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Hemoglobinopathies and thalasemias – genetic basis and molecular diagnosis**

Hemoglobinopatie i talasemie – podłoże genetyczne oraz diagnostyka z zastosowaniem technik biologii molekularnej

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S u m m a r y

Hemoglobinopathies and thalasemias are genetic disorders connected with the presence of mutations in genes coding globin proteins, mainly in genes coding α and β globin. Hemoglobinopathies are connected with haemoglobin protein alterations. Thalasemias are caused by decreased production of specific globin chains. Mutations in γ globin gene cause an increase in haemoglobin F concentration in adults, and this condition was called hereditary persistence of fetal haemoglobin (HPFH). Taking into consideration the fact that this disorders are presently common in the areas (including Poland), where they have not been previously often detected, there is increasing need to develop better diagnostic methods, evaluating genes coding globin proteins. They should detect not only common mutations, but also rare and *de novo* mutations. Their application is also important in prenatal diagnosis, particulary in populations with high frequency of thalasemias and hemoglobinopathies.

Key words: haemoglobinopathy, thalassemia, haemoglobin structure, haemoglobin types, molecular diagnosis

S t r e s z c z e n i e

Hemoglobinopatie i talasemie są chorobami genetycznymi, które mają związek z mutacjami w genach kodujących białka globiny, najczęściej w genach α i β globiny. Hemoglobinopatie wynikają z zaburzeń struktury białka hemoglobiny. W talasemii dochodzi do obniżenia syntezy określonych łańcuchów globiny. Mutacje dotyczące genu γ globiny prowadzą do wzrostu stężenia hemoglobiny płodowej u dorosłych, a taki stan nazwano zespołem wrodzonego przetrwania hemoglobiny płodowej (HPFH). W związku z występowaniem hemoglobinopatii i talasemii w rejonach świata, w których wcześniej nie były wykrywane, w tym w Polsce, rośnie potrzeba opracowania coraz lepszych metod diagnostycznych oceniających geny kodujące białka globiny. Powinny one wykrywać powszechne mutacje, ale także mutacje rzadkie i powstające *de novo*. Ich zastosowanie jest również ważne w diagnostyce prenatalnej, szczególnie w populacjach, w których talasemie i hemoglobinopatie są częste.

Słowa kluczowe: hemoglobinopatia, talasemia, budowa hemoglobiny, rodzaje hemoglobiny, diagnostyka molekularna

INTRODUCTION

Anaemias are heterogeneous group of disorders, connected with red blood cell (RBC) count and/or haemoglobin concentration decrease. A large group are haemolytic anemias – congenital or acquired diseases caused by various intra- and extracellular factors. Each one of this disorders is connected with shorten lifespan of RBCs and accelerated removal of this cells. They may be the result of haemoglobin synthesis disturbances, caused by mutations in one or more genes coding globing chains. Congenital haemolytic anaemias connected with this variations are divided into two groups. The first one contains disorders in which mutation causes disturbance in aminoacid sequence of globin polypeptide chain and that gives abnormal type of haemoglobin with impaired functions; this group of diseases is called haemoglobinopathies (1). The second group are thalassemias, in which mutation in one or more globin genes leads to absence or decrease in globin protein synthesis. Excessive globin chains damage red blood cells, and that in consequence shortens RBCs lifespan (2, 3).

It was long believed, that haemoglobinopathies and thalassemias are found only in tropical and subtropical regions. High degree of carrier-state in this areas was connected with mechanisms protecting against Plas-

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modium and malaria (4). In recent years it was noticed that frequency of this disorders is increasing in different regions, like North America, Great Britain, Australia (5) and Germany (1). Cases of thalassemia α and β are also more often described in Poland (6-10). It is related to accelerated migration and increased percentage of mixed marriages, but also to improved diagnostic methods detecting this disorders. It is important to diagnose thalassemia and describe the number and the type of mutations not only to begin the appropriate therapeutic action, but also to determine the carrier state in patients in reproductive age. This strategy is particularly important in regions, where thalassemias are very common and the risk of having a partner with globin disorder is high (11, 12).

HAEMOGLOBIN STRUCTURE AND CODING OF GLOBIN CHAINS

Haemoglobin (Hb) is red blood cell protein, and its basic function is oxygen transport. Haemoglobin particle is built of four subunits covalently bound. Each subunit is composed of polipeptyde chain, globin molecule, bound with haeme. The haeme group is the same in all Hb types and is built of porphyrin ring with iron atom in the centre (13). Different kinds of haemoglobin contain various protein chains. Particular globin types are named with Greek alphabet letters- α, β, γ, δ, ε and ζ. The correct haemoglobin kinds in adults are HbA (2α2β, 97%), HbA_2 (2α2δ, 2%) and remains of foetal Hb, HbF (2α2γ, 1%) (3). The regular set of haemoglobin types during human life and pathology in thalassemias and haemoglobinopathies is shown in table 1.

All genes coding globin chains are gathered in two clusters: cluster α on chromosome 16, containing genes coding α and ζ globin; cluster β on chromosome 11, containing genes coding β, γ, δ and ε globin (14). Expression of all genes in each cluster is controlled by one regulatory element, α-MRE (major regulatory element) controlling α and ζ genes, and β-LCR (locus control region) controlling β, γ, δ and ε genes. The regulatory element affects the promoter of one of the genes in the cluster and enhances its expression. Expression of other genes in this cluster remains very low or is entirely inhibited (14-17). During foetal life occurs double haemoglobin synthesis switching (17). Around 10 week of foetal life stops the expression of ε and ζ globin, and synthesis of embryonic haemoglobins (Gower I, Gower II and Portland), and the main Hb becomes foetal haemoglobin (HbF). The second haemoglobin switching occurs shortly before birth. Then the γ globin gene expression is silenced and β globin gene expression is enhanced. As a result, synthesis of γ globin and HbF decreases, and synthesis of β globin and HbA increases (18). This process lasts until around six month after birth.

GENETIC BASIS OF HAEMOGLOBINOPATHIES AND HEREDITARY PERSISTENCE OF FETAL HEMOGLOBIN

Haemoglobinopathies are diverse group of disorders, with different clinical symptoms and genetic background. Mutations occur usually in β globin gene, less frequently in α globin and other genes. In every case mutation leads to synthesis of a new protein, in varying degrees different from the correct globin chain. A large group of haemoglobinopathies are anaemias connected with point mutations in β globin gene. The examples are haemoglobinopathies C, D and E, in which irregular forms of haemoglobin are produced, respectively, HbC, HbD and HbE (19).

Table 1. The types of regular and abnormal haemoglobin in healthy human and in the most common thalassemias and haemoglobinopathies (according to [22, 36]).

	Haemoglobin content			
	HbA (2α2ß)	**HbA_2 (2α2δ)**	**HbF (2α2γ)**	**Other Hb**
Embryo	absent	absent	absent	Gower I (2ζ2ε) Gower II (2α2ε) Portland (2ζ2γ)
Healthy foetus	about 15%	absent	about 85%	absent
Healthy adult	about 97%	about 2%	about 1%	absent
α thallasemia trait HbH disease Hydrops fetalis related to Hb Bart's	85-95% 60-95% absent	about 2% 2% or less absent	about 1% 1% or less absent	Hb Bart's (4γ) up to 10% at birth HbH (4β) 5-30% Hb Bart's up to 30% at birth HbH 5-10% Hb Bart's 90-95%
Thallasemia β minor Thalassemia β intermedia Thalassemia β major	80-95% 30-50% 0-20%	3-7% 0-5% 0-13%	1-5% 50-70% 80-100%	absent absent absent
HbE heterozygotes HbE homozygotes	60-65% absent	2-3% about 5%	1-2% 5-10%	HbE 30-35% HbE up to 95%
HbC heterozygotes HbC homozygotes	60-70% absent	slight increase slight increase	slight increase 5-10%	HbC 30-40% HbC up to 95%
HbD heterozygotes HbD homozygotes	50-65% 1-5%	1-3% absent	1-5% 1-3%	HbD 45-50% HbD up to 95%
Sickle cell anaemia heterozygotes Sickle cell anaemia homozygotes	55-70% absent	about 3% about 3%	about 1% about 7%	HbS 30-45% HbS up to 90%

In heterozygotes there is about 30-50% of this abnormal haemoglobin, while in homozygotes they can present as much as 90% of total Hb. HbE is most frequent in South-East Asia, where percentage of carriers reaches 60% in some countries (20), HbC in West Africa (1), HbD in South Asia (21). Heterozygotes are carriers of one damaged gene and have no clinical symptoms. Homozygotes suffer from haemolysis, but it is usually mild and does not require therapy (22). Interesting case of haemoglobinopathy is congenital methaemoglobinaemia or haemoglobinopathy M. Mutation occurs in α or β globin gene, and protein is altered in the region next to the iron atom. Iron is stabilized in Fe^{3+} form and that gives abnormal form of Hb- methaemoglobin, incapable of carrying oxygen (23).

Mutations causing haemoglobinopathies are not only point mutations. There are described cases of large deletions and the example is haemoglobinopathy connected with presence of Hb Lepore. It is abnormal haemoglobin, with no regular function and is a result of deletion of large regions of β and δ globin genes. The polypeptide chain of Hb Lepore is coded by the remains of this two genes. Homozygotes have symptoms similar to signs of β thalassemia major described below, heterozygotes have mild symptoms similar to β thalassemia minor or intermedia (24). A large group of haemoglobinopathies are anaemias connected with presence of unstable haemoglobins (for instance Hb Bristol and Hammersmith). There are described more than 220 mutations leading to this kind of haemoglobinopathies, and most of them is a consequence of mutation in β globin gene. Unstable haemoglobins are denaturated in high temperature and globin chains are precipitated as so-called Heinz bodies. Red blood cells have less flexibility and shorten lifespan, and that gives anaemia of varying degrees of severity (22).

The most common haemoglobinopathy and one of the most common results of congenital RBC defects is sickle cell anaemia, SCA. This disease was first described in 1910 and it was one of the first disorders described on molecular level. Sickle cell anaemia is inherited in the autosomal recessive way. Mutation occurs in β globin gene, the second nucleotide of the sixth codon, adenine, is replaced by thymine. As a consequence, in β globin protein chain, in 6 position, glutamic acid is replaced by valine. Exchange of one aminoacid results in formation of abnormal form of haemoglobin- HbS, which may present as much as 90% of total Hb in homozygotes. The HbS particle is more "sticky" than the correct HbA molecule. Polypeptide chain of HbS tends to create long polymers, damaging red blood cells. Forming of sickle RBCs is a typical symptom of SCA. This erythrocytes are elongated and stiff, are unable to perform their function and have significantly shorten lifespan. They also tend to form blockages in small blood vessels, and that results in tissues anoxia and strong pain (22).

Disorders of γ globin synthesis are primarily connected with deletions in β cluster or point mutations in γ globin gene promoter (17). They lead to significant increase in foetal haemoglobin concentration in adults. This state was named hereditary persistence of foetal hemoglobin (HPFH) (25). HbF may be distributed in all red blood cells in the same concentration (pancellular HPFH) or be present only in some population of erythrocytes (heterocellular HPFH) (22). This cells are called F cells (FC). Hereditary persistence of foetal hemoglobin should be differentiated with other anaemias, in which increase in HbF concentration is observed, for example thalassemia δβ or sickle cell anaemia. In this disorders there are observed irregular parameters of MCV (mean corpuscular volume), MCH (mean corpuscular hemoglobin) and Hb concentration, while in HPFH red blood cells have correct morphological parameters (25).

MOLECULAR BASIS OF THALASSEMIA

The second group of haemolytic anaemias connected with abnormal haemoglobin synthesis are thalassemias. There are described various types of this diseases related to mutations in different globin genes, including composed variants, like thalassemia δβ and γβ (22). The most common forms of thalassemias are connected with mutations in α globin gene and they are named α thalassemias, in which lack or decrease in α globin synthesis is observed. Thalassemia β is connected with decrease or complete inhibition of β globin synthesis (13).

Thalassemia α is most frequent in South-East Asia, the Mediterranean region (mainly in Greece and Cyprus) and Middle East (26). In humans there are 4 α globin genes, two on both chromosomes 16. Mutation may concern one of the genes on a chromosome (thalassemia α^+) or both genes, which is connected with complete lack of α globin expression on this chromosome (thalassemia α°) (27). These mutations are usually large deletions, removing the entire gene or its large fragment, and sometimes both genes of a cluster or their fragments (27). There are also described variants of α thalassemia related to deletion of regulatory region (α-MRE). In this cases, α globin genes are correct, but their expression is inhibited (28). Cases of α thalassemia connected with point mutations are significantly less common (29). Some types of mutations are also related to splicing (the process of RNA cutting and exons joining after transcription) and translation (protein synthesis) or are connected with altered protein stability (27).

Due to the presence of four functional α globin genes, various clinical variants of α thalassemia are possible. Lack of one gene (αα/α-) gives no signs in so-called silent carrier of α thalassemia. Lack of two α globin genes may occur in two configurations – homozygous, one gene removed from each chromosome (α-/α-) or heterozygous, both genes removed from one chromosome (αα/--). This state is called α thalassemia trait. It is usually asymptomatic or connected with mild anaemia and microcytosis. Lack of three α globin genes (α-/--)

leads to so-called haemoglobin H disease. It is connected with significant α globin deficiency, and excessive β globin chains create nonfunctional HbH tetramers. Dependly on the type of mutation, anaemia of varying degrees of severity is observed. In the most severe, lethal variant of α thalassemia, due to mutation in all four genes (--/--) and lack of α globin protein, foetal haemoglobin synthesis is entirely inhibited. Chains of γ globin create nonfunctional tetramers, so-called haemoglobin Bart's. It leads to hydrop fetalis and foetal death, and due to significant placenta enlargement, mothers are vulnerable to postnatal complications (22, 27).

Thalassemia β occurs most frequently in the Mediterranean region (particularly in Cyprus and Sardinia), the Middle East, South-East Asia and North Africa (30). There are two forms of this disorder. In one of them, β globin chains synthesis is entirely inhibited (thalassemia β°), in the second, their production is decreased (thalassemia β^{+}) (5). Molecular basis of β thalassemia is less complex than in the case of α thalassemia, due to presence only two β globin genes, one on each chromosome 11. Mutations connected with β thalassemia are mainly point mutations, but there are described large deletions removing β globin gene or its regulatory element. Point mutations occur within the β globin gene or next to this gene and they are divided into three groups: transcriptional mutations, mutations connected with splicing and translation (5).

Pathophysiology of β thalassemia is different from this of α thalassemia. Anaemia is related not only to accelerated elimination of damaged red blood cells and decreased level of regular haemoglobin, but also to erythropoiesis disorder. Excessive α globin chains precipitate, create so-called inclusion bodies and destroy erythroblasts and mature RBCs (31, 32). Dependly on the number and the type of mutations, there are three clinical variants of β thalassemia. The heterozygous form (with one damaged gene) is called β thalassemia trait or β thalassemia minor and is asymptomatic or connected with mild anaemia (31). Lack of two genes, depending on the type of mutations, leads to β thalassemia intermedia or major. The form intermedia leads to anaemia of varying degrees of severity and different transfusion requirement. People with β thalassemia major need regular blood transfusions and simultaneous treatment for iron overload (32).

LABORATORY DIAGNOSTICS OF HAEMOGLOBINOPATHIES AND THALASSEMIAS USING MOLECULAR TECHNIQUES

Large progress in laboratory diagnostics of haemoglobinopathies and thalassemias is related to developing and improving molecular techniques. For the first time they were used in thalassemia diagnostics in 1974 (33) and in prenatal diagnosis of thalassemia α two years later (12). **This techniques are used primarily in the case of disorders with high frequency.** In populations, where α and β thalassemias are most common, diagnostic methods based on DNA are routinely applied. Commercial tests were developed and they detect the most common mutations, since usually only limited number of mutations is present in particular population, for example 4-5 characteristic mutations cause 90% of all β thalassemia cases in given population (5).

Southern blotting (or Southern blot) is one of the first methods based on DNA, applied in diagnostics of haemoglobinopathies and thalassemias. It allows to detect specific DNA fragments, after electrophoretic separation, transfer to the nylon or nitrocellulose membrane and incubation with labeled probes, binding wanted DNA sequences. This method is usually connected with RFLP technique (restriction fragment length polymorphism), in which restriction enzymes are used, cutting DNA at specific sequences. When the mutation in the globin gene removes recognition site in DNA sequence, the pattern of DNA fragments is different from the pattern of healthy person's DNA. In order to visualize DNA fragments separation, electrophoresis in agarose gel or Southern blotting is performed (33).

Among methods based on PCR (polymerase chain reaction) the most popular is technique using oligonucleotides probes, ASO (allele-specific oligonucletide probes). It may be performed in two ways. One approach consists in hybridization of labeled oligonucleotides probes to amplified DNA fixed to nylon membrane. The second involves the labeled amplified DNA hybridization to the probes fixed to the membrane. Labeling is usually based on horseradish peroxidase reaction – tested DNA reacts with complementary probe, which is accompanied by enzymatic reaction with colour product. The pattern of bands is compared with the standard and mutation (or mutations) is identified. The latter technique is more popular, is getting more automated and many commercial tests were developed, becoming the primary diagnostic tool in laboratories routinely examinating suspected thalassemia cases (9, 33, 34).

Other PCR-based techniques are less common. Multiplex PCR is a relatively simple and fast technique, used in populations, where only limited number of well-characterized mutations is present. This method allows to perform a number of PCR reactions with various primers, detecting different mutations, in one tube, which means in the same conditions. The PCR products are separated by electrophoresis and the number and the type of mutations, as well as the carrier state (homo- or heterozygote) is determined. It is important not only in evaluation of the disease, but also in prenatal diagnosis (11). There is increasing interest in the use of real-time PCR technique, using fluorescently labeled oligonucleotide probes. This method allows to amplify and simultaneously quantify DNA, and is very precise and fast (12). In order to detect deletion, gap-PCR technique can be applied. Primers are complementary to the DNA regions that flank the deletion. This method allows to diagnose various deletions causing

haemoglobinopathies and thalassemias, including mutations leading to Hb Lepore and HPFH (34).

In molecular diagnostics of haemoglobinopathies most of the described methods can be used, for example RFLP, ASO or gene sequencing (34). In the case of sickle cell anaemia, RFLP technique is the most popular. Mutation in the sixth codon of β globin gene removes the recognition site for several resctriction enzymes. One of the first described is MstII, cutting DNA in the sequence CCTNAGG (where N represents any nucleotide). Replacing adenine by thymine, removes the site recognized by MstII restrictase. After separation of DNA fragments resulting from enzyme cutting, the number of bands indicates the number of mutations. In SCA homozygotes there is one long fragment (mutation in both gene and no cutting), in healthy homozygotes two short fragments (result of restrictase cutting of both genes), and in heterozygotes (SCA carriers) all three fragments are present (one gene intact and one mutated) (33).

To detect rare or the novo mutations, another techniques must be applied. The best choice is globin gene sequencing – reading the nucleotide sequence and searching for mutations (33, 34). Less common variants of thalassemias may be connected with mutations in genes regulating expression of α and β globin genes, placed outside the globin clusters or even on different chromosomes. In such cases, α and β globin genes sequencing will not reveal the molecular basis of the disease. In order to identify this kind of mutations, it is necessary to better understand the mechanisms of regulation of globin genes expression (33, 35).

Due to the high frequency of haemoglobinopathies and thalassemias in some populations prenatal diagnosis is performed. DNA is isolated from the chorion collected by biopsy, and the presence of the disorder may be detected using any method of the techniques described above. Application of two different methods is recommended (12).

Haemolytic anaemias are investigated by our Department as a part of the project, in which congenital and acquired mechanisms of RBCs destruction are studied. Of them, α thalassemia is of our large interest. This disorder is rarely diagnosed in Poland using molecular techniques and at the same time it seems to be more frequent in Polish population, than it was expected.

BIBLIOGRAPHY

1. Kohne E, Kleihauer E: Hemoglobinopathies: a longitudinal study over four decades. Dtsch Arztebl Int 2010; 107: 65-71.
2. Weatherall DJ: The thalassemias. BMJ 1997; 314: 1675-1678.
3. Metcalfe SA, Barlow-Stewart K, Campbell J, Emery J: Genetics and blood-haemoglobinopathies and clotting disorders. Aust Fam Physician 2007; 36: 812-819.
4. López C, Saravia C, Gomez A et al.: Mechanisms of genetically--based resistance to malaria. Gene 2010; 467: 1-12.
5. Thein SL: Genetic modifiers of beta – thalassemia. Haematologica 2005; 90: 649-660.
6. Adamowicz-Salach A, Zdebska E, Burzyńska B et al.: Talasemia α przyczyną niedokrwistości mikrocytarnej w Polsce – opis przypadków. Pediatr Pol 2007; 82: 151-155.
7. Maciag M, Adamowicz-Salach A, Siwicka A et al.: The use of real-time PCR technique in the detection of novel protein 4.2 gene mutations that coexist with thalassaemia alpha in a single patient. Eur J Haematol 2009; 83: 373-377.
8. Zdebska E, Krawcewicz A, Adamowicz-Salach A et al.: β-Talasemia w Polsce. I. Mutacje śródziemnomorskie. Pol Merk Lek 2006; 20 (115): 53-56.
9. Jackowska T, Wagiel E, Zdebska E et al.: Niedokrwistość mikrocytarna u dzieci w Polsce jako objaw talasemii. Przegląd Pediatryczny 2007; 37 (2): 221-226.
10. Maciag M, Pochocka D, Adamowicz-Salach A et al.: Diversity of Thalassemia Variants In Poland – Screening by Real-Time PCR. Acta Haematol 2008; 120 (3): 153-157.
11. Bergstrome JA, Poon A: Evaluation of a single-tube multiplex polymerase chain reaction screen for detection of common alpha-thalassemia genotypes in a clinical laboratory. Am J Clin Pathol 2002; 118: 18-24.
12. Patrinos GP, Kollia P, Papadakis MN: Molecular diagnosis of inherited disorders: lessons from hemoglobinopathies. Hum Mutat 2005; 26: 399-412.
13. Muncie HL, Campbell JS: Alpha and beta thalassemia. Am Fam Physician 2009; 80: 339-344.
14. Pace BS, Zein S: Understandin mechanisms of γ-globin gene regulation to develop strategies for pharmacological fetal hemoglobin induction. Dev Dyn 2006; 235: 1727-1737.
15. Bank A: Regulation of human fetal hemoglobin: new players, new complexities. Blood 2006; 107: 435-443.
16. Jarman AP, Wood WG, Sharpe JA et al.: Characterisation of the major regulatory element upstream of the human alpha-globin gene cluster. Mol Cell Biol 1991; 11: 4679-4689.
17. Bauer DE, Orkin SH: Update on fetal hemoglobin gene regulation in hemoglobinopathies. Curr Opin Pediatr 2011; 23: 1-8.
18. Peterson KR: Hemoglobin switching: new insights. Curr Opin Hematol 2003; 10: 123-129.
19. Kohne E: Hemoglobinopathies. Dtsch Arztebl Int 2011; 108: 532-540.
20. Vichinsky E: Hemoglobin E syndromes. Hematology Am Soc Hematol Educ Program 2007: 79-83.
21. Taghavi Basmanj M, Karimipoor M, Amirian A et al.: Co-inheritance of hemoglobin D and β-thalassemia traits in three Iranian families: clinical relevance. Arch Iran Med. 2011; 14: 61-63.
22. Provan D, Singer CRJ, Baglin T, Lilleyman J: Hematologia kliniczna; red. naukowy tłumaczenia Hołowiecki J Warszawa Wydawnictwo Lekarskie PZWL 2008.
23. Da-Silva SS, Sajan IS, Underwood JP 3rd: Congenital methemoglobinemia: a rare cause of cyanosis in the newborn – a case report. Pediatrics 2003; 112: 158-61
24. Chaibunruang A, Srivorakun H, Fucharoen S et al.: Interactions of hemoglobin Lepore (deltabeta hybrid hemoglobin) with various hemoglobinopathies: a molecular and hematological characteristics and differential diagnosis. Blood Cells Mol Dis 2010; 44: 140-145.
25. Musielak M: Genetycznie uwarunkowane stany zwiększonej ilości hemoglobiny płodowej (HbF) u ludzi zdrowych i chorych. Pol Merkur Lekarski 2011; 30: 62-65.
26. Vichinsky EP: Alpha thalassemia major – new mutations, intrauterine management, and outcomes. Hematology Am Soc Hematol Educ Program 2009; 35-41.

27. Harteveld CL, Higgs DR: Alpha – thalassemia. Orphanet J Rare Dis 2010; 5: 13.
28. Ribeiro DM, Sonati MF: Regulation of human alpha-globin gene expression and alpha-thalassemia. Genet Mol Res 2008; 7: 1045-1053.
29. Higgs DR, Weatherall DJ: The alpha thalassemias. Cell Mol Life Sci 2009; 66: 1154-1162.
30. Galanello R, Origa R: Beta-thalassemia. Orphanet J Rare Dis 2010; 5: 11.
31. Thein SL: Patophysiology of beta thalassemia – a guide to molecular therapies. Hematology Am Soc Hematol Educ Program 2005: 31-37.
32. Danjou F, Anni F, Galanello R: Beta-thalassemia: from genotype to phenotype. Haematologica 2011; 96: 1573-1575.
33. Gu X, Zeng Y: A review of the molecular diagnosis of thalassemia. Hematology 2002; 7: 203-209.
34. Old JM: Screening and genetic diagnosis of haemoglobinopathies. Scand J Clin Lab Invest 2007; 67: 71-86.
35. Trent RJ, Webster B, Bowden DK et al.: Complex phenotypes in the haemoglobinopathies: recommendations on screening and DNA testing. Pathology 2006; 38: 507-519
36. Hillman RS, Ault KA, Leporrier M, Rinder HM: Hematology in clinical practice. The McGraw-Hill Companies, Inc 2011.

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