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THALASSEMIA: MOLECULAR GENETIC DIAGNOSTIC METHODS

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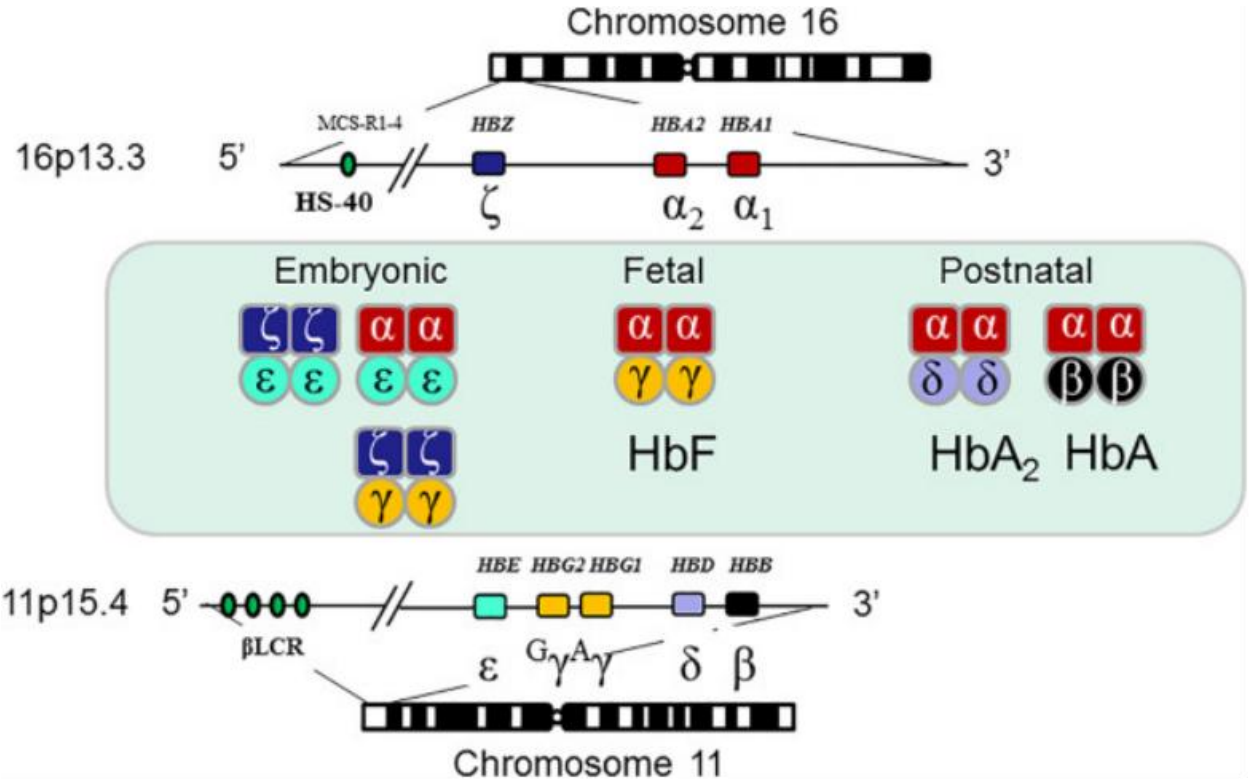
INTRODUCTION

Thalassemias can be defined as α- or β-thalassemias depending on the defective globin chain and the underlying molecular defects. The article presents the current molecular genetics methods of investigation used in clinical-laboratory and research practice. Carriers of α- and β-thalassemia (heterozygotes) exhibit microcytic hypochromic parameters with mild or no anemia. Although hematological research methods and Southern blotting are still considered the main research methods in routine clinical practice used in the diagnosis of thalassemia, molecular analysis was not mandatory until recently to confirm the diagnosis of β-carrier. However, molecular genetic testing is necessary to confirm α-thalassemia carrier status. Molecular diagnostics are needed to predict severe transfusion-dependent cases and intermediate and mild non-transfusion-dependent cases. Chorionic villus DNA testing is a prenatal diagnostic method and the methods are the same as for detecting mutations, depending on laboratory equipment and experience. Thalassemia syndromes are a heterogeneous group of hemoglobin disorders caused by decreased or absent production of normal globin chains. These are the most common recessive diseases worldwide, with an estimated 1–5% of the world's population carrying the thalassemia genetic mutation [1, 10]. Thalassemia is most common in Southeast and South Asia, the Middle East, Mediterranean countries, and North and Central Africa. However, due to ongoing population migration, thalassemia is now becoming increasingly common in Northern Europe and North America [3,7]. These changes have challenged health professionals and policymakers throughout the region to ensure equitable access to quality services for the prevention, diagnosis and adequate treatment of thalassemia.

Studies of thalassemia patients have been instrumental in identifying numerous causative mutations in globin genes, upstream and downstream untranslated regions, and regulatory elements controlling the expression of α- and β-globin gene families and hence hemoglobin switching. Cell lines and mouse models have allowed the identification of a diverse collection of interacting transcription factors and other protein complexes involved in the regulation of the expression of these genes [2, 5, 6]. Despite the many animal models described to date, the study of natural deletions and point mutations in carriers and patients remains important for understanding expression regulation and disease mechanisms. In the present review, we will highlight aspects of the molecular basis of α-thalassemia that will provide insight into how we can understand the mechanisms underlying α-thalassemia [16, 17].

Human hemoglobin is a tetrameric protein consisting of two alpha-like and two beta-like globin chains, each forming a pocket containing a heme group for oxygen binding. The globin genes are

located in two distinct gene clusters at different chromosomal loci in the order of their expression during development (Fig. 1). Expression is regulated by complex interactions of transcription factors and regulatory elements (promoters and enhancers) to turn genes on and off in a stage-specific and tissue-specific manner.



Pic. 1 . Schematic representation of the chromosomal location of the α- and β-globin gene clusters on 16p and 11p, respectively [2].

Note: Embryonic and fetal genes are indicated by open rectangles (in green and yellow). Genes that remain active throughout postnatal life are highlighted in purple and black. Shown are the different types of hemoglobins expressed during the embryonic period, from left to right: Hb Gower-1 (ζ2ε2), Hb Gower-2 (α2ε2) and Hb Portland (ζ2γ2), fetal hemoglobin (HbF) and those expressed in the postnatal period (HbA and HbA) . [2b]

Globin gene disorders (hemoglobinopathies) are characterized by either abnormal variants of globin chains, such as sickle cell disease, or decreased synthesis of globin chains in erythroid cells (thalassemia) during hematopoiesis [18]. Hemoglobinopathies are inherited predominantly in an autosomal recessive manner. Reduction or absence of α-globin chains leads to an excess of unpaired beta-(β)-like globin chains that form insoluble homotetramers, leading to intracellular precipitation, ineffective erythropoiesis and acute hemolytic anemia typical of severe forms of α-thalassemia [14, 19].].

Thalassemias can be broadly characterized as α- or β-thalassemias, depending on the defective globin chain and the underlying molecular defects; they are a recessive trait; thus, clinically significant phenotypes result from homozygosity or double heterozygosity for various globin

gene defects. They may also result from co-inheritance of thalassemia traits and hemoglobin structural variants such as hemoglobin S, C and E. Several forms of hemoglobin E/β-thalassemia, S/β-thalassemia and hemoglobin C/β-thalassemia are common, but these forms require molecular analysis because they have unique characteristics and features. Without treatment, the hallmark of thalassemia syndromes is an imbalance in the α/β-globin chain ratio, leading to ineffective erythropoiesis. Unstable tetramers of free globin chains precipitate in erythroid cells, leading to premature cell death inside and outside the bone marrow (peripheral hemolysis). The resulting clinical manifestations are dilation of the bone marrow, increased splenic function (splenomegaly), and chronic hemolytic anemia.

There are more than 200 known β-thalassemia mutations, most of which are single nucleotide substitutions, insertions, or short deletions. Several large β gene deletions have been identified, and most of them can be diagnosed using Gap-PCR. Fortunately, a limited number of point mutations are common among different ethnic groups; Therefore, for any given ethnic region, a PCR method is initially used, designed to simultaneously detect a general specific mutation. With this approach, more than 80% of cases can be identified for most ethnic groups. For β-thalassemia mutations, the reverse dot blot technique, in which amplified DNA is hybridized to a panel of mutation-specific probes attached to a nylon strip, is widely used. Some laboratories also use an amplification-resistant mutation system: it allows you to quickly, economically and conveniently test several mutations simultaneously. Denaturing gradient gel electrophoresis is an alternative to the above methods and is useful in countries where a very wide range of β-mutations occurs. Almost 90% of β mutations can be detected by a shift in the band structure towards normal. However, improvements and decreasing costs of DNA sequencing have made the β gene direct sequence the most widely used in many laboratories, allowing analysis of the exact nucleotide sequence of a gene or the gene region being assessed. The original method described by Sanger et al. was modified in 1977, and several types of automatic sequencers are available today. Direct sequencing also allows the identification of unknown mutations [2].

Alpha thalassemia is an inherited autosomal recessive disease characterized by microcytic hypochromic anemia. This is one of the most common monogenic gene disorders among the world population. Clinical severity ranges from almost asymptomatic to mild microcytic hypochromia and a fatal hemolytic condition called Barth fetal hydrops syndrome. The molecular basis is usually deletions and, less commonly, point mutations affecting the expression of one or more duplicated α genes. Clinical variations and increasing disease severity are directly associated with decreased expression of one, two, three, or four copies of the α-globin genes. Deletions and point mutations in α-globin genes and their regulatory elements have been studied extensively in carriers and patients, and these studies have provided insight into whether α-globin genes are regulated. By looking at naturally occurring deletions and point mutations, our knowledge of globin gene regulation and expression will continue to expand and lead to new therapeutic targets. [12].

Alpha thalassemias arise mainly from deletions of varying lengths and can be detected predominantly by reverse dot blot and Gap-PCR. Primer sequences have been published for the diagnosis of several types of α + or α thalassemias [8, 13, 15]. Amplification of sequences in the α-globin gene cluster is more difficult than in the β-globin gene cluster due to significant sequence homology within the α-globin gene cluster. For unknown α-deletions, some laboratories still use Southern blotting, but more recently multiplex ligation-dependent probe amplification has been introduced into research practice. This method is now used instead of

Southern blotting due to its sensitivity and reliability, without the need for the former radioactive detection method [12].

After the era of Sanger sequencing, next generation sequencing (NGS) represents a new principle of sequencing technology, as this technology increases sequencing throughput from a few hundred base pairs to several thousand in a single analysis. NGS, based on massively parallel sequencing of clonally amplified DNA molecules, coupled with sufficient computing power and appropriate software for efficient data analysis, has allowed genetic diagnostics to enter clinical practice at an affordable cost [11]. It can be applied to the entire genome, the entire exon, and to specific target regions of the genome. Currently, there is no literature data on sequencing globin genes using this approach. There is only one commercial sequencing panel available (TruSight Inherited Disease, Illumina Inc.) that reports that the HBB and HBA1 genes are included, but sequencing only two genes does not justify the cost of the entire procedure for one patient.

The most important step of NGS is the experimental development of a set of probes that will be used to capture DNA: the high level of homology between genes in the alpha and beta clusters makes the NGS approach to thalassemia very complex, time-consuming and expensive. although not impossible. Another important point about NGS is that it is useful for detecting single nucleotide substitutions and insertions or small deletions (depending on the type of study), but is less accurate for other types of genomic variations. Although bioinformatics research is now also working on tools to test for the presence of complex rearrangements, large deletions or triplications are currently missed, and especially in the case of alpha thalassemia, this renders the entire procedure completely useless.

In conclusion, it is important to emphasize that DNA testing for globin gene mutations is not required to diagnose most carrier status. It is advisable to confirm the diagnosis of TDT by knowing the parental mutations, and in cases of NTDT, where co-inheritance of different globin gene defects may be responsible for the different expression of the phenotype.

Some cases are hematologically and clinically difficult to diagnose even with family hematological workup due to the presence of mild mutations or α- and β-interactions; Therefore, in such cases, it is convenient to carry out molecular analysis before starting treatment. Chorionic villus DNA testing is a prenatal diagnostic method and the methods used are the same as for detecting mutations, depending on laboratory equipment and experience. An important step in prenatal diagnosis is the removal of any maternal tissue, since the presence of residual maternal tissue may lead to diagnostic error. To exclude maternal contamination, the presence of short tandem repeats or variable numbers of tandem repeats in fetal and parental DNA samples by PCR is important. Details of the methods commonly used for molecular diagnosis of thalassemia are described in the International Thalassemia Federation publication Prevention of Thalassemia and Other Hemoglobin Disorders [9].

Literature:

1. Brancaleoni et al. Laboratory diagnosis of thalassemia //International journal of laboratory hematology, 2016; 38: 32-40.
2. Cardiero G. et al., Alpha-Thalassemia in Southern Italy: Characterization of Five New Deletions Removing the Alpha-Globin Gene Cluster, International Journal of Molecular Sciences, 2023; 3(24): 2577,

3. Daidone R. et al. A Particular Focus on the Prevalence of α- and β-Thalassemia in Western Sicilian Population from Trapani Province in the COVID-19 Era, International Journal of Molecular Sciences, 2023; 5(24): 4809.
4. Farashi S., Harteveld C. L. Molecular basis of α-thalassemia //Blood Cells, Molecules, and Diseases, 2018; 70: 43-53.
5. Ferih Kh., et al. Applications of Artificial Intelligence in Thalassemia: A Comprehensive Review, Diagnostics, 10.3390/diagnostics13091551, 2023; 9 (13):1551.
6. Saad H. K. M., et al. , HBB Gene Mutations and Their Pathological Impacts on HbE/β-Thalassaemia in Kuala Terengganu, Malaysia, Diagnostics, 2023; 7(13): 1247.
7. Krishnevskaya E. et al. New-Generation Ektacytometry Study of Red Blood Cells in Different Hemoglobinopathies and Thalassemia, Thalassemia Reports, 10.3390/thalassrep13010007, 2023; 1(13): 70-76.
8. Molchanova T.P. et al. The differences in quantities of alpha 2- and alpha 1-globin gene variants in heterozygotes Br. J. Haematol., ,1994; 88: 300-306
9. Noulsri E. et al. Diagnostic Performance of 10 Mathematical Formulae for Identifying Blood Donors with Thalassemia Trait, Laboratory Medicine, 2023.
10. Panja A. et al. The Key Genetic Determinants Behind the Phenotypic Heterogeneity of HbE/β-thalassemia Patients and the Probable Management Strategy, Thalassemia Syndromes - New Insights and Transfusion Modalities [Working Title], 2023: 109999.
11. Peng C. et al. Analysis of rare thalassemia genetic variants based on third-generation sequencing, Scientific Reports, 2022: 1: 12.
12. Ruengdit Ch. et al. Multiplex Quantitative Real-Time Polymerase Chain Reaction and High-Resolution Melting Analysis for Identification of a Couple At-Risk of Having a Newborn with Severe Thalassemia, Hemoglobin, 2022; 5(45):309-313.
13. Shakin S.H., Liebhaber S.A. Translational profiles of alpha 1-, alpha 2-, and beta-globin messenger ribonucleic acids in human reticulocytes J. Clin. Invest., 1986;78: 1125-1129.
14. Shinar E., Rachmilewitz E.A. Lux S.E. Differing erythrocyte membrane skeletal protein defects in alpha and beta thalassemia J. Clin. Invest., 1989; 83: 404-410.
15. Smetanina N.S. et al. The relative levels of alpha 2-, alpha 1-, and zeta-mRNA in HB H patients with different deletional and nondeletional alpha-thalassemia determinants Biochim. Biophys. Acta, 1316 (1996), pp. 176-182.
16. Tariq Z. et al. Thalassemia and Nanotheragnostics: Advanced Approaches for Diagnosis and Treatment, Biosensors, 2023; 4(13): 450.
17. Vijian D., et al., Gene Mutation Spectrum among Alpha-Thalassaemia Patients in Northeast Peninsular Malaysia, Diagnostics, 2023; 5(13): 894.
18. Weatherall D.J., Clegg J.B. The Thalassemia Syndromes (4th ed.), 2001.
19. Yuan J. , A. Bunyaratvej, S. Fucharoen, C. Fung, E. Shinar, S.L. Schrier The instability of the membrane skeleton in thalassemic red blood cells Blood, 1995; 86: 3945-3950