pathogenesis, inheritance and clinical manifestation have not been firmly established. Enolase deficiency is known to be associated with chromosome 1p locus mutations (1 pter-p36.13) and to cause chronic nonspecific hemolytic anemia (Boulard-Heitzmann et al., 1984). Lachant et al. (1986) described four generations of a Caucasian family with hereditary erytrocytes enolase deficiency. Stefanini (1972) described chronic hemolytic anemia associated with erythrocyte enolase deficiency exacerbated by ingestion of nitrofurantoin.

2.2.9 Point mutation in pyruvate kinase

Pyruvate kinase (PK) catalyses the last step of the Embden–Meyerhof metabolic pathway, in which an ATP molecule is produced. Among the four PK isozymes present in humans (M1, M2, L and R), both PK-L (found in the liver, kidney and gut) and PK-R present in erythrocytes are encoded by the same gene, which is localised on hromosome 1q21. The respective expression of these two isozymes is under the control of specific promoters leading to structural differences in the N-terminal part of the protein. PK-R is a 574 amino acid-long protein, which associates into tetramers according to a double dyad symmetry pattern, resulting in allosteric enzymatic kinetics. PK deficiency is the most frequent red cell enzymatic defect responsible for hereditary nonspherocytic hemolytic anemia and is transmitted according to a recessive autosomal mode. Based on the gene frequency of the 1529A mutation in the white population and on its relative abundance in patients with hemolytic anemia caused by PK deficiency, the prevalence of PK deficiency is estimated at 51 cases per million white population (Beutler et al., 2000). The degree of haemolysis varies widely, ranging from very mild or fully compensated forms, to life-threatening neonatal anemia and jaundice necessitating exchange transfusions. Heterozygous carriers usually display very mild symptoms. Therefore, the defect is frequently ignored and its prevalence is difficult to establish. Severe disorders are described in homozygous or compound heterozygous patients (Zanella et al., 2005). According to the most recent database, more than 180 mutations have been reported on the PK-LR gene. Two mutations, both located in exon 11, are recurrent (Arg510 → Gln, Arg486 → Trp). Arg510→ Gln is the most frequent mutation found in northern Europe, central Europe and the USA (Pissard S. at al., 2006, as citated Wang et al., 2001) and Arg486 → Trp in southern Europe (Spain, Portugal and Italy) and in France (Pissard et al., 2006, as citited Zanella et al, 1997; Zarza et al, 1998; Pissard et al., 2006). The most frequent mutations of PKLR gene in the Indian population appear to be 1436G→A (19.44%), followed by 1456C→T (16.66%) and 992A→G (16.66%) (Kedar et al.,

2009). Erythrocyte PK plays an important role as an antioxidant during erythroid differentiation. Glycolytic inhibition by erythrocyte PK gene mutation augmented oxidative stress, leading to activation of hypoxia-inducible factor-1 as well as downstream proapoptotic gene expression (Aisaki et al., 2007). Extended molecular analysis is useful for studying how several interacting gene mutations contribute to the clinical variability of pyruvate kinase deficiency (Perseu et al., 2010).

2.2.10 Point mutation in glucose-6-phosphate dehydrogenase

Glucose 6-phosphate dehydrogenase (G6PD) is a ubiquitous enzyme, which is critical in the redox metabolism of all aerobic cells. It catalyzes the first, rate-limiting step of the pentose phosphate pathway, coupled to NADPH synthesis and to ribose availability which is essential for the production of nucleotide coenzymes and replication of nucleic acids (Sodiende O., 1992). The pentose phosphate pathway is the unique source of NADPH, which enables erytrocytes to counterbalance the oxidative stress triggered by several oxidant agents preserving the reduced form of glutathione. GSH protects the sulfhydryl groups in hemoglobin and in the red cell membrane from oxidation (Mason et al., 2007). G6PD is a dimer and each subunit contains a single active site. G6PD-enzyme is encoded by a human X-linked gene (Xq2.8) consisting of 13 exons and 12 introns, spanning nearly 20 kb in total. G6PD gene is probably the most polymorphic locus in humans, with over 400 allelic variants known (Minucci et al., 2009). G6PD, the most common enzyme deficiency worldwide, causes a spectrum of disease including neonatal hyperbilirubinemia, acute hemolysis, and chronic hemolysis. Persons with this condition also may be asymptomatic. Approximately 400 million people are affected worldwide. Homozygotes and heterozygotes can be symptomatic, although the disease typically is more severe in persons who are homozygous for the deficiency. Different gene mutations cause different levels of enzyme deficiency, with classes assigned to various degrees of deficiency and disease manifestation. Acute hemolysis is caused by exposure to an oxidative stressor such as infection, some foods (fava beans), drugs or various chemicals. The variant that causes chronic hemolysis is uncommon because it is related to sporadic gene mutation rather than the more common inherited gene mutation (Frank, 2005). About 160 mutations have been reported, most of which are single-base substitutions leading to amino acid replacements (Minucci et. al., 2009). Mutations are classified into four types, according to their clinical effects. Several variants, such as the the Mediterranean variant, reach the polymorphism (Wajcman et. al., 2004). The Mediterranean variant of G6PD deficiency is due to the C563CT point mutation, leading to replacement of Ser with Phe at position 188, resulting in acute haemolysis triggered by oxidants (Ingrosso et. al., 2002). Individuals with such mutations seem to have enjoyed a selective advantage because of resistance to falciparum malaria. Different mutations, each characteristic of certain populations are found.The most common African mutation G6PD is 202A376G. G6PD Mediterranean (563T) is found in Southern Europe, the Middle East and in the Indian subcontinent (Beulter, 1996).

2.2.11 Point mutation in 6-phosphogluconate dehydrogenase

The 6-phosphogluconate dehydrogenase (6PGDH) is the third enzyme of the oxidative branch of the pentose phosphate pathway. This pathway has two major functions: the production of ribulose 5-phosphate which is required for nucleotide synthesis, and the

generation of NADPH which provides the major reducing power essential to protect the cell against oxidative stress and a variety of reductive biosynthetic reactions, particularly lipid production. Thus, 6PGDH plays a critical role in protecting cells from oxidative stress (He et al., 2007). Few cases of erytrocytes 6PGD deficiency in humans have been described. The episodic hemolytic events with jaundice in patients with 6PGD deficiency may be the result of a defective erythrocytes ability to counteract conditions of marked oxidative stress as happens at birth and following traumatic events. The presence of 6PGD deficiency could be mistaken for a partial G6PD deficiency if the assay of G6PD activity was performed without correcting for 6PGD activity (Vives Corrons et al., 1996; Caprari et al., 2001).

2.3 Point mutation in glutathione synthetase

Glutathione (GSH) is the most abundant intracellular thiol in living aerobic cells. GSH is present in millimolar concentrations in most mammalian cells and it is involved in several fundamental biological functions, including free radical scavenging, detoxification of xenobiotics and carcinogens, redox reactions, biosynthesis of DNA, proteins and leukotrienes, as well as neurotransmission/neuromodulation. It has been assigned several critical functions: protection of cells against oxidative damage; involvement in amino acid transport; participation in the detoxification of foreign compounds; maintenance of protein sulfhydryl groups in a reduced state; and as a cofactor for a number of enzymes. GSH is found in low levels in diseases in which increasing evidence implicate oxidative stress in the development of the disease, for example retinopathy of prematurity, necrotizing enterocolitis, bronchopulmonary dysplasia, patent ductus arteriosus and asthma. GSH is metabolised via the gamma-glutamyl cycle, which is catalyzed by six enzymes (Polekhina at al., 1999; Njålsson et al., 2005; Norgren et al., 2007). GSH is synthesized from glutamate, cysteine and glycine. The final step in its synthesis is catalysed by the enzyme glutathione synthetase (GS) The human GS enzyme is a homodimer with 52 kDa of subunits containing 474 amino acid residues, encoded by a single-copy gene located on chromosome 20q11.2 (Webb et al., 1995; Njålsson et al., 2005).

GS deficiency is a rare autosomal recessive disorder. Since the human genome contains only one GS gene, the various clinical forms of GS deficiency reflect different mutations or epigenetic modifications in the GS gene. Thus GSH acts as a feedback inhibitor of the initial step in its biosynthesis, in patients with hereditary deficiency of GS the lack of GSH leads to the formation of increased amounts of g-glutamylcysteine which is converted into 5-oxoproline by g-glutamyl cyclotransferase and excreted in massive amounts. Shi et al. identified seven mutations at the GS locus on six alleles: one splice site mutation, two deletions and four missense mutations and in patients with 5-Oxoprolinuria (pyroglutamic aciduria) resulting in GS deficiency and homozygous missense mutation in an individual affected by a milder-form of the GS deficiency, which is apparently restricted to erythrocytes and only associated with haemolytic anaemia (Shi, et al., 1996). Japanese patients with chronic nonspherocytic hemolytic anemia were found to have decreased GS activity and the others were moderately deficient in GCS. Hemolytic anemia was their only manifestation, and neither 5-oxoprolinemia nor 5-oxoprolinuria, which are usually associated with to generalized type of glutathione synthetase deficiency, was noted in patients. (Hirono et al., 1996). Dahl N. at al. described thirteen different point mutations. In vitro analysis of

naturally occurring missense mutations showed that mutations could affect the stability, catalytic capacity and substrate affinities of the enzyme. Four mutant cDNAs were investigated with the mutations resulting in Leu188→Pro, Tyr270→Cys, Tyr270→His and Arg283→Cys, respectively. Each of the four mutations resulted in a considerable decrease of enzymatic activity to levels corresponding to 1 to 12% of the wild-type control value, confirming that these mutations were pathogenic. Clinically affected patients present with severe metabolic acidosis, 5-oxoprolinuria, increased rate of hemolysis, hemolytic anemia, neonatal jaundice and defective function of the central nervous system. A milder form of GS deficiency apparently restricted to erythrocytes, is associated with decreased erythrocyte GSH levels and hemolytic disease, which is usually well compensated. Complete loss of function of both GS alleles is probably lethal. Missense mutations will account for the phenotype in the majority of patients with severe GS deficiency (Dahl et al., 1997). A 141-bp deletion corresponding to the entire exon 4, whilst the corresponding genomic DNA showed a G491→A homozygous splice site mutation, and a C847→T (Arg283→ Cys) mutation in exon 9 are described in patients with GS deficiency and Fanconi nephropathy (Al-Jishi et al., 1999). A homozygous state for 656 A→G, a 808 T→C mutation of GS gene in patients with chronic haemolysis and markedly reduced erythrocytes was found in Spain (Corrons, et al., 2001). Patients with GS deficiency can be divided into three groups. Mildly affected patients have mutations affecting the stability of the enzyme, causing a compensated haemolytic anaemia; moderately affected patients have, in addition, metabolic acidosis; and severely affected patients also develop neurological defects and show increased susceptibility to bacterial infections. Moderately and severely affected patients have mutations that compromise the catalytic properties of the enzyme. 5-Oxoprolinuria appears in all three groups, but is more pronounced in the two latter groups (Njålsson .et al., 2005). 5-Oxoproline is able to promote both lipid and protein oxidation, to impair brain antioxidant defenses and to enhance hydrogen peroxide content, thus promoting oxidative stress, and is a mechanism that may be involved in the neuropathology of GS deficiency (Pederzolli et al., 2010). Approximately 25% of patients with hereditary GS deficiency die during childhood. Even though the correlation between phenotype and genotype in these patients is complex, an indication of the phenotype can be based on the type of mutation involved (Njålsson et al., 2005). Severe GS deficiency is associated with progressive retinal dystrophy of the rod-cone type, affecting the central retina with advanced macular edema in adulthood. The retinal degenerative changes in GS deficiency may be the result of the increased oxidative stress accumulated generally in the retina and also apparent in the macular area, and an insufficient level of the free radical scavenger GSH. Patients with GS deficiency may represent a model of the retinal response to oxidative stress in humans (Burstedt et al., 2009). Recently 30 different mutations in the GSS gene have been identified (Njålsson et al., 2005). The severe form of GS deficiency usually present in the neonatal period, is characterized by acute metabolic acidosis, hemolytic anemia and progressive encephalopathy (Iyori et al., 1996; Yapicioğlu et al., 2004). Diagnosis of GS deficiency is made by clinical presentation and detection of elevated concentrations of 5-oxoproline in urine and low GS activity in erythrocytes or cultured skin fibroblasts. Diagnosis can be confirmed by mutational analysis. The most important determinants for outcome and survival in patients with GS deficiency are early diagnosis and early initiation of treatment. Presently, GS deficiency is not included in newborn screening programmes in Europe. As

outcome depends significantly on early start of treatment, routine inclusion of this disorder in newborn screening panels should be considered. Treatment consists of the correction of acidosis, blood transfusion, and supplementation with antioxidants (Simon et al., 2009). Patients with GS deficiency are given vitamins C and E to boost their antioxidant levels, and bicarbonate to correct metabolic acidosis (Jain et al.,1994; Ristoff et al., 2001; Njålsson et al., 2005).

2.4 Point mutation in glutathione reductase

Glutathione reductase (GR) is a key enzyme required for the conversion of oxidized glutathione (GSSG) to reduced glutathione (GSH) concomitantly oxidizing reduced nicotinamide adenine dinucleotide phosphate (NADPH). GR is a homodimeric flavoprotein with a subunit Mr of 52.4 kDa. Its 2 identical redox active sites are formed by residues from both subunits, implying that monomeric GR is not active. Human GR is encoded by a single gene, located on chromosome 8p21.1 and consisting of 13 exons. GR consists of apoglutathione reductase (apoGR) and flavin adenine dinucleotide (FAD) as a prosthetic group (Kamerbeek et al., 2007).

Acquired FAD deficiency due to low amounts of riboflavin (vitamin B2) in the diet (or failure to convert it sufficiently to FAD) may result in inactive apoGR. In that case GR activity can be restored by riboflavin administration. Due to inherited mutations, the GR protein can be absent or exhibit low catalytic activity. Whereas inherited glutathione reductase deficiency is rare, FAD deficiency is common in malnourished populations. The clinical symptoms of GR deficiency include reduced lifespan of erythrocytes, cataract, and favism (hemolytic crises after eating fava beans). A 2246-bp deletion in DNA, which results in unstable and inactive GR and a premature stop codon on one allele and a substitution of glycine 330, a highly conserved residue in the superfamily of NAD(P)H-dependent disulfide reductases, into alanine on the other allele were described in the GR gene in patients with clinical GR deficiency (Kamerbeek et al., 2007). GR deficiency may alter the clinical manifestation of an unstable hemoglobinopathy (Mojzikova at al., 2010) and may be the cause of neonatal hyperbilirubinemia (Casado et al., 1995). GR deficiency state can be asymptomatic as the residual enzyme activity might be sufficient to maintain the reduced glutathione level to prevent oxidative stress (Nakashima et al., 1978). A study on 1691 individuals from Saudi Arabia to determine the overall frequency of GR deficiency has been conducted. The overall frequency of genetic GR deficiency was 24.5% and 20.3% in males and females respectively. In addition, 17.8% of males and 22.4% of females suffered from GR deficiency due to riboflavin deficiency. This could be easily corrected by dietary supplementation with riboflavin. No cases of severe GR deficiency were identified (el-Hazmi et al., 1989; Warsy et al., 1999).

2.5 Point mutation in glutathione peroxidase

Glutathione peroxidase (GPx) is the general name of an enzyme family with peroxidase activity whose main biological role is to protect the organism from oxidative damage. There are eight well-characterized mammalian selenoproteins, including thioredoxin reductase and four isozymes of glutathione peroxidase. GPx1 is a homotetrameric selenoprotein and one of a family of peroxidases that reductively inactivate peroxides using glutathione as a

source of reducing equivalents. GPx1 is found in the cytoplasm and mitochondria of all cell types, whose preferred substrate is hydrogen peroxide (Dimastrogiovanni et al., 2010).

GPx1 has been implicated in the development and prevention of many common and complex diseases, including cancer and cardiovascular disease (Lubos at al., 2011). The T allele of the GPx1 rs1050450 (C→ T) gene variant is associated with reduced enzyme activity. Significant association between the T allele and peripheral neuropathy in subjects with diabetes is observed (Tang et al., 2010). Takapoo at al. using a murine model of GPx1 deficiency (Gpx1(+/-)) found elevated hydrogen peroxide levels and increased secretion of the pro-inflammatory immunomodulator cyclophilin A (CyPA) in both arterial segments and cultured smooth muscle cells as compared to wild type. Reduction in vascular cell GPx1 activity and the associated increase in oxidative stress cause CyPA-mediated paracrine activation of smooth muscle cells. These findings identify a mechanism by which an imbalance in antioxidant capacity may contribute to vascular disease (Takapoo et al., 2011). Mice with a disrupted GPx1 gene (*Gpx1* 0/0) developed myocarditis after coxsackievirus B3 infection, whereas infected wild-type mice (*Gpx1* +/+) were resistant. Thus, GPx1 provides protection against viral-induced damage in vivo due to mutations in the viral genome of a benign virus (Beck et al., 1998). The deficiency of GPx1 promotes atherogenesis (Lubos et al., 2011). Severe acute hemoglobinemia and hemoglobinuria were described as a result a hereditary heterozygous GPx deficiency in Japan (Gondo et al., 1992). Patients with reduced GPx activity are at a high risk of developing carbamazepine-induced hemolytic crisis and/or aplastic crisis (Yamamoto et al., 2007).

2.6 Point mutation in peroxiredoxin

Peroxiredoxin (Prx) is a scavenger of hydrogen peroxide and alkyl hydroperoxides in living organisms. Six distinct mammalian Prx isozymes, types 1 to 6, have been detected in a wide range of tissues, and these have been shown to have strong antioxidant activities in vitro. In addition to their antioxidant activity, Prxs have been implicated in a number of cellular functions (Lee et al., 2003). Prx2 is an antioxidant enzyme that uses cysteine residues to decompose peroxides. Prx2 is the third most abundant protein in erythrocytes, and competes effectively with catalase and glutathione peroxidase to scavenge low levels of hydrogen peroxide, including that derived from hemoglobin autoxidation (Low, et al., 2008). Mice lacking Prdx1 are viable and fertile but have a shortened lifespan owing to the development of severe haemolytic anaemia and several malignant cancers, both of which are also observed at increased frequency in heterozygotes. The haemolytic anaemia is characterized by an increase in erythrocyte reactive oxygen species, leading to protein oxidation, haemoglobin instability, Heinz body formation and decreased erythrocyte lifespan (Neumann et al., 2003). Point mutations in gene Prx2 in humans are not described.

2.7 Point mutation in superoxide dismutase 1

Superoxide dismutase 1 (SOD1) is a primarily cytosolic enzyme of the cellular oxidative defense and acts as a protein homodimer with each monomer containing one complexed copper and zinc ion. Point mutations scattered throughout the sequence of Cu,Zn superoxide dismutase 1 (SOD1) cause a subset of amyotrophic lateral sclerosis (ALS) cases. ALS is a progressive neurodegenerative disorder affecting motor neurons (Ip et al.,

2011). The 140 Cu,Zn SOD1 gene mutations associated with ALS is described (Giannini et al., 2010). Variable penetrance and predominant lower motor neuron involvement are common characteristics in patients bearing mutations in exon 3 of the SOD1 gene (del Grande et al., 2011). Some mutations are associated with a long survival time, while others are linked to a very rapid progression (Syriani et al., 2009). With mild mechanical trauma which causes no major tissue damage, the G93A-SOD1 gene mutation alters the balance between pro-apoptotic and pro-survival molecular signals in the spinal cord tissue, leading to a premature activation of molecular pathways implicated in the natural development of ALS (Jokic et al., 2010). Mitochondria have shown to be an early target in ALS pathogenesis and contribute to the disease progression. Morphological and functional defects in mitochondria were found in both human patients and ALS mice overexpressing mutant SOD1. Axonal transport of mitochondria along microtubules is disrupted in ALS (Shi et al., 2010). Abnormal neuronal connectivity in primary motor cortex resulting from the G93A-SOD1 mutation might extend to adjacent regions and promote development of cognitive/dementia alterations frequently associated with ALS (Spalloni et al. 2011). Mutant SOD1 can alter cell cycle in a cellular model of ALS. Modifications in cell cycle progression could be due to an increased interaction between mutant G93A SOD1 and Bcl-2 through the cyclin regulator p27 (Cova et al., 2010). The D90A mutation has been identified in recessive, dominant, and apparently sporadic cases. A→C exchange at position 272 in the SOD1 gene is detected. This mutation results in an amino acid substitution of alanine for aspartate at position 90 (D90A). D90A in heterozygous state may cause predominant upper motor neuron phenotype with very slow progression (Luigetti et al., 2009). Oxidative stress markers have been found in nervous and peripheral tissues of familial and sporadic ALS patients (Cova et al., 2010). Lipid peroxidation in the erythrocytes of ALS patients was significantly increased with respect to controls (Babu et al., 2008). Recently described chronic, but moderate regenerative, haemolytic anaemia of aged SOD1-knockout mice is associated with erythrocytes modifications and sensitivity to both intra- and extra-vascular haemolysis (Starzyński et al., 2009). Deficiency of the SOD1 gene causes anemia and autoimmune responses against erythrocytes. Severity of anemia and levels of intracellular reactive oxygen species are positively correlated. Oxidation-mediated autoantibody production may be a more general mechanism for autoimmune hemolytic anemia and related autoimmune diseases. Shift in glucose metabolism to the pentose phosphate pathway and decrease in the energy charge potential of erythrocytes, increase in reactive oxygen species due to SOD1 deficiency accelerate erythrocytes destruction by affecting carbon metabolism and increasing oxidative modification of lipids and proteins. The resulting oxidation products are antigenic and, consequently, trigger autoantibody production, leading to autoimmune responses (Iuchi et al., 2007, 2009, 2010).

2.8 Point mutation in catalase

Catalase is an important anti-oxidant enzyme and physiologically maintains tissue and cellular redox homeostasis, thus plays a central role in defense against oxidative stress, it is the main regulator of hydrogen peroxide metabolism. Catalase is a tetramer of four polypeptide chains, each over 500 amino acids, it contains four porhyrin heme (iron) groups that allow the enzyme to react with hydrogen peroxide (Uchida et al., 2011).

Catalase deficiency in blood is known as acatalasemia. Deficiency of catalase may cause high concentrations of hydrogen peroxide and increase the risk of the development of pathologies for which oxidative stress is a contributing factor. Hydrogen peroxide at high concentrations is a toxic agent, while at low concentrations it appears to modulate some physiological processes such as signaling in cell proliferation, apoptosis, carbohydrate metabolism, and platelet activation. Benign catalase gene mutations in 5' noncoding region and intron 1 have no effect on catalase activity and are not associated with a disease. Decreases in catalase activity in patients with tumors is more likely to be due to decreased enzyme synthesis rather than to catalase mutations. Acatalasemia, the inherited deficiency of catalase has been detected in 11 countries (Góth et al., 2004). The molecular defects in the catalase gene, levels of m-RNA and properties of the residual catalase studied by scientists are reviewed in human (Japanese, Swiss and Hungarian) and non-human (mouse and beagle dog) acatalasemia. Japanese acatalasemia-I, the G to A transition at the fifth position of intron 4 of the catalase gene, limits the correct splicing of the mRNA, resulting in trace quantities of catalase with normal properties. The bicistronic microRNA miR-144/451 can influence gene expression by altering the activity of a key transcriptional program factor, impacting anti-oxidant-encoding genes like catalase (Yu et al., 2010). Hungarian acatalasemia type C showed a splicing mutation. In the Japanese acatalasemia II and the type A and B of Hungarian acatalasemia, deletion or insertion of nucleotides was observed in the coding regions, and a frame shift altered downstream amino acid sequences and formed truncated proteins. In the Hungarian acatalasemia D, substitution of an exon nucleotide was found. In mouse and beagle dog acatalasemia, substitution of nucleotides in the coding regions was also observed. Studies of residual catalase in Swiss mouse and beagle dog acatalasemia showed that aberrant catalase protein degrades more quickly than normal catalase in cells (Ogata et al., 2008). Japanese-type acatalasemia (Takarara disease) is characterized by the almost total loss of catalase activity in erythrocytes and is often associated with ulcerating oral lesions (Hirono et al, 1995). Polymerization of hemoglobin and aggregation of the acatalasemic erythrocytes observed upon the addition of hydrogen peroxide can be the mechanism for the onset of Takarara disease (Masuoka et al., 2006). Catalase deficiency in Hungary has been reported to be associated with increased frequency of diabetes mellitus (Vitai et al, 2005). That is human acatalasemia may be a risk factor for the development of diabetes mellitus. Catalase plays a crucial role in the defense against oxidative-stress-mediated pancreatic beta cell death (Kikumoto et al., 2009). Exon 2 and neighboring introns of the catalase gene may be minor hot spots for type 2 diabetes mellitus susceptibility mutations (Vitai et al., 2005). The catalase gene was selected as a candidate gene because of the reduction of catalase enzyme activity and concomitant accumulation of excess hydrogen peroxide observed in the entire epidermis of vitiligo patients. One of three catalase genetic markers studied was found to be informative for genotypic analysis of Caucasian vitiligo patients and control subjects. Both case/control and family-based genetic association studies of the T/C single nucleotide polymorphism (SNP) in exon 9 of the catalase gene, which is detectable with the restriction endonuclease BstX I, suggest possible association between the catalase gene and vitiligo susceptibility. The observations that T/C heterozygotes are more frequent among vitiligo patients than controls and that the C allele is transmitted more frequently to patients than controls suggest that linked mutations in or near the catalase gene might contribute to a quantitative deficiency of catalase activity in the

epidermis and the accumulation of excess hydrogen peroxide (Casp et al., 2002). The increased plasma homocysteine and inherited catalase deficiency together could promote oxidative stress via hydrogen peroxide. The patients with inherited catalase deficiency are more sensitive to oxidative stress of hydrogen peroxide than the normocatalasemic family members (Góth et al., 2003). The normal activity of glutathione peroxidase could prevent the lysis of the erythrocytes in acatalasemic patients. In the presence of extremely high levels of hydrogen peroxide acute hemolysis may not be excluded; therefore, follow-up of these patients is required (Góth et al., 1995). Patients with low (inherited and acquired) catalase activities who are treated with infusion of uric acid oxidase because they are at risk of tumour lysis syndrome may experience very high concentrations of hydrogen peroxide. They may suffer from methemoglobinaemia and haemolytic anaemia which may be attributed either to deficiency of glucose-6-phosphate dehydrogenase or to other unknown circumstances. Data have not been reported from catalase deficient patients who were treated with uric acid oxidase. It may be hypothesized that their decreased blood catalase could lead to an increased concentration of hydrogen peroxide which may cause haemolysis and formation of methemoglobin. Blood catalase activity should be measured for patients at risk of tumour lysis syndrome prior to uric acid oxidase treatment. (Góth et al., 2007). Acatalasic erythrocytes metabolized glucose through the hexosemonophosphate shunt at three times the normal rate and increased this rate many times when exposed to levels of peroxide-generating drugs that had negligible effect on normal erythrocytes. When erythrocytes lacked both their hexosemonophosphate shunt and catalase, oxidative damage was greater than with either deficiency alone (Harry Jacobt et al., 1965). Under acatalasemic conditions, it was suggested that NAD(P)H is an important factor to prevent oxidative degradation of hemoglobin (Masuoka et al., 2003).

2.9 Point mutation in adenylate kinase and pyrimidine 5'-nucleotidase

Erythrocytes adenylate kinase (AK) deficiency is a rare hereditary erythroenzymopathy associated with moderate to severe nonspherocytic hemolytic anemia and, in some cases, with mental retardation and psychomotor impairment. To date, diagnosis of AK deficiency depends on demonstration of low enzyme activity in erythrocytes and detection of mutations in AK1 gene. Five variants of AK1 isoenzyme-bearing mutations (118G→A, 190G→A, 382C→T, 418-420del, and 491A→G) are found in AK-deficient patients with chronic hemolytic anemia (Abrusci et al., 2007). Pyrimidine 5' -nucleotidase (P5'N-1) deficiency is one of the frequent enzyme abnormalities causing hereditary nonspherocytic hemolytic anemia. The disease is transmitted as an autosomal recessive trait. The degree of hemolysis is generally mild-to moderate. The structural human gene for P5'N-1 is now available and fifteen different mutations have been identified so far. Some patients exhibit high residual P5'N-1 activity, suggesting that P5'N-1 deficiency is compensate by other nucleotidases and/or alternative pathways in nucleotide metabolism. No specific therapy for P5'N-1 deficiency is now available (Kondo, 1990; Chiarelli et al., 2006).

3. Conclusion

The optimum metabolism of erythrocytes depends on activity of glucose transporter type 1, glycolysis enzymes, glutathione synthetase, glutathione reductase, glutathione peroxidase,

peroxiredoxin 2, superoxide dismutase 1, catalase and nucleotide metabolism. To date, all of the enzyme-deficient variants which have been investigated were caused by point mutations. Most mutations are located in the coding sequences of genes.

Expression of mutant glucose transporter 1 (GLUT1) resulted in a significant decrease in transport activity. Impaired glucose transport across brain tissue barriers results in epilepsy, mental retardation and motor disorders. Recently, mutations in GLUT1 gene have been identified as a cause in some patients with autosomal dominant paroxysmal exercise-induced dyskinesias, which in certain cases was accompanied by hemolytic anemia with echinocytosis. The causative mutations for some forms of hereditary stomatocytosis have been found to result from mutations in SLC2A1, encoding GLUT1. Stomatocytosis is associated with a cold-induced cation leak, hemolytic anemia and hepatosplenomegaly but also with cataracts, seizures, mental retardation and movement disorder.

Erythrocytes glucose metabolism only depends on hexokinase 1 (HK1) activity. HK1 deficiency is a very rare disease with a clinical phenotype of hemolysis. Glucose-6-phosphate isomerase (GPI) deficiency is one of the most common cause of congenital nonspherocytic hemolytic anemia. Patients with inherited GPI deficiency present with nonspherocytic anemia of variable severity, and with neuromuscular dysfunction. Homozygous GPI deficiency in human is responsible for chronic nonspherocytic hemolytic anemia. GPI deficiency can become clinically relevant consequence to the administration of drugs. GPI deficiency can lead to the impairment of the system that removes free radicals generated by amoxicillin, thereby resulting in oxidation of hemoglobin and destabilization of erythrocytes membranes, with acute hemolysis and severe hemoglobinuria. Phosphofructokinase (PFK) deficiency was the first recognized disorder that directly affects glycolysis. Several mutations in PFKM cause type VII glycogen storage disease (GSDVII), which is a rare disease described by Tarui (Tarui's disease). GSDVII is characterized by the coexistence of muscle disease and hemolytic process. PFK deficiency include isolated hemolytic anemia, compensated hemolysis or asymptomatic state. Portal and mesenteric vein thrombosis in patient with a known case of PFK deficiency has been described.

Aldolase A deficiency has been reported as a rare, autosomal recessive disorder. Clinical manifestations of aldolase A deficiency included transfusion-dependent anemia, increasing muscle weakness and rhabdomyolysis. Hemolytic crisis in patients with aldolase A deficiency can be provoked by fever, upper respiratory infections.

Recent evidence suggests that glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is a multifunctional protein displaying diverse activities distinct from its conventional metabolic role. GAPDH has been referred to as a "housekeeping" protein and based on the view that GAPDH gene expression remains constant. Evidence of an impairment of GAPDH glycolytic function in Alzheimer's and Huntington's disease subcellular fractions despite unchanged gene expression is reported.

Phosphoglycerate kinase (PGK) deficiency is generally associated with chronic hemolytic anemia, although it can be accompanied by either mental retardation or muscular disease.

In human, phosphoglycerate mutase (PGAM) is present in three isozymes. The homodimer BB is present in erythrocytes. Only one PGAM BB deficiency has been reported. In a patient with clinical diagnosis of Hereditary Spherocytosis and partial deficiency (50%) of erythrocytes PGAM activity, a homozygous point mutation have recently been reported.

Erytrocytes enolase deficiency is rare, and its pathogenesis, inheritance and clinical manifestation have not been firmly established. Enolase deficiency causes chronic nonspecific hemolytic anemia. Chronic hemolytic anemia associated with erythrocyte enolase deficiency exacerbated by ingestion of nitrofurantoin has been described.

Pyruvate kinase (PK) deficiency is one of the most frequent red cell enzymatic defect responsible for hereditary nonspherocytic hemolytic anemia. According to the most recent database, more than 180 mutations have been reported on the PK-LR gene. Erythrocytes PK plays an important role as an antioxidant during erythroid differentiation. Glycolytic inhibition by erythrocytes PK gene mutation augmented oxidative stress, leading to activation of hypoxia-inducible factor-1 as well as downstream proapoptotic gene expression.

Glucose 6-phosphate dehydrogenase (G6PD) deficiency, the most common enzyme deficiency worldwide, causes a spectrum of diseases including neonatal hyperbilirubinemia, acute hemolysis, and chronic hemolysis. Persons with this condition also may be asymptomatic. Approximately 400 million people are affected worldwide ahd about 160 mutations have been reported. Acute hemolysis is caused by exposure to an oxidative stressor such as infection, some foods (fava beans), drugs or various chemicals.

Few cases of erytrocytes 6-phosphogluconate dehydrogenase (6PGD) deficiency in human have been described. The episodic hemolytic events with jaundice in patients with 6PGD deficiency may be the result of a defective erythrocytes ability to counteract conditions of marked oxidative stress as happens at birth and following traumatic events.

Glutathione synthetase (GS) deficiency is a rare autosomal recessive disorder. Clinically affected patients present with severe metabolic acidosis, 5-oxoprolinuria, increased rate of hemolysis, hemolytic anemia, neonatal jaundice and defective function of the central nervous system. A milder form of GS deficiency apparently restricted to erythrocytes, is associated with decreased erythrocyte GSH levels and hemolytic disease, which is usually well compensated. Complete loss of function of both GS alleles is probably lethal.

The clinical symptoms of glutathione reductase (GR) deficiency include reduced lifespan of erythrocytes, cataract, and favism (hemolytic crises after eating fava beans). GR deficiency may alter the clinical manifestation of an unstable hemoglobinopathy and may be the cause of neonatal hyperbilirubinemia. GR deficiency state can be asymptomatic as the residual enzyme activity might be sufficient to maintain the reduced glutathione level to prevent oxidative stress. Whereas inherited glutathione reductase deficiency is rare, acquired GR deficiency due to low amounts of riboflavin in the diet is common in malnourished populations.

Severe acute hemoglobinemia and hemoglobinuria has been described as a result a hereditary heterozygous glutathione peroxidase (GPx) deficiency in Japan. Patients with reduced GPx activity are at a high risk of developing carbamazepine-induced hemolytic crisis and/or aplastic crisis. Point mutations in gene peroxiredoxin 2 in human are not described.

Deficiency of the SOD1 gene causes anemia and autoimmune responses against erythrocytes. Severity of anemia and levels of intracellular reactive oxygen species are positively correlated. Oxidation-mediated autoantibody production may be a more general mechanism for autoimmune hemolytic anemia and related autoimmune diseases. Shift in glucose metabolism to the pentose phosphate pathway and decrease in the energy charge potential of erythrocytes, increase in reactive oxygen species due to SOD1 deficiency

accelerates erythrocytes destruction by affecting carbon metabolism and increase oxidative modification of lipids and proteins. The resulting oxidation products are antigenic and, consequently, trigger autoantibody production, leading to autoimmune responses.

Acatalasemia, the inherited deficiency of catalase has been detected in 11 countries. Japanese-type acatalasemia (Takarara disease) is characterized by the almost total loss of catalase activity in erythrocytes and is often associated with ulcerating oral lesions. Polymerization of hemoglobin and aggregation of the acatalasemic erythrocytes observed upon the addition hydrogen peroxide can be the mechanism for the onset of Takarara disease. The patients with inherited catalase deficiency are more sensitive to oxidative stress to hydrogen peroxide. In the presence of extremely high levels of hydrogen peroxide acute hemolysis may not be excluded. Patients with low inherited catalase activities who are treated with infusion of uric acid oxidase because they are at risk of tumour lysis syndrome may experience very high concentrations of hydrogen peroxide. Inherited adenylate kinase deficiency and pyrimidine 5'-nucleotidase deficiency causes hemolytic anemia.

Under physiological conditions, changes in the activity of proteins and enzymes of erythrocytes owing to point mutations may not be appreciable, however under certain conditions for example, the neonatal period, activation of oxidative stress such as during nfection, a hypoxemia-ischemia, an acidosis, reception of some medicament, influence of toxins, point mutations in proteins and enzymes of erythrocytes can lead to premature destruction of erytrocytes, development of intravascular hemolysis and hemolytic anemia. Hence, erytrhrocyte enzyme deficiency should be considered in patients with hemolytic anaemia. Extended molecular analysis is useful for studying how several interacting gene mutations contribute to the clinical variability of erytrocytes enzymes deficiency.

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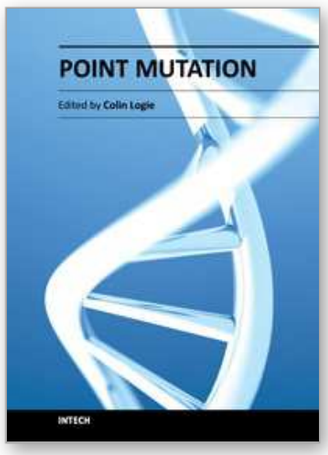

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This book concerns the signatures left behind in chromosomes by the forces that drive DNA code evolution in the form of DNA nucleotide substitutions. Since the genetic code predetermines the molecular basis of life, it could have been about any aspect of biology. As it happens, it is largely about recent adaptation of pathogens and their human host. Nine chapters are medically oriented, two are bioinformatics-oriented and one is technological, describing the state of the art in synthetic point mutagenesis. What stands out in this book is the increasing rate at which DNA data has been amassed in the course of the past decade and how knowledge in this vibrant research field is currently being translated in the medical world.

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