CHAPTER 1

GENERAL INTRODUCTION

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1.1 Anemia

Anemia is a common clinical condition characterized by low levels of hemoglobin (Hb). The main causes of anemia include blood loss or insufficient production of red blood cells and/or Hb as a result of a pathological condition. Most common are iron or vitamins deficiencies (Folic acid and B12), which are generally easy to treat. More problematic can be anemia secondary to severe infections and hemolysis or to clinical conditions associated with disrupted bone marrow function. Anemia can be hereditary and caused by red cell enzyme defects, such as glucose-6 phosphate dehydrogenase deficiency (G6PD), or red cell membrane defects, such as congenital spherocytosis. However, the most common hereditary conditions causing anemia worldwide are the Hemoglobinopathies which are caused by abnormal synthesis of hemoglobin, the main focus of this thesis.

1.2 Hemoglobin

Hemoglobinopathies (HBP) are diseases characterized by changes in the production and function of the vital protein hemoglobin, one of the most essential proteins that transport oxygen from the lungs to all tissues, supporting oxidative metabolism. The hemoglobin molecule (Hb) is the main component of the red blood cells (RBC) and is a tetramer, consisting of two pairs of β-like and α-like globin chains. To fulfill the oxygen transport function, Hb tetramers have 4 oxygen binding prosthetic groups (hemes) each containing one iron atom (1) (Figure 1.1). The production of globin chains is coded by globin genes that have a strictly regulated pattern of expression in different tissues and stage of development (2, 3).

During early embryonic development, erythropoiesis takes place in the yolk sac, transitioning to the fetal liver and finally to the bone marrow as development proceeds through fetal and then postnatal life (4) (Figure 1.2). Furthermore with respect to hemoglobin expression there are also two main switches during prenatal development. The first switch from embryonic-to-fetal hemoglobin expression takes place early in gestation. The second from fetal-to-postnatal hemoglobin expression starts during the last months of gestation and is completed during the following 12 months of postnatal life (4).

At birth, the average content of the red blood cells consists of approximately 80% fetal hemoglobin Hb F ($\alpha_2\gamma_2$) and 20% postnatal hemoglobin Hb A ($\alpha_2\beta_2$).

After the age of 2 years and in normal conditions, HbA will make up »97% of the total circulating Hb, and HbA_2, a second postnatal Hb tetramer ($\alpha_2\delta_2$), will be expressed at a much lower level (»2.5%) while HbF expression will have almost disappeared, although it remains detectable at a very low levels (<1%).

All postnatal hemoglobins are synthesized in the bone marrow in the erythroid red cell precursors. After maturation erythroid cells loses their nucleus and are released in the peripheral circulation where, as young red cells (reticulocytes), they quickly mature into regular red cells and carry out their tasks for an average of 120 days before being replaced by new red cells.

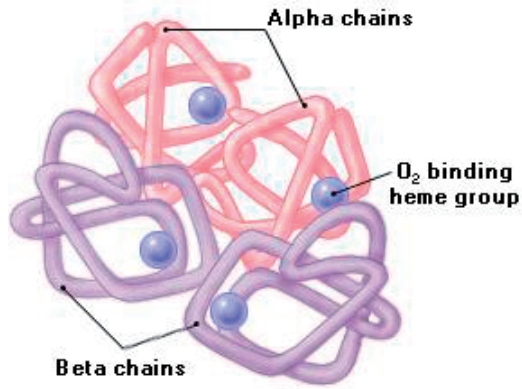

Figure 1.1. Schematic representation of the hemoglobin molecule consisting of two β and two α subunits, each containing an oxygen binding heme group. (Figure adapted from http://resources.med.fsu.edu/gsm/hp/program/section4/4ch5/asidpg16.htm).

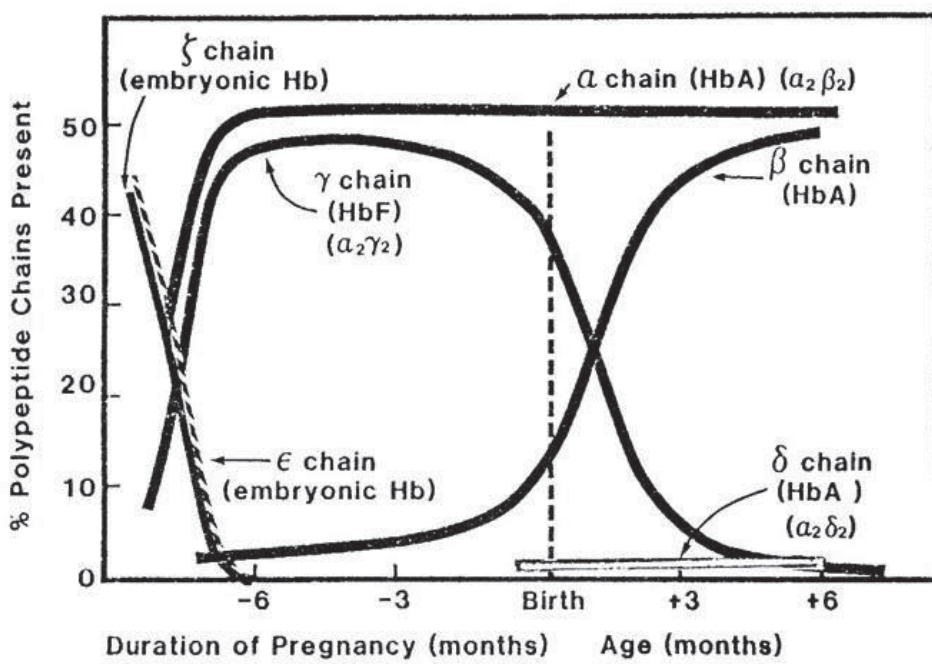

Figure 1.2. Depicting the expression of the globin genes during the switch from embryonic, fetal and postnatal stage. (Figure adapted from http://www.ilym.org/tiki-index.php?page=Plummer1983).

1.3 Hemoglobin evolution

The basic structure and function of the hemoglobin protein is highly conserved among different species making it an interesting model for evolutionary and biochemical investigation. Gametic mutations are the key of the evolutionary mechanism of all living organisms. The mechanism is based upon selection of random *de novo* mutations passed to the progeny to be tested for their advantage or disadvantage. Hemoglobin in its current form has a long evolutionary history. Globin genes coding for the basic protein in vertebrates are believed to be older than 500 million years (5). The relationship between the primary sequences and the three-dimensional structures shows that the human α- and β-globin genes family is derived from a monomeric myoglobin, the oxygen storage and delivery proteins still present in our muscular tissues (6). During the last 200 million years splitting between ancestral alpha and beta like genes, duplications and rare point mutation events have differentiated the ancestral proto-myoglobin gene into the current alpha- and beta- like genes present in the human gene clusters (7) (Figure 1.3).

1.4 Malaria and globin gene selection

As briefly mentioned above, evolution depends on many factors among which the most important are the adaptation to the environment and the fitness of the individuals in providing healthy progeny (8). Hemoglobinopathies (HBP) are caused by mutations on the globin genes that had probably not been particularly selected for until man developed agriculture and had to share the environment with malaria mosquitoes. Carriers of a globin gene mutation were advantaged in malarial-infested areas (9,10,11).

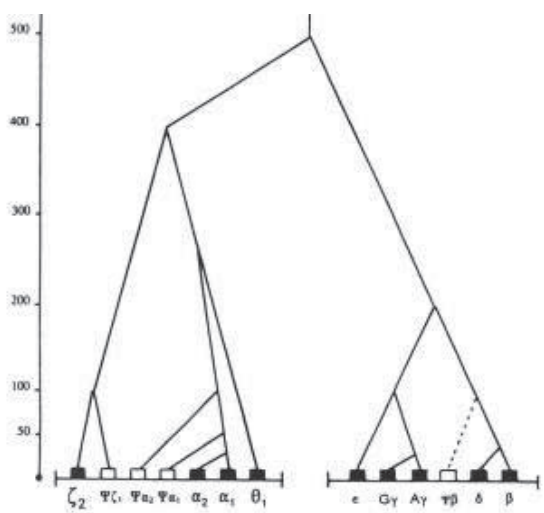

Figure 1.3. Evolution of 500 million years of the human globin gene clusters from proto-myoglobin to the actual α and β-like genes (From Giordano PC. Dissertation 1998). Active genes are in black, open boxes are non-active pseudo (ψ) genes.

Haldane observed that the geographical distribution of HBP and the distribution of malaria greatly overlap, and published the hypothesis that individuals carrying sickle cell disease (SCD) or thalassemia must have a selective advantage over non-carriers in terms of survival and reproduction in malarial-infested areas (12,13,14,15).

Regarding the protecting mechanisms, it has been postulated that the sickle cell hemoglobin (HbS) mutation confers a protection against malarial infection because the deoxy HbS cells infected with malaria become sickled and are subsequently destroyed by the T-cells and macrophages, reducing acute malarial infection in the carriers (16,17). Similarly, it has been shown that α-thalassemia protects against severe malaria by the same mechanism as HbS, ameliorating the pro-inflammatory effects of cytoadherence (18). In vitro studies have shown that there is reduced malarial growth in erythrocytes and enhanced removal of parasitized cells by the immune system in individuals that are thalassemia carriers or heterozygote for Hb S (19).

1.5 The human hemoglobin genes

The human globin genes are clustered on different chromosomes, the β- like genes on chromosome 11 and the α- like genes on chromosome 16. The beta cluster contains an inactive pseudo beta gene (ψβ), the embryonic epsilon (ε) gene as well as the fetal G-gamma (Gγ) (*HBG2*) and A-gamma (Aγ) *(HBG1*) and adult delta (δ) (*HBD*) and beta (β) (*HBB*) genes (20). As mentioned above, the main genes responsible for the hemoglobin composition in postnatal life (HbA) are the β and the α genes. The beta gene is 1.6 Kb in length. About 50 to 70 kb upstream of the beta globin gene is the locus control region (LCR) which regulates the activation and expression of the β globin gene cluster (21, 22).

The alpha cluster located on the telomeric region of chromosome 16, contains several inactive pseudo genes (ψζ, ψα2, ψα1) and a θ gene of unknown function, an embryonic zeta (ζ) gene and two identical α-globin genes (α_2 and α_1) (*HBA2* and *HBA1*) both 1.2 kb long. The main regulatory element (MRE) of the alpha globin genes is located about 60 kb upstream (23). All alpha- and beta- like genes consists of three coding regions (exons) and two introns known as intervening sequences (IVS) (Figure 1.4). The genes are linearly arranged 5' to 3' in the order expressed during development (Figure 1.5) (4). While the beta globin chains are needed for the formation of HbA only, the α-globin chains are involved in the embryonic hemoglobins Gower II (α_2/ε_2), the fetal hemoglobin (HbF (α_2/γ_2), and in both postnatal HbA_2 (α_2/δ_2) and HbA (α_2/β_2). This difference explains some of the postnatal pathology of beta gene defects and the pre- and post-natal pathology of alpha gene defects. The hemoglobin tetramers formed during embryonic, fetal and adult development are summarized in Table 1.1.

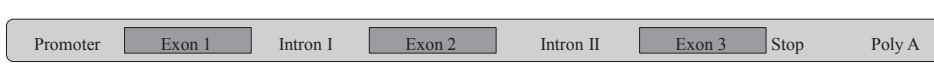

Figure 1.4. Schematic representation of the basic structure of the globin genes, showing the promoter, coding exons and introns and stabilizing polyA tail.

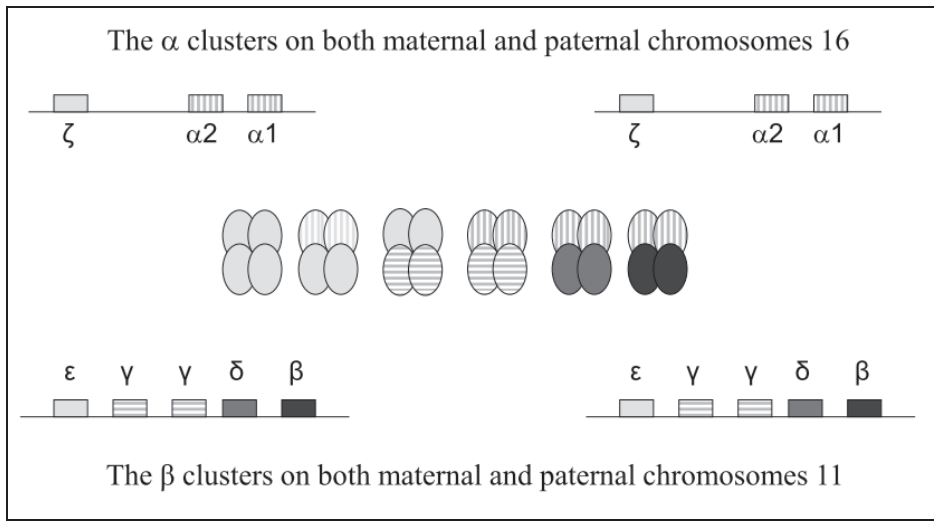

Figure 1.5. Schematic representation of the globin gene clusters on chromosome 16 and 11 respectively with the embryonic ζ and ε genes, the fetal γ genes, the embryonic, fetal and postnatal α genes and the postnatal δ and β genes. The corresponding Hb tetramers, Hbs Gower-I and -II, Hb Portland, Hb F, Hb A_2 and Hb A are depicted from left to right with their different globin chain compositions. Pseudo genes are not shown (Giordano PC, 2012, reference 24).

Table 1.1. Summary of the different hemoglobin synthesized during normal human developmental stages.

Embryonic hemoglobins	Fetal hemoglobins	Adult (post-natal) hemoglobins
Hb Gower I (ζ_2/ε_2) Hb GowerII (α_2/ε_2) Hb Portland (ζ_2/γ_2)	Hb F (α_2/γ_2)	Hb A (α_2/β_2) Hb A2 (α_2/δ_2)

1.6 The Hemoglobinopathies

Hemoglobinopathies (HBP) are the most frequent inherited autosomal recessive disorder in man. In an autosomal recessive disorder two copies of an abnormal gene must be present in order for the disease to develop. Both males and femailed can be carriers or affected.

A carrier couple will transmit the disease to their children in a Mendelian fashion, which means that statistically speaking, half of the progeny will be carriers of one of the two parental mutations and be unaffected like their parents (heterozygous), ¼ will not be carriers (normal) and ¼ will have both parental mutations and be affected by the disease (Figure 1.6). The severity of the disease will depend on the type of mutation and/or complex gene combinations and present with variable form of thalassemia.

As mentioned above, HBP are caused by mutations on the globin genes that, due to a selective advantage in the presence of malaria, have become endemic in the tropical and

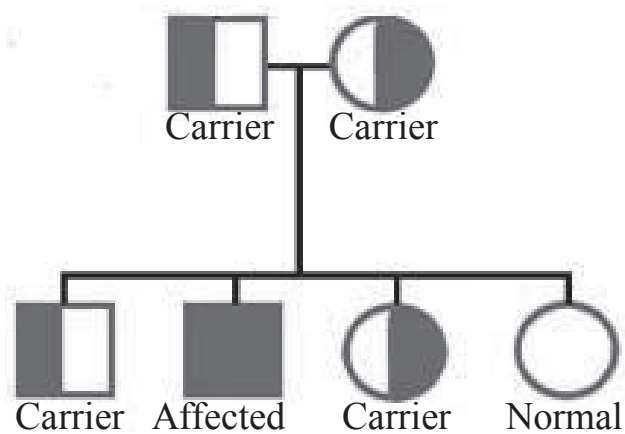

Figure 1.6. Inheritance of an autosomal recessive mutation causing SCD or BTM following a Mendelian fashion.

subtropical areas of the world. From there, they spread by ancient migrations and slavery and today, due to recent massive migrations, are found in many non-endemic areas worldwide (25).

Mutations on the globin genes can change the primary structure of the gene products causing abnormal hemoglobins, can decrease the expression of the gene causing thalassemia and anemia and finally some are silent (polymorphisms).

Most mutation events are limited to one or few nucleotides (point mutations) while other involves larger defects. Large deletions which eliminate parts of genes or even entire genes or locus control regions are associated with a reduced synthesis of the relevant globin chain and hence an expression of thalassemia or it can result in an elevated expression of Hb F (e.g HPFH deletions – section 1.13).

Point mutations can be associated with either thalassemia or abnormal hemoglobins, the latter typically caused by changes on the coding regions of the genes (exons). Point mutations associated with reduced globin synthesis may occur in all parts of the globin genes starting from the locus control regions, the promoter regions, the initiation and stop codons, the coding exons, the exon/intron splice junction sites, deeper intronic sequences, the 5' and 3' untranlsated regions and finally the poly A addition site. Point mutations may generate a stop codon directly or indirectly because of a frame shift due to a deletion or an insertion event of more than one nucleotide causing thalassemia. Exonic point mutations that change a particular amino acid lead to an abnormal hemoglobin, which may or may not have phenotypic consequences. Rarely exonic mutations affect the normal splicing of genes (for example HbE). Not all mutations affect the structure or function of the hemoglobin molecule and it is important to define the correlation between the mutation and the phenotype to predict the severity of the conditions in homozygous or compound heterozygous form.

Hundreds of point mutations causing Hemoglobinopathy have been characterized (26).

A minority are common in specific geographical areas or ethnic groups while many are rare and may or may not be associated with an ethnic origin. We investigated the common HBP's in Oman in chapter 4 to reaveal the common spectrum of HBP mutations.

1.7 The structural and the expression defects

As mentioned above, hemoglobin disorders are divided into two main groups: the structural (hemoglobin variants) and the expression defects (the thalassemias).

The structural hemoglobin variants result in most of the cases from point mutations causing single amino-acid substitutions mainly in the β or α globin chains (27) and have generally little or no adverse effect in the carriers. However, structural mutations may alter in some cases, the stability, the expression or the functional properties of the hemoglobin tetramer and lead to a clinical disorder in the carrier as well.

More than thousand structural hemoglobin variants have been described but the most common worldwide are Hb S, Hb C, Hb E and Hb D^{Punjab}.

Although all recessive, the homozygous state for Hb S as also the combination of HbS/β-thalassemia and HbS/HbC, E or D^{Punjab} variants results in sickle cell disease (SCD) (Table 1.2). The compound heterozygous state for Hb S and Hb C is associated with a clinically milder SCD while the other combinations are usually severe. The HbE variant, although mild in the carrier and in the homozygous state, it may result in both severe SCD and thalassemia major in combination with HbS and β-thalassemia mutations respectively (28) (Table 1.2).

Table 1.2. Cross table showing the combination of HbS and of other common β gene defects (double-lined squares) and of the common α gene traits and the genetic risk deriving from their combinations. (For rare, unknown Hb X variants: ? stands for unknown risk and ?! for possible risk.). Hb S: [β6(A3)Glu→Val, GAG>GTG; *HBB*: c.20A>T]; Hb E: [β26(B8)Glu→Lys, GAG>AAG; *HBB*: c.79G>A]; Hb C [β6(A3)Glu→Lys, GAG>AAG; *HBB*: c.19G>A]; Hb D: Hb D-Punjab [β121(GH4)Glu→Gln, GAA>CAA; *HBB*: c.364G>C] (courtesy of P.C Giordano).

Carrier of	β-Thal	HbS	HbE	HbC	HbD	α^+-Thal (- α/αα)	α°-Thal (- -/αα)	HbX
β-Thal	β-Thal major							
HbS	SCD	SCD						
HbE	β-Thal major	SCD	β-Thal minor					
HbC	β-Thal minor ?	SCD	β-Thal minor	HbC disease				
HbD	β-Thal minor	SCD	β-Thal minor	β-Thal minor	Normal			
α^+-Thal (- α/αα)	β/α^+ Thal minor	α^+-Thal minor	β/α^+ Thal minor	α^+-Thal minor	α^+-Thal minor	α^+/α^+ Thal minor		
α°-Thal (- -/αα)	β/α° Thal minor ?	α°-Thal minor	β/α° Thal minor ?	α°-Thal minor	α°-Thal minor	HbH disease	α°-Thal major	
HbX	?!	?!	?!	?	?	?	?	?

Expression defects of the β-globin genes, most of them recessive in the carriers, are virtually all associated with severe or intermediate forms of β-thalassemia, with SCD being the main concern for public health and prevention in endemic and immigration countries.

Due to the presence of 4 active alpha globin genes, structural mutations of these genes express at a lower percentage (<25%) and their phenotype is usually mild. Expression defects of the alpha genes (deletion or point mutations) express in mild to severe phenotypes depending from the number of genes affected. Loss of expression of all 4 alpha genes results in a lethal condition usually incompatible with post-natal life. When 3 alpha genes are not expressed the phenotype is severe or intermediate (HbH disease). Loss of expression of one or two alpha genes results in a very mild phenotype. Exceptions are those mutations causing unstable α-globin products or mutated Poly A addition signal sequences resulting in severe HbH diseases when in homozygous or hemizygous forms. An example of a possibly severe homozygous alpha-thalassemia case is described in Chapter 6.

1.8 Sickle cell disease: history and clinical condition

The disease long known in Africa with several local names (Abotutuo, Chwechweechwe, Nwiiwii or Nuiduidui), was firstly reported in 1910 by Herrick (29) as a severe hereditary condition affecting young children of African origin. Sickle cell disease or anemia (SCD or SCA) has probably expanded in West Africa, most likely before the desertification of the Sahara, which took place about 10,000 years ago (30). Herrick proposed the association with a cellular abnormality because of the peculiar elongated sickle like shape of the red cells observed in the blood smear of the patients.

In the 1930's, it was shown that the sickle shape was induced by low oxygen pressure and was reversible after oxygenation. Pauling et al. separated in 1949 the abnormal hemoglobin fraction and because of the sickle shape of the cells they called this hemoglobin HbS (31). Later, methods such as the starch gel electrophoresis, allowed an easy detection of HbS and of the other common Hb variants.

In the middle of the 50's, Ingram demonstrated, by separation and analysis of tryptic peptides on combined paper electrophoresis and chromatography (fingerprinting), that the glutamic acid residue at position 6 in the N-terminal peptide of the β chain was replaced by a valine (32). More than twenty years later DNA analysis and the identification of the β globin gene (33) allowed the characterization of the single amino acid substitution resulting from an adenine to thymine transversion at codon 6 (G*A*G→G*T*G) which results in the formation of hemoglobin S when the abnormal β-chains combine with the α-globin chains. To date, more than 1000 hemoglobin variants have been reported and new ones keep coming. The pathological implications of the common variants are well known and that of the rare ones is a matter of continuing research. One century later and the diseases caused by these recessive hereditary traits are quite well treated but not yet cured and therefore carrier identification and primary prevention are the main issues faced by public health.

Being a recessive condition, HbS carriers are mainly asymptomatic and their red cells do not sickle to any significant degree at normal venous oxygen tension. Only at a very low oxygen tension the erythrocytes will temporarily sickle when a carrier is exposed to high altitude (34), extreme exercise along with dehydration (17), and hypothermia in normoxic and hypoxic

conditions (35). Under environmental stress, HbS carriers are prone to a reduced red blood cell (RBC) deformability, which is thought to increase blood viscosity, raising the risk for vaso-occlusion events (36). Although it is very rare for sickle cell trait (SCT) subjects to experience any of these complications, it is of great importance that SCT individuals are aware of their carrier state to avoid potential genetic risk and risk of complications during anesthesia and pregnancy (37).

Patients affected with SCD suffer of a cascade of pathological events causing chronic and acute infarctions, excruciating painful crises, progressive organ and tissues damage and hemolysis (38), drastically shortening the RBC life span (39). HbS tend to polymerize when deoxygenated, resulting in sickle shaped cells causing chronic and acute infarctions (Figure 1.7). As a consequence of vaso-occlusion, dactylitis is one of the first symptoms observed in infants, affecting about 30% of patients in the first year of life (40). Other severe symptoms include splenic sequestration, acute chest syndrome, leg ulcers, avascular necrosis and gallstones. The disease is caused by different β-globin genotypes (always including at least one Hb S allele) and modulated by quite a few genetic and external causes. Therefore not all SCD patients have the same severe outcome of the disease and it is not easy to predict a genotype / phenotype correlation.

Chapters 9, 10 and 11 of this thesis are based on correlation studies between clinical severity of SCD with identical beta genotype and haplotype, subhaplotypes, XmnI polymorphism and response to hydroxyurea drug and finally in relation to alpha-thalassemia genotypes. These studies were conducted in order to draw associations between genotype and phenotype for better risk assessment and selection for the best treatment.

The life expectancy of well-treated SCD patients is considerably shorter than that of the general population and the survival greatly depends on the genotype. If well treated, male and female patients with intermediate SCD genotypes are reported to have a median life

Figure 1.7. Schematic representation of the vaso-occlusion caused by the formation of sickle cells in the post capillary veins under oxygen deprivation (adapted from http://www.cixip.com/index.php/page/content/id/559).

Table 1.3. Summary of the common and rare mutations in the *HBB* gene, including the common variants HbS, HbC, HbD^{Punjab}, HbE that may cause SCD or TM. The phenotype prediction is either described (!) or presumed (?). Adapted from Giordano, 2013 (reference 45).

Traits	Combinations	Predictions
HbS	S/S or S/β-thal	!
HbC	C/S intermediate	!
HbE	E/S severe or intermediate or E/β-thal	!
HbD^{Punjab}	D/S severe	!
HbO^{Arab}	O/S severe	!
Hb Lepore	L/S or L/β-thal severe or L/E	!
HbS Antilles (HbS + β23)	Dominant in carrier, severe in combination	!
HbC Ziquichor (HbS + β58)	S/S, C/S, D/S, E/S... or S/β-thal severe	!
HbC Harlem (HbS + β73)	S/S, C/S, D/S, E/S... or S/β-thal severe	!
HbS Providence (HbS + β82)	S/S, C/S, D/S, E/S... or S/β-thal severe	?
HbS Oman (HbS + HbO^{Arab})	Dominant in carrier, severe in combination	!
HbS South End (HbS + β132)	S/S, C/S, D/S, E/S... or S/β-thal severe	?
HbS-Sao Paulo (HbS + β65)	S/S, C/S, D/S, E/S... or S/β-thal severe	?
Hb Ndjamena (HbS + β37)	S/S, C/S, D/S, E/S... or S/β-thal intermedia	?
HbS Travis (HbS + β142)	S/S, C/S, D/S, E/S... or S/β-thal severe	?
HbS Cameroon (HbS + β90)	S/S, C/S, D/S, E/S... or S/β-thal severe	?
HbS Wake (HbS + β139)	S/S, C/S, D/S, E/S... or S/β-thal severe	?
HbS Jamaica Plain (HbS + β68)	S/S, C/S, D/S, E/S... or S/β-thal severe	?
HbS Clichy (HbS + β8)	S/S, C/S, D/S, E/S... or S/β-thal severe	?
HbS san Martin (HbS + β105)	S/S, C/S, D/S, E/S... or S/β-thal severe	?
$HbS/thal^{+}$ (HbS + -88C>T)	S/S or S/β-thal intermedia	?
HbC Rothschild (HbC + β37)	C/S intermediate	?
Hb Arlington Park (HbC + $HbN^{Baltimore}$)	C/S intermediate	?
Hb Corbeil (HbE + β104)	E/S intermediate, E/β-thal severe	?
$HbT^{Cambodja}$ (HbE + HbO^{Arab})	E/S intermediate, E/β-thal severe	?
Hb Cleveland (HbD^{Punjab} + β92)	D/S severe	?
Hb Korle-Bu	KB/S intermediate	?
All $β^{0}$-thalassemia defects	S/β-thalassemia severe	!
All $β^{+}$-thalassemia defects	S/β-thalassemia severe or intermediate	!?
All β-variants with thalassemic effect	S/β-thalassemia severe or intermediate	!?
GγAγδβ-deletions	Del/S, Del/E, Del/β-thalassemia severe	!?
Aγδβ-deletions	Del/S, Del/E, Del/β-thalassemia intermediate	!?
εγδβ-deletions	Del/S, severe	!?
δβ-deletions	Del/S, Del/E, Del/β-thalassemia intermediate	!?
HPFH point mutations	S/HPFH, β-thal/HPFH, HbE/HPFH mild/silent	!?

expectancy of 42 and 48 years respectively (41) whereas if untreated, the disease is usually lethal in childhood (42).

As shown in Table 1.2, patients with SCD can either be homozygous for the HbS allele (HbS/S), compound heterozygous with other common Hb variants or hemizygous with a β thalassemia mutation (HbS/β). Severe conditions can also result from less common combinations such as HbO^{Arab} (43) or with a list of rare traits (Table 1.3). Inheritance of HbS with β° thalassemia mutations results in absence of HbA and in the presence of HbS % similar to HbS/S condition but generally associated with marked microcytic anemia. When HbS is combined with a β^+ mutation, HbA is synthesized at a reduced rate, and with milder beta defect the SCD condition can be less severe (44). We describe an example of a mild beta-thal nucleotide transversion in association with HbS in chapter 6.

1.9 The thalassemias

As mentioned above, the thalassemias are defects caused by globin gene mutations affecting gene expression. Consequently, thalassemias are classified according to the particular globin chains ineffectively synthesized (46). The pathological conditions associated to thalassemia can be very variable. While generally asymptomatic and only associated with a mild microcytic anemia in the carrier, thalassemia can be lethal in utero and/or very severe in postnatal life depending from upon the genotype at the β-gene locus and to some extent other globin and non-globin genes. Because of the prenatal expression of the alpha genes, alpha thalassemias can present symptoms before birth in mild, severe or lethal forms, while beta thalassemias are usually asymptomatic until about six months after birth, at the time when the postnatal HbA normally takes over from the fetal HbF. In addition, patients may present with different phenotypes due to the kind of mutation or defect combinations. As previously mentioned, the majority of beta thalassemias are caused by point mutation defects. The majority of alpha- and a few beta- thalassemias are caused by large deletions. Alpha deletions may affect one or both alpha genes of the specific allele and homozygosity for two fully deleted genes is not compatible with life. Some large deletions of the beta genes may include the fetal and the embryonic genes in the beta globin gene cluster and in the heterozygous form may be severe in utero but transforming to a mild beta thalassemia heterozygosis in adult life (47).

1.10 The beta thalassemia: history and clinical conditions

How old thalassemia can be was shown by J. Lawrence Angel who in 1964 found porotic hyperostosis and thickening of the diploic space prominent in skeletal remains of children from the Bronze Age in Cyprus and Greece as a result of thalassemia major (9,48).

The disease has remained clinically unclassified until the 20th century probably due to the general high infant mortality and to the fact that the condition was not associated with the humoral theory of Greek medicine developed by Hippocrates which was rather philosophical but not that far from the truth.

Greeks believed that not only the blood itself but blood vessels carried air, food and emotions, while Romans associated all kind of unhealthy state with the stinking marshes that surrounded the city giving the actual name of the disease Malaria, meaning “bad air”.

Only in the 20th century two publications both published in 1925, one in Italy and the other in the USA, defined the severe form of the disease.

Rietti called the disease IERGA (Ittero Emolitico con Resistenza Globulare Aumentata) because of jaundice, hemolysis and the classical increased osmotic resistance of the red cells (49). Cooley and Lee reported the same pathology in children of Italian and Greek immigrants in the USA (50). Subsequently the disease was given many names such as Rietti-Greppi-Micheli disease, Cooley anemia, erythroblastic anemia and all this became confusing until the definition "Thalassemia" from the Greek word for sea was proposed in 1932 by Whipple and Bradford, due to the high incidence of the disease in Mediterranean populations (51).

At the same time, population geneticist J.B.S. Haldane, had elaborated the theory of malaria selection for thalassemia and his hypothesis was published with the first mathematical models for evolution due to selection (52). Between 1944 and 1947 Silvestroni and Bianco studied many affected families and defined the hereditary character of the disease (53). In 1955 Kunkel and Vallenius separated the HbA_2 fraction (54) and in 1957 Silvestroni and Bianco again demonstrated that an elevated HbA_2 fraction was present in the parents of affected children (55). From that time on, many developments have followed at the biochemical and molecular level and today thalassemia is perhaps the most well studied recessive disorder in man. As mentioned above, beta-thalassemia (β-thal) is mainly caused by prevalent or less common point mutations in the β-globin gene due to substitutions or deletional/ insertional defects. Defects can either lead to reduced synthesis of the β-globins (β^+) or complete absence (β^o). In distinction to the classical and common recessive forms of beta-thalassemia, some rare dominant beta globin mutations may result in the synthesis of extremely unstable beta globin variants causing intermediate thalassemia phenotype in the heterozygous state (56). Usually mutations in the promoter and 5' UTR affect gene transcription while mutations in the splice junction, 3' UTR and polyadenylation site affect mRNA processing and mutations in initiation codon or frame shift mutations affect mRNA translation. The very mild or silent mutations are associated with normal red blood cell indices and normal or borderline HbA_2 (57) raising difficulty in carrier screening at the hematological level. We describe in chapter 6 an example of a mild beta-mutation associated with near borderline HbA_2 %.

Clinically and hematologically, thalassemia is classified into three conditions of increasing severity; the mild beta-thalassemia carrier state, the thalassemia intermedia, and the severe thalassemia major.

As mentioned above, carriers of β-thalassemia are usually asymptomatic with a hematological parameters characterized by a mild anemia, reduced MCV and MCH values (60–70 fL and 19–23 pg), a raised level of HbA_2 (3.5–9.0 %), and normal or slightly elevated HbF (58).

Homozygous or compound heterozygous for severe mutations become severely anemic around six months of age, once the delayed expression of the normal HbF has become insufficient in absence of HbA. Children are usually diagnosed within the first two years of life due to severe anemia, hemolysis and failure to thrive. The hematological picture of a non-transfused patient will show a severe erythromorphology with numerous of erythroblasts, very low Hb, MCV and MCH values, absence of HbA with HbF and HbA_2 as the only Hb fractions present. In these conditions, patients will usually require an urgent blood

transfusion that will maintain the Hb value at a reasonable level for 2-3 weeks when the need for a continuous transfusion regime will become evident. Subsequently these patients will accumulate transfusional iron up to a toxic level and thus require continuous iron chelation therapy. Other complications occurring in transfusion-dependant patients include growth retardation, delay or failure of sexual maturation, cardiac disease and complications in liver or endocrine glands (59). Individuals who are regularly transfused and get state of the art management may survive beyond the age of 40. Cardiac complications are the cause of death in 71% of the patients with thalassemia major (60). The clinical presentation of thalassemia major is listed in Table 1.4.

Beta-thalassemia intermedia is a milder conditions that includes a very heterogeneous group ranging in severity from a transfusion free carrier-like state to the delayed severe transfusion-dependent type (61). Intermediate cases with the severe forms present severe clinical symptoms usually between the ages of 2 and 6 years. The mild condition may present with complications later in life or may go on without requiring blood transfusion till adulthood or during pregnancy. The intermediate state result from complex genotypes involving coinheritance of homozygous or compound heterozygous mild β-thalassemia alleles and may also result from the co-inheritance of alpha- and beta-thalassemia mutations or co-inheritance of additional alpha globin gene genotypes (triplicated or quadruplicated alpha globin gene rearrangements) when interacting with typical heterozygous beta-thalassemia (62).

Beta thalassemia subjects with no functional beta globin synthesis may present as beta intermedia due to amelioration by high Hb F expression caused by HPFH deletions (63) or rare mutations in the beta-LCR regulatory sequence (64). Essentially, the clinical severity of beta thalassemia major is related to the extent of imbalance between the alpha globin and non alpha globin chains (62). When the beta globin chains are reduced or absent, the free alpha chains precipitate and lead to oxidative damage of the cell membrane, hemolysis and ineffective

Table 1.4. List of the variable clinical presentation possibly seen in a β-thalassemia major individual divided per different age groups.

Age group	Clinical phenotype
6-24 months	Pallor and jaundice Failure to thrive Feeding problems Diarrhea Fever Spleen and liver enlargement
2+ years	Growth retardation Poor musculature Hepatosplenomegaly Leg ulcers Skeletal deformities Gallstones Thrombosis

erythropoesis (65). Although differentiation between thalassemia major and thalassemia intermedia is not always easy, it is needed to plan state of the art management and to avoid starting unnecessary transfusions (66).

In conclusion, analysis of both the alpha and beta genotypes and testing for the presence of ameliorating genetic factors is essential for risk prediction, treatment and / or prevention of the hemoglobinopathies. In order to define these factors in the Omani population we have described the beta-thalassemia spectrum in the country in an extensive study presented in chapter 5.

1.11 The alpha thalassemias

While humans have only two beta globin genes, one on the maternal and one on the paternal chromosome 11, the alpha gene cluster on chromosome 16 is characterized by two active alpha genes, two inherited on the maternal allele and two on the paternal, resulting in a total of four alpha globin genes.

As already mentioned, most α thalassemias are caused by deletions that may remove one or both α-globin genes of each allele. In the first case, when one of the two genes is still expressed, the condition is called α^+ thalassemia. When both α genes of the same allele are deleted the condition is called α° thalassemia. The presence of 4 alpha genes and the occurrence of partial expression of an affected allele make risk assessment somewhat complex. In general, carriers of α^+ or α° thalassemia will show mild anemia if any, with minimal to evident microcytosis (reductions in MCV values) and hypochromia (low MCH values) and sometimes a slight reduction in HbA_2 levels. However, the genetic risk of the two conditions will be very different. While an individual homozygous for α^+ thalassemia will not be affected and present with mild anemia, homozygosis for α° thalassemia will be a lethal condition and combinations of α° with α^+ conditions will result in the intermediate forms of variable severity called HbH disease.

The phenotype of Hb H patients is variable depending on the nature of the mutation. HbH caused by deletional mutations results in a moderately severe hypochromic microcytic anemia but clinically these patients are less severely affected than patients with HbH caused by point mutations who have a more severe manifestation and may require frequent hospitalization and recurrent blood transfusions (67).

The severe form α° homozygosis (Hb Bart's Hydrops Fetalis), which results from four defective α-globin genes, is a severe conditions leading to perinatal death. The common genotypes of these conditions are summarized in Table 1.5.

Most cases of alpha thalassemia trait in the world are caused by relatively large deletions rather than point mutations. The most common alpha thalassemia mutations are the $-\alpha^{3.7}$ kb rightward deletion (RW) followed by the $-\alpha^{4.2}$ kb leftward deletion (LW) which are both α^+ defects. The former involves the deletion of the 3' part of (*HBA2*) gene and 5' of (*HBA1*) gene, forming a hybrid gene while the latter consists of full removal of *HBA2* gene and 5' of the *HBA1* gene. Both defects are α+ conditions.

Other large deletions involving the alpha cluster include the $-\alpha^{20.5}$, the SEA, the Med I, the Thai and the Fil (68) (Figure 1.8). These deletions involve both alpha genes and are therefore called the α° thalassemia conditions associated with the severe forms of the disease. Non-

Table 1.5. The symbolic indication of the different alpha genotypes and the accompanied phenotype.

Genotype	No. of defective genes	Phenotype
(-α/αα)	1	Silent or mild carrier α^+
(αα/--)	2	Mild α° carrier
(-α/-α)	2	Mild homozygous α^+
(-α/--)	3	Intermediate (HbH)
(--/--)	4	Severe Hb Bart's (hydrops fetalis)

deletional alpha thalassemia mutations are also regularly found. Among the most common are the penta-nucleotide 5nt deletion (HBA2: c.95+2_95+6delTGAGG), the polyadenylation tail (+94) AATAAA>AATAAG mutation (HBA2: c.*94A4G) and Hb Constant Spring (CS) (HBA2:c.427T>C) (69). We have studied the spectrum of alpha-thalassemia in Oman including deletional and non-deletional mutations in chapter 7 to decipher the common and rare alpha mutations in the population.

Carrier identification of alpha thalassemia is important as it has a similar hematological picture to iron deficiency anemia (70). Carriers of alpha thalassemia are often wrongly diagnosed and empirically treated with iron therapy where in fact they only need information and eventually folic acid supplement.

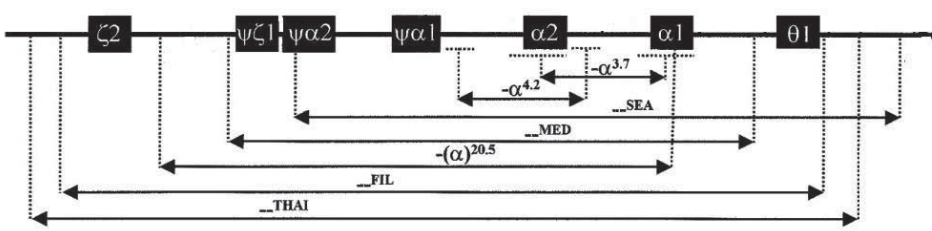

Figure 1.8. The common deletions of the alpha-globin gene cluster. The extent of each deletion is represented by an arrow line. Adapted from Tan et al, 2001 (reference 71).

1.12 Delta thalassemia

The delta globin gene coding for the globin chains needed to form hemoglobin A_2 (δ_2/α_2) is located on chromosome 11 between the β- and the γ-globin genes (see section 1.5). The δ gene (*HBD*) is similar to the β-gene but with a much less active promoter. Therefore, the δ gene expression in normal individuals is approximately 2.5–3.5% of the total hemoglobin resulting in a similar level of expression of HbA_2. Defects of the δ genes have no clinical significance but may compromise the diagnosis of β-thalassemia trait. Thalassemic or structural defects of the δ may reduce HbA_2 expression (<2%) and this in co-inheritance with a β-thal allele, may lead to diagnostic mistakes due to the reduction to normal ranges of the HbA_2 level that should

have been found elevated in that particular β-thalassemia carrier (72,70). Similarly, structural mutations of the delta globin genes may cause the splitting of HbA_2 in two fractions, and if the abnormal fraction is overlooked, it many cause a wrong estimation of the HbA_2 level as well.

As in β-thalassemia, also in δ-thalassemia, each population has its own spectrum of common mutations which should be taken into consideration when confirming or excluding a presumed carrier of β-thalassemia with less elevated or normal HbA_2 levels (73). For that we investigated cases with low HbA_2 readings to define the spectrum of δ-thalassemia in the Omani population in chapter 8.

1.13 Hereditary persistence of fetal hemoglobin (HPFH) and δβ-thalassemia

The clinical definition of hereditary persistence of fetal hemoglobin (HPFH) goes back to the time when molecular analysis of gene deletions was not yet available and in fact most of these defects are mild δβ-thalassemias resulting from large deletion in the β-gene cluster enhancing the HbF expression. Today, the real HPFH defects are considered those caused by non-deletion events, that is by point mutations in the promoters of the γ globin genes.

More than 40 different types of HPFH like δβ deletions with varying 5' and 3' breakpoints have been reported usually all mild in the carriers (74). These disorders are characterized by high HbF levels and can be distinguished from one another by comparing their clinical and hematological parameters and deletion break points (75). Individuals heterozygous for HPFH deletions present with elevated red blood cell (RBC) counts and with HbF levels ranging from (15-30%) (76). Rare cases homozygous for HPFH deletions have been described with near 100% HbF and very high RBC counts (77).

The elevated RBC counts are due in these cases to the higher oxygen affinity of the HbF tetramer which is delivering lesser O_2 to the tissues causing hypoxia in spite of the elevated Hb levels of these patients. Depending on the level of the HbF expression, deletions in combinations with β-thalassemia can be associated with thalassemia intermedia phenotypes (75) and in general compound heterozygous with β-thalassemia are clinically mild due to the high levels of γ-globin synthesis compensating for absence of β-globin chains, thus reducing the level of excess alpha globin chains.

1.14 Hb Lepore

Hemoglobin Lepore (Hb Lepore) defects are caused by unequal cross over between homologous regions on the δ and the β genes resulting in several δβ-fusion genes with β^+ thalassemia expression. Several Hb Lepore mutations have been described of different kind of δβ-globin hybrid chains (78). The reason why the δβ-globin hybrid chain is transcribed at a decreased rate is because it is controlled by the δ gene (*HBD*) promoter which is normally less efficient than the β gene (*HBB*) promoter. Due to decreased quantity of the δβ hybrid chains produced, the clinical phenotype in the heterozygote is associated with mild hypochromic, microcytic anemia with a normal or reduced HbA_2 and eventually elevated HbF level and a relatively low percentage of Hb Lepore (6-15%). Cases with homozygosity or compound heterozygosity of Hb Lepore and β-thalassemia, may present with a severe thalassemia major or intermedia phenotype (79). Therefore, molecular characterization is essential, especially in cases of risk prediction.

1.15 The classic modulating factors (β-globin cluster haplotypes, high HbF, XmnI polymorphisms and coexisting alpha thalassemia)

Thalassemia and sickle cell disease risk prediction is a complex matter. Different thalassemia mutations may have different reduction in expression, compensation with higher HbF expression can be present or absent, and coexisting alpha thalassemia or alpha gene triplications and duplications may ameliorate or aggravate the phenotype in both patients and carriers. As these and other still incompletely defined factors can either ameliorate or exacerbate the phenotype, genetic analysis of modulating factors helps the geneticist and the clinicians to predict the severity and prognosis of the disease. We have approached these elements in chapters 9, 10 and 11 of this thesis.

1.16 Haplotype

The β-globin haplotypes on which the mutation is carried was originally defined based on the pattern generated by restriction enzymes – so-called restriction fragment length polymorphisms or RFLPs (30). In the case of sickle cell disease (SCD), the HbS mutation has been described on different haplotype backgrounds, named after the regions in which they were first identified (80). The Asian (Arab-Indian) haplotype was first found in the Middle East and India (81). The Benin haplotype was first reported from central and West Africa whiles the Central African Republic (CAR) or Bantu haplotype from Central Africa around Angola and Congo. The Senegal haplotype was described on the Atlantic coast of West Africa (80).

Differential HbF expression is associated with the different haplotypes and may influence the clinical course of SCD. Thus analysis of β^S haplotype aids in predicting disease severity. The Senegal and Asian haplotypes are associated with higher levels of Hb F and a milder disease (82), while the CAR haplotype is associated with a more severe disease and the Benin and Cameroon haplotypes are intermediate in severity (30). Although several studies have been conducted to establish a clear relation between β^S-haplotypes and disease, the correlation remains unclear (83). Therefore we have charted the haplotypes and sub-haplotypes in homozygous Omani S/S patients and we have studied the correlation between different haplotype genotypes and SCD phenotype in chapter 9.

1.17 HbF

Hb F ($\alpha_2\gamma_2$) is the major ameliorating factor of beta thalassemia and sickle cell disease (84). Post-natal reactivation of fetal hemoglobin expression has been the aim of many studies. However, so far none means has been identified which permanently reactivates the expression of γ genes, to levels sufficient for therapeutic effect in postnatal life. Temporary reactivation however has been obtained to some extent by continuous use of different cytotoxic drugs, among which hydroxyurea (HU) is the only one without significant adverse effects, and is currently used routinely in the clinic (85).

The HbF expression is mainly regulated by elements linked to the β-globin gene cluster that switch off expression after birth. Some elements associated with the promoter sequences of the gamma globin genes are known to enhance HbF expression in postnatal life, among which the XmnI polymorphism on the promoter of the Gγ gene is the most important (86). High HbF

levels have also been observed significantly elevated in a number of bone marrow malignancies and slightly elevated during a pregnancy or at the laboratory which are likely due to a variety of mechanisms (87).

In case of sickle cell disease, high HbF concentration in the red cell dilutes the concentration of Hb S, increases the oxygen tension and inhibits Hb S polymerization (88), improving the phenotype significantly. In one study, reduced HbF level was associated with higher risk of stroke (89) and increased risk of brain infarcts in young children (90). However, these studies are often not sufficiently categorized; mixing ethnicity, genotypes, age of the patients, sample sizes and analytical approaches, all factors that are bound to bias the observations.

In our studies presented in chapters 9 and 10, we have tried to overcome this problem, categorizing patients more precisely and showing the correlation between genotype, haplotype and phenotype and the response to hydroxyurea treatment in the presence and absence of the XmnI polymorphism.

1.18 XmnI polymorphism and other natural beta cluster enhancers

As mentioned above, XmnI genotype variability can explain the considerable difference in HbF levels among patients and the variable responses to hydroxyurea in sickle cell disease and beta-thalassemia patients. As mentioned above, the XmnI -158 C>T mutation on the G-gamma gene promoter is one of the main single nucleotide polymorphism associated with high HbF%. It is usually silent in normal subjects but may become active in beta-thalassemia heterozygote under some hematopoietic stress and even fully active in cases of beta-thalassemia major and sickle cell disease, sometimes leading to notably raised levels of HbF production (91). Other HbF determinants not linked to the beta globin gene cluster have also been reported such as *HBS1L-MYB* intergenic region chr6q23, *BCL11A* mutations chr2p15, and *KLF1* mutations chr19p13.2 (92). The less common genetic variants influencing Hb F levels that are linked to the beta globin gene cluster are summarized in Table 1.6.

1.19 Coexisting alpha – thalassemia

Alpha-thalassemia may also modulate the phenotype of sickle cell disease (SCD) patients (82) by reducing the intracellular concentration of HbS, which in turn decreases the chance of polymerisation, cellular damage and hemolysis (93). Alpha thalassemia also lowers the MCV value and this should favor the rheology of the red cells and reduce the chance of HbS polymerization, the number of irreversibly sickled cells and the chance of infarctions (94). The modulating effect of α-thalassemia has been proposed to be proportional to the number of deleted α-globin genes (93) and the coinheritance of α-thalassemia has been reported to modify the phenotype of SCD by decreasing hemolytic rate, risk of stroke, pulmonary hypertension and leg ulceration while increasing the frequency of acute painful vaso-occlusive episodes and acute chest syndrome (95). However, also for alpha thalassemia the correlation studies are controversial and in our opinion this could be due to the fact that the studied cohorts are insufficiently categorized at the genotype level.

In our study presented in chapter 11 we have tried to overcome this problem by studying the effect of alpha thalassemia on well categorized cohorts of patients showing the correlation between genotype, haplotype and phenotype and the presence and absence of alpha thalassemia.

Table 1.6. New and known molecular determinants associated with elevated HbF. *=new mutations, **=common polymorphism present on many haplotypes (adapted from Amato et al, 2014, reference 87).

Mutation	Gene	Ethnic prevalence	HbF% in carrier
−4 bp (−225/−222)	Aγ	African	6–7
−202 C>G	Gγ	African	15–20
−202 C>T	Aγ	African	3
−198 T>C	Aγ	English	4–12
−196 C>T	Aγ	Italian/Chinese	21–15
−197 C>T*	Aγ	Italian	6
−195 C>G	Aγ	Brazilian	4–5
−175 T>C	Aγ	Afro American	17–38
−175 T>C	Gγ	Afro American, English, Italian	28–29
−161 G>A	Gγ	African	1–2
−158 C>T	Gγ	African and multiethnic	<1 unless stress
−158 C>T** (Xmn-I)	Gγ	Afro American	2–5
−117 G>A	Aγ	Mediterranean	8–10
−114 C>G	Gγ	Australian?	8.6
−114 C>T	Gγ	Japanese	11–14
−114 C>T	Aγ	Afro American	3–6
−114 C>G	Gγ	Australian ?	8.6
−13 bp (−114/−102)	Aγ	African	30
−113 A>G*	Aγ	Italian	6.5
−110 A>C	Gγ	Czechoslovakian	1%

By this we can again underline that the analysis of genotype, haplotype and modulating factors can help define the prognosis and the genetic risk allowing better counseling and rational based interventions before the onset of organ damage. Nevertheless, besides genetic and molecular modifiers, environmental factors such as physical activity, diet, toxins and socioeconomic status may also influence the clinical course of SCD (82). Understanding both the environmental and the genotype factors associated with the clinical variability between patients could dramatically improve patient care, and prevention.

1.20 Diagnosis of hemoglobinopathies

Hematology laboratories play an essential role in the diagnosis of sickle cell and thalassemia carriers and affected individuals. The two main basic methods of HBP diagnosis which are used in almost all laboratories are a) the complete blood count (CBC) which can detect hypochromic microcytic parameters, and b) separation and estimation of the hemoglobin fractions by high performance liquid chromatography (HPLC) or capillary electrophoresis (CE). These methods can putatively detect heterozygosis, homozygosis or compound heterozygosis of the common conditions with great sensitivity and specificity.

Since carriers of the common HbS, C and D variants are often normochromic, CBC should not preclude Hb separation but should be done in parallel. By measuring the main hemoglobins; HbF, HbA and HbA_2, all carriers of "high HbA_2 beta thalassemia" will be diagnosed. Any other Hb fraction putatively identified, as HbS, C, E or D will detect carriers of the variant and/or combination of these traits. The CBC should always match the putative HPLC/CE diagnosis and if not, further examination should be done at the molecular level (96). Alpha thalassemia will be characterized by microcytosis in the presence of a slightly reduced HbA_2 but will need molecular confirmation, particularly in case of risk assessment. Only HbS can be confirmed by much simpler techniques such as sickle or solubility tests (24).

Although the provisional diagnosis of the common variants made by CBC and Hb separation are reliable, molecular confirmation is essential to decipher the underlined genotype, haplotype and modulating factors. The main method for molecular diagnosis is based on polymerase chain reaction (PCR) technique. The so-called GAP PCR (using primers complimentary to the breakpoint sequences amplifying a deletion-specific fragment that spans the deletion if present) is used for the common alpha deletions. Basic PCR reactions using specifically targeted gene primers followed by direct DNA sequencing are used to detect point mutations. Analysis of large deletions/insertions within the alpha and beta clusters can be performed by multiplex ligation-dependent probe amplification (MLPA). Figure 1.9 shows the basic flow chart of the methods used during HBP screening diagnosis in multi ethnic countries.

We have shown the validity of these technologies in our publications on the molecular spectrum in Oman reported in chapters 4 and 5. We have shown the possibilities of advanced screening technologies in our paper reported in chapter 12.

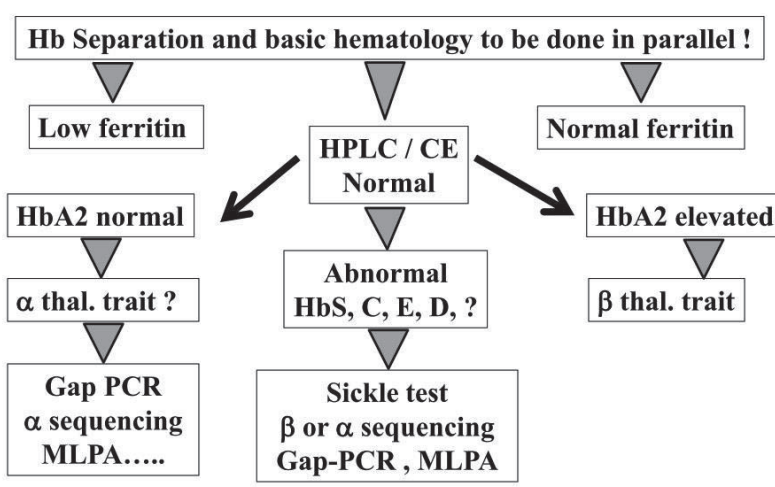

Figure 1.9. Flow chart of HBP screening/diagnosis (courtesy of P.C. Giordano).

1.21 Epidemiology in endemic countries (Worldwide epidemiology)

The World Health Organization (WHO) estimates that at least 5.2% of the world population is at risk for having children with a severe hemoglobin disorder (97), that over 7% of the pregnant women carry a hemoglobin variant (97) and that 300–400 thousand babies with severe forms of these diseases are born each year (98) (Figure 1.10). Although these conditions occur at their highest frequency in Africa and other tropical regions, population migrations have ensured that these conditions are now encountered in many non-endemic countries.

The most wide spread hemoglobin disorder is sickle cell disease (SCD). Thousands of children with this severe disease are born worldwide each year, largely in Sub-Saharan Africa (85%) (97), the Middle East and parts of the Indian sub-continent, where carrier frequencies are ranging from 5 to 40% (98). In India, around 40,000 SCD children are born each year. In America and Eastern Mediterranean the figure is estimated at 10,000 while 2000 are born in Europe (99). It is calculated that 10,000-15,000 SCD patients are living in the United Kingdom and France (100) while it is estimated that worldwide 1.1% of the couples are at risk of having children with a severe hemoglobin disorder, and that 2.7 per 1,000 conceptions are affected (101).

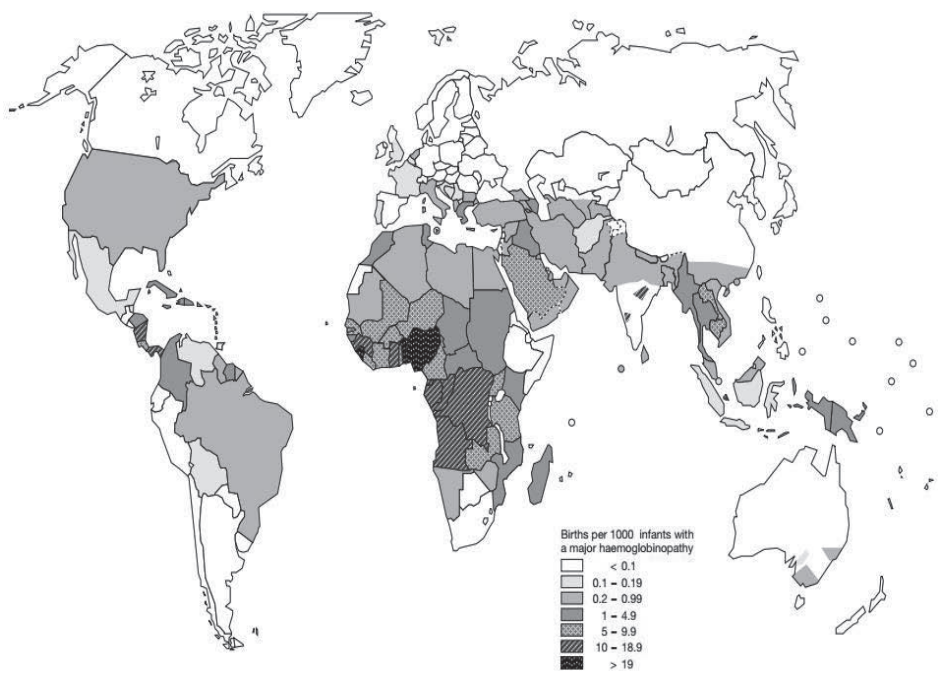

Figure 1.10. Geographical map showing the distribution of HBP disorders around the world in terms of expected affected infants per 1000 births. Adapted from (http://www.who.int/genomics/public/Maphaemoglobin.pdf).

Globally, a total of 269 million persons are estimated to carry thalassemia (β-thalassemia and α-thalassemia) (98). High incidences of thalassemia are reported for the Mediterranean region, parts of Africa, the Middle East, the Indian sub-continent, the Pacific Islands and in South-East Asia where carrier frequency for β-thalassemia and α-thalassemia ranges from 1 to 20% and from 10 to 20% respectively (98). However, α thalassemia do not pose a huge global health problem as β-thalassemia (98) because 20% of the world population carry α+ thalassemia (99) which is a less problematic condition for global public health than the α° thalassemias (due to large deletion of the α-globin cluster) which has a restricted distribution, occurring mainly in South-East Asia and the Mediterranean basin.

High prevalence of beta-thalassemia is present in Mediterranean populations, the Middle-East, Central Asia, India and Far East with the highest incidences reported in Cyprus, Sardinia and South East Asia (102). Due to migration, β-thalassemia is now common in Europe, North and South America, Caribbean, and Australia.

1.22 Treatment

Beta- thalassemia major patients rely entirely on regular blood transfusions to avoid the complications of severe anemia. Blood transfusions allows normal development throughout childhood but lead rapidly to iron overload that can be fatal in the second decade of life. For that reason, iron-chelating therapy has been developed. A drug named Deferoxamine mesylate was first introduced in the 1960s as a standard iron chelating therapy to improve the survival of thalassemia patients (103).

The pathophysiology in SCD is quite different to that of Thalassemia Major. In sickle cell disease children, immunizations through vaccination are the key to prevent the body from infections due to their damaged spleens that are unable to protect the body from bacteria. Much effort has been and continues to be directed to design drugs that could promote HbF synthesis, prevent red cell dehydration, and inhibit HbS polymerization. Only hydroxyurea has been proven thus far to be a useful drug to alleviate the symptoms of SCD such as acute vaso-occlusive complications, episodes of acute pain, acute chest syndrome, along with blood transfusion in highly symptomatic patients (104). Besides raising HbF %, hydroxyurea also reduces red cell–endothelial interaction and decreases erythrocyte density (105). However, it is estimated that 40% of patients do not respond to hydroxyurea treatment at all (106). Furthermore, long-term exposure to hydroxyurea treatment still raises concerns due to its side effects such as myelosuppression and the potential risk of malignancies (106).

Bone marrow transplantation (BMT) is the only cure available for severe Hemoglobinopathy (HBP), but it is only used in patients when a sibling donor with identical human leukocyte antigen (HLA) is matched. Its use is limited by the toxicity and morbidity associated with the procedure, the difficulty in finding a suitable family donor (107) and the availability of transplant centers. Bone marrow transplantation has been carried out from unrelated donor, as long as the HLA genotype is near-compatible, and some patients have become transfusion free following BMT (108). Cord blood transportation from an unaffected HLA compatible newborn also offers a good chance of curing an affected sibling (109). Moreover, hematopoietic stem cells and homologous recombination techniques are being actively investigated using affected mouse models with beta-thalassemia to correct the molecular

defect by transferring a normal gene via a suitable vector or transfecting the embryonic stem cells from the affected mice with a DNA fragment containing a normal and active beta globin gene construct (110).

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