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REVIEW ARTICLE

EXPLORING THE IMPACT OF GENETICS IN THE DIAGNOSIS AND MANAGEMENT OF THALASSEMIA: A NARRATIVE REVIEW

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Abstract

Thalassemia refers to a collection of inherited hemoglobin disorders resulting from mutations in the α- or β-globin genes, which causes impaired hemoglobin production and a range of clinical symptoms. Grasping the genetic foundations of thalassemia is crucial for precise diagnosis, risk assessment, and the creation of tailored treatment plans. This narrative review seeks to examine the influence of genetics on the identification and treatment of thalassemia, emphasizing progress in molecular diagnostics, treatment strategies, and new gene-targeted therapies. An extensive literature review was performed utilizing PubMed, Scopus, Web of Science, and Google Scholar to locate research on the genetics of thalassemia, molecular diagnosis, treatment approaches, and gene therapy. Both original research and review articles published in English until October 2025 were included. Essential themes were identified and categorized into genetic mechanisms, diagnostic instruments, treatment methods, and future perspectives. Genetic knowledge has revolutionized thalassemia treatment, allowing for accurate mutation identification, strategic transfusion preparation, and focused therapies. Molecular methods like PCR, MLPA, and next-generation sequencing enable precise diagnosis and identification of carriers. Progress in hematopoietic stem cell transplantation and gene therapy, featuring lentiviral and CRISPR/Cas9 techniques, presents possible curative solutions. Genetic modifiers, including co-inherited α-thalassemia and polymorphisms that promote fetal hemoglobin, greatly affect disease severity and treatment needs. Incorporating genetic insights into clinical practice improves the diagnosis and tailored treatment of thalassemia. New gene-based therapies offer hope for cures, and continuing research on genetic modifiers can enhance patient results. This review offers an extensive framework connecting genetic mechanisms to current and emerging therapeutic approaches.

Keywords: Diagnosis and management, genetic, hemoglobinopathy, thalassaemia.

INTRODUCTION

Thalassemia refers to a collection of genetic hemoglobin disorders marked by impaired production of one or more globin chains, resulting in ineffective erythropoiesis, ongoing anemia, and various clinical issues. Worldwide, thalassemia presents a considerable public health challenge, showing high rates of prevalence in the Mediterranean, Middle East, Southeast Asia, and certain regions of Africa. The clinical spectrum varies from asymptomatic carriers to patients with severe anemia who rely on transfusions, experiencing organ damage and decreased life expectancy[1]. Thalassemia's molecular foundation consists of mutations in the α- or β-globin genes. Alpha-thalassemia is generally attributed to deletions or point mutations in the HBA1 and HBA2 genes, while beta-thalassemia arises from mutations in the HBB gene that diminish (β^+) or completely eliminate (β^0) β-globin synthesis. The intensity of the disease is affected not only by the main mutations but also by genetic modifiers, such as the co-inheritance of α-thalassemia and variations that influence fetal hemoglobin (HbF) synthesis. Grasping these genetic factors is crucial for precise diagnosis, risk assessment, and creating tailored treatment plans[2]. Progress in molecular diagnostics, including polymerase chain reaction (PCR), multiplex ligation-dependent probe amplification (MLPA), and next-generation sequencing (NGS), has permitted accurate mutation identification,

carrier detection, and prenatal diagnosis. These advancements have revolutionized clinical practice, enabling healthcare providers to customize treatment strategies according to unique genetic characteristics and more accurately predict disease complications[3]. This narrative review seeks to thoroughly examine the influence of genetics on the diagnosis and treatment of thalassemia.

METHODS

This manuscript was prepared as a narrative review aimed at synthesizing current knowledge on the role of genetics in the diagnosis and management of thalassemia. A structured literature search was conducted using databases including PubMed, Scopus, Web of Science, and Google Scholar. Keywords used included "thalassemia," "alpha-thalassemia," "beta-thalassemia," "genetics," "molecular diagnosis," "gene therapy," "hematopoietic stem cell transplantation," and "personalized therapy."

Articles written in English until October 2025 were taken into account. Included were both primary research studies and pertinent reviews to offer a thorough insight into the genetic mechanisms, diagnostic methods, and treatment approaches for thalassemia. Reports of cases, abstracts from conferences, and studies with inadequate information were omitted. The chosen literature underwent critical analysis, with major themes identified and categorized into these areas: genetic foundation of thalassemia, molecular diagnostic methods, treatment options including gene therapy, and new research trajectories. Figures and tables were created to condense intricate genetic data and clinical management techniques to improve reader understanding. This storytelling method facilitated the combination of molecular knowledge with clinical viewpoints, emphasizing both traditional methods and novel treatments while pinpointing areas for future investigation.

Haemoglobinopathies

Haemoglobinopathies are genetic disorders that affect the synthesis or shape of the haemoglobin molecule. Different types of abnormal haemoglobin are categorized according to the impaired component. Genetic alterations in haemoglobin constitute the most common type of genetic disorders. A variety of genetic variations in globin chain synthesis result in either diminished globin chain production (referred to as thalassaemia) or changes in the structure of globin chains (called haemoglobinopathies)[3].

Structural haemoglobin variants

Sickle-cell disease is the most well-known type of haemoglobinopathy, characterized by structural alterations in haemoglobin. This condition results from a mutation in a single base of the b-globin gene, leading to the substitution of valine for glutamate at the sixth position of the b-globin chain. In people who are homozygous for this mutation, both b-globin genes are impacted, resulting in sickle-cell anaemia. This leads to a reduced P50 compared to HbA, resulting in a leftward shift of the oxyhaemoglobin dissociation curve. In contrast, people with the heterozygous variant display sickle-cell trait, which is usually milder. Various additional haemoglobinopathies are present, such as HbC (Table 1)[1,4].

Table 1: Molecular structure of normal haemoglobin.

Hemoglobin Type	Globin Chains Composition	Oxygen Affinity / Function	Normal Range (%)	Notes
HbA (Adult Hemoglobin)	2 α + 2 β	Standard oxygen transport	95–98%	Major adult hemoglobin; primary transporter of oxygen in adults
HbA2	2 α + 2 δ	Slightly lower oxygen transport efficiency than HbA	2.2–3.5%	Minor adult hemoglobin; elevated in β-thalassemia carriers
HbF (Fetal Hemoglobin)	2 α + 2 γ	Higher oxygen affinity than HbA	<1% (adults); 50–95% at birth	Facilitates oxygen transfer from mother to fetus; persists in certain hemoglobinopathies
HbE	2 α + 2 ε	Embryonic hemoglobin; high oxygen affinity	<0.1%	Expressed only in embryonic stage
HbGower 1	2 ζ + 2 ε	Embryonic hemoglobin	Rare in adults	Early embryonic hemoglobin; replaced by HbF during fetal development
HbGower 2	2 α + 2 ε	Embryonic hemoglobin	Rare in adults	Early embryonic hemoglobin; replaced by HbF during fetal development
HbPortland	2 ζ + 2 γ	Embryonic hemoglobin	Rare	Transient during embryogenesis; precursor of HbF

Thalassemia

Thalassemia is the most common genetic condition impacting red blood cells, resulting in anemia from faulty genes that produce globin proteins. Based on their genetic composition, people may be carriers or impacted by thalassemia. Thalassemia has two main forms: alpha (α) and beta (β), with alpha thalassemia being the most common internationally, particularly in Southeast Asia[5]. The word "thalassemia" comes from Greek terms that translate to "sea" and "blood". The particular form of thalassemia syndrome is designated according to the affected globin chain; for instance, β-thalassemia arises from abnormalities in the β-globin gene, whereas α-thalassemia is caused by mutations in

the α-globin gene. In β-thalassemia, normal α-globin chain synthesis persists, leading to the buildup of unmatched α-globin in erythroid precursors. A characteristic feature of β-thalassemia is defective erythropoiesis caused by the problematic buildup of unbound α-globin chains that fail to create haemoglobin tetramers. Rather, these chains aggregate, forming inclusion bodies in the bone marrow and leading to the demise of erythroid precursors. Individuals with β-thalassemia major often encounter iron overload from inadequate erythropoiesis and frequent blood transfusions; they need regular transfusions to sustain an acceptable quality of life[6]. Excessive iron can negatively impact essential organs such as the heart, kidneys, lungs, liver, and endocrine system[7]. The alpha thalassemia trait is prevalent in the Indian subcontinent, Southeast Asia, and some parts of the Middle East. This condition is marked by slight anemia and reduced red blood cell indices caused by the deletion or inactivation of three alpha globin genes, leading to an excess of beta chains. Patients often show signs of jaundice, splenomegaly, marked anaemia, and irregular red blood cell indices. In instances of alpha thalassemia major, the serious type arises when all alpha genes are removed from both sets of chromosome 16[8].

Types of thalassemia

It is caused by a decrease in the production of one or more globin chains. The types that affect either beta or alpha-chain production are the most important.

1. Alpha- thalassemia

Multiple forms of alpha thalassemia exist, with the silent carrier alpha thalassemia being among the most prevalent types. In this variant, two alpha-type genes exist on each chromosome 16, but one alpha gene is absent at the start of the chromosome, yielding three active genes. People with silent carrier alpha thalassemia typically maintain normal hematological health but may sometimes show slightly reduced red blood cell levels. The condition referred to as "alpha thalassemia" typically arises from the loss of one or two alpha genes from either or both chromosome 16.

There are three categories to determine the severity of this disorder:

a. Silent carrier: Of four possible alleles, those with the silent carrier are damaged or absent (αα, oo). The RBC is lower than usual, but there are no additional complaints.

b. Alpha-thalassemia trait/carrier: Two of the four alleles are missing or have been taken off one chromosome (αα, oo) or both (αo and I). He, with this characteristic, suffers from mild anaemia.

c. Haemoglobin H: This form deletes all three alleles, leaving only one functional allele. Individuals with haemoglobin H confront moderate to severe anaemia, and occasionally, exposure to specific chemicals or medications can make their condition worse.

Alpha-thalassemia major: This condition can lead to hydrops fetalis syndrome or significant anaemia in cases where both alleles (oo, oo) are missing. The individual may exhibit prominent hepatosplenomegaly and skeletal abnormalities and face an increased risk of causing foetal death[9].

Two forms of alpha thalassaemia may cause health problems:

a. Haemoglobin H (HbH) disease: Alpha thalassaemia is a mild condition that can lead to mild to moderate anaemia. Treatment might involve blood transfusions, either intermittently or regularly. Additional characteristics of this condition include an enlarged spleen, which is responsible for filtering blood, and jaundice, which causes a yellowing of the skin and eyes.

b. Haemoglobin barts hydrops fetalis (HbBarts) syndrome: A serious form of alpha thalassaemia arises when surplus fluid gathers in a fetus during gestation as a result of severe anaemia. Regrettably, the infant frequently does not endure long following birth. The mother of an affected infant may also face risks, such as dangerously high blood pressure, referred to as preeclampsia. Moreover, one can be a genetic carrier of alpha thalassaemia, known as alpha plus thalassaemia trait or alpha zero thalassaemia trait, but these traits typically do not result in considerable health problems independently.

2. Beta-thalassemia

The beta-globin gene is located on chromosome 11, setting it apart from the duplication linked to alpha thalassemia. Beta thalassemia can present in various forms. The minor or silent carrier variant of beta thalassemia arises from a mild mutation, resulting in patients usually exhibiting no symptoms or minor variations in the quantity or size of their red blood cells. This version is the most prevalent. The intermediate form, referred to as the beta-thalassemia trait, is marked by decreased beta-globin production. Studies indicate that people with the beta-thalassemia trait might possess some defense against myocardial infarctions, advancing coronary artery disease, and ischemic strokes. As a result of these protective benefits, these patients usually experience lower blood pressure, reduced blood viscosity, and decreased serum cholesterol levels. Additionally, there are signs suggesting that beta-thalassemia could serve as a protective factor against Alzheimer's disease[10]. Beta thalassemia is the result of a specific gene, the beta-globin (HBB) gene located on chromosome 11, and can be divided into four categories.

a. Thalassemia minor: Individuals with thalassemia minor typically exhibit low red blood cell counts and often do not show any symptoms. They may experience mild anaemia from time to time, which is identified as either β/β or β/β thalassemia.

Thalassemia intermediate: People with thalassemia intermedia have either one absent allele or a mutated β/β or β/β allele. The particular kinds of mutations affect the seriousness of the condition. The majority of patients with this intermediate form have mild anaemia and typically do not need ongoing blood transfusions; however, in more severe situations, lifelong transfusions may be essential. Symptoms may appear in children from six months to two years old; although they might require fewer transfusions, their growth and cognitive development could be negatively impacted. Complications may occur, like the generation of enucleated red blood cells (RBCs) from heightened

RBC proliferation. This may result in reduced RBC production, leading to anaemia or potentially early death. Additional clinical concerns that might occur include thrombophilia, leg ulcers, splenectomy, iron overload, and infertility. Additional complications may include osteoporosis, joint discomfort, and cardio-vascular problems resulting from bone abnormalities, with heart disease being a major cause of mortality.

b. Thalassemia major: Cooley's anaemia or thalassemia major involves the deletion or insufficient production of the beta globulin chain (β/β/). If both parents are affected by the disease or are carriers, children with thalassemia may be diagnosed in their first year. Symptoms include pallor, jaundice, deformities in facial bones, and, in some cases, growth failure at birth, as they cannot produce normal adult haemoglobin[11].

c. Dominant β-thalassemia: This is a rare condition where an individual exhibits thalassemia like symptoms despite having only one mutated allele. Those affected may experience symptoms such as an enlarged spleen, jaundice, or varying degrees of anaemia, ranging from mild to severe.

Pathophysiology of thalassemia

An insufficient production or lack of a particular globin chain leads to decreased haemoglobin levels, smaller red blood cells (RBCs), hypochromia, various target cells (codocytes), ovalocytes, and basophilic stippling. This discrepancy in the production of α or β globin chains disturbs the α/β ratio. Reduced lifespan of RBCs contributes substantially to the onset of anaemia. Forming unique combinations of polypeptides results in the production of haemoglobin[12]. In β-thalassemia, the surplus of unpaired α chains builds up in RBCs and harms their membranes, leading to their elimination by macrophages in the bloodstream or bone marrow. The premature demise of RBCs in the bone marrow is known as ineffective erythropoiesis, where the bone marrow strives to generate RBCs but is unable to release functional ones into circulation, or the released cells are eventually eliminated in the spleen. Anaemia in β-thalassemia arises from ineffective erythropoiesis and heightened RBC destruction. People with β-thalassemia typically exhibit no symptoms during fetal development and until approximately 6 months old because of the predominance of fetal hemoglobin (HbF, α2γ2) and the lack of transition from the γ to β chain. Symptoms generally manifest between 6 to 24 months of age after the transition from γ to β chain has finished[13]. In α-thalassemia, decreased alpha-globin production leads to a lower number of alpha chains, causing an excess of β chains in adults and γ chains in infants. The surplus β chains create unstable tetramers referred to as haemoglobin H (Hb H), made up of four beta chains. In contrast, the surplus γ chains create tetramers that are ineffective at transporting oxygen because of their excessively high affinity for O2, which obstructs dissociation in peripheral tissues. Homozygous α0 thalassemia, characterized by elevated γ4 levels without any α-globin (commonly referred to as HbBarts), often leads to death shortly after birth[14].

Symptoms of thalassemia

Due to reduced red blood cells in thalassemia, individuals may experience symptoms such as low blood count or anaemia. Common signs of anaemia include feelings of fatigue or weakness, dizziness, shortness of breath, a fast heartbeat, headache, leg cramps, difficulty in concentrating, pale skin.

Impact of genetic on the diagnosis and treatment of thalassemia

1. Molecular analysis for α and β-thalassemia mutations

The introduction of PCR has simplified the screening process for single-based mutations[15]. The majority of mutations associated with thalassemia are point mutations, which are typically classified as single-base substitutions, small insertions, or deletions. The following is an overview of some standard DNA techniques: allele-specific PCR, reverse dot blot (RDB) analysis, real-time PCR with melting curve analysis, and DNA sequencing[16].

a. Allele-specific PCR: This technique employs two identical primers differing only at the 3' end base; one primer is intended to attach to the wild-type sequence, while the other binds to the mutant sequence. Moreover, a standard primer for the complementary DNA strand is required. For Taq polymerase-mediated primer extension, which does not have 3' to 5' exonuclease (proofreading) activity, perfect base pairing with the DNA template is required at the 3' end of the primer. In a typical person, PCR outcomes reveal a product exclusively with the wild-type primer set. An individual with heterozygous genes will generate bands using both the wild-type and the mutant primer sets, while a homozygous mutant individual will yield no product with the wild-type primer set but will test positive with the mutant primer set[17].

b. Reverse dot-blot analysis: The proposed mutation can be identified by hybridizing an allele-specific oligonucleotide (ASO) DNA probe to a PCR product fixed on a membrane filter sheet in a dot format. This ASO probe can be either radiolabelled with 32P for autoradiography or conjugated to reporter groups (such as biotin, digoxigenin, or an enzyme like horseradish peroxidase) that allow visualization through chemil-uminescent or colorimetric reactions. The presence of commercial kits has made these non-radioactive detection methods more commonplace. Two hybridization tests are required for every mutation: one using the probe for the mutant sequence and another employing the probe for the normal sequence. Optimising the hybridisation stringency for every ASO probe is crucial. Reverse dot-blot analysis has been established[18,19]. When the ASO probes have an amino group at the 5' terminal base, enabling them to be covalently attached to a nylon membrane strip. This strip is subsequently hybridized with amplified DNA tagged with biotin for colorimetric detection. An average person will yield favorable outcomes with all wild-type probes and none with mutant probes. Heterozygous individuals will display a positive outcome with one mutation dot and the usual dots. Conversely, homozygous mutations will produce a bright dot with the mutant probe but not with the

matching normal sequence, along with positive dots for other standard probes. An essential aspect of this method is optimizing the washing temperature for every probe, which can be accomplished by modifying the length of each ASO probe.

c. Real-time PCR with melting curve analysis: Traditional PCRs provide a distinct outcome, yet they demand labor-intensive and time-consuming steps after PCR processing. Real-time PCR, also known as quantitative PCR (qPCR), is commonly employed to identify, characterize, and measure nucleic acids. It features high throughput, automation, and a minimal risk of post-PCR contamination. Presently, the use of real-time PCR with melting curve analysis for diagnosing thalassemia relies on two primary methods: intercalating dye assays and probe-based assays, generating a fluorescent signal from the synthesis of products in PCR. The initial method relies on fluorescent DNA intercalating dyes like SYBR Green I, which attach to double-stranded DNA (dsDNA) and experience a conformational alteration that enhances their fluorescence. In the absence of single-stranded DNA (ssDNA) or the dyes that are free in the solution, they will not emit fluorescence. Upon finishing the amplification reaction, the thermal cycler protocol produced a melt curve by gradually raising the temperature in slight increments and tracking the fluorescent signal at each stage. When 50% of dsDNA is separated into ssDNA, this is known as the melting temperature (Tm). The varying size or GC content of PCR products showed the difference in Tm peaks. Consequently, the multiplex GAP-PCR combined with melting curve analysis was created for genotyping α-thalassemia. The primers were created to specifically amplify two deletion fragments, the –SEA and –THAI deletions, along with two normal fragments and the α2-globin gene. The melting curve analysis is capable of differentiating α-thalassemia 1 heterozygote, α-thalassemia 2 homozygotes, Hb H disease, and α-thalassemia 1 homozygote (Hb Bart's hydrops fetalis)[28]. The second probe-based tests are now commonly employed for identifying point mutations. TaqMan assays consist of oligonucleotide probes that are labelled with fluorescence. The TaqMan assay utilized the 5'-exonuclease function of heat-resistant Taq polymerases. The probe consists of a fluorescent reporter at the 5' end and a quencher at the 3' end. The reporter's fluorescence is quenched due to its proximity to the quencher. However, during the annealing/ extension step in the PCR reaction, the probehybridises to the target region. The 50 to 30 exonuclease activity of Taq will cleave off the reporter, resulting in a fluorescence signal proportional to the amount of PCR product in the sample. This technique can be applied to β-thalassemia diagnosis, the multiplex probe-based fluorescence melting curve analysis (FMCA), a powerful tool for point mutation detection based on the Tm generated by thermal denaturation of the probe-target hybrid[20,21].

d. Direct DNA sequencing: Mutations can be identified by sequencing the PCR product, usually through Sanger's dideoxy termination technique, requiring a single DNA strand as a template. Various techniques exist to create this individual strand. One alternative is to take some amplified DNA and perform another PCR cycle using just one primer. Alternatively, the initial PCR product can be denatured and rapidly cooled to maintain the separation of the two strands. An alternative method includes phosphorylating a primer strand at the 5'-end and subjecting the PCR product to lambda exonuclease, which will eliminate the 5'-phosphorylated strand in the double-stranded DNA. Furthermore, adding biotin to the 5'-end of one primer enables the PCR product to adhere to streptavidin coated magnetic beads, which can subsequently be processed to isolate the duplexes and eliminate the non biotinylated strands. An alternative is to subclone the PCR products into a sequencing vector; however, this approach poses the risk of detecting PCR artefacts[22,23].

e. Multiplex ligation-dependent probe amplification: Multiple ligation-dependent probe amplification (MLPA) is a multiplex PCR method that identifies deletions or duplications in particular segments of DNA. Following the use of conventional techniques, this approach has successfully detected both known and unknown deletions in difficult cases. MLPA is easy to set up and only needs a thermocycler and capillary electrophoresis. The procedure starts with the denaturation and hybridization of DNA, followed by mixing it with a collection of MLPA probes. These probes consist of two different oligonucleotides (LPO and RPO) that bind to adjacent target sequences. Subsequently, the probes participate in a ligation reaction. During the PCR process, only the amplified ligated probes are present, and the amount of these ligated probes reflects the number of target sequences in the sample. The amplification products obtained are examined via capillary electrophoresis.

Next-generation sequencing: The progress of sequencing technology greatly aids in the characterization of the human genome. NGS technologies have acquired the ability to sequence the whole human genome with unprecedented throughput, scalability, and speed that surpasses what is achievable with Sanger sequencing technology. The majority of NGS platforms consist of three main stages: preparing the library through random DNA fragmentation and ligating with specific linkers. Second, library enhancement through clonal amplification techniques and PCR. Third, sequencing utilizes the incorporation of fluorescently labeled nucleotides by DNA polymerases or ligation methods. NGS has allowed scientists to identify and comprehend intricate diseases via whole-genome sequencing, exome sequencing, or focused gene panels[34]. Recently, NGS has been utilized for thalassemia screening[21]. The targeted NGS strategy was developed to encompass all coding regions of globin genes, their crucial regulatory areas, and modifiers like KLF1, BCL11A, HBS1L, and MYB. Initial findings indicate that NGS could be significantly more precise than traditional thalassemia diagnosis through complete blood count (CBC), hemoglobin analysis, hemoglobin typing, and selection for genotyping.

Treatment approach for thalassemia

1. Current approaches

Alongside conventional therapies such as consistent blood transfusions and iron-chelating drugs, a potential strategy for treating thalassemia includes pharmacologically activating the gamma-globin gene. Regular blood transfusions may result in an accumulation of iron in the body, which presents dangers to both the heart and liver. Several pharma-ceutical alternatives exist that can increase HbF levels to aid in the effective management of thalassemia. For example, Hydroxyurea is an affordable treatment that improves the activity of various signaling pathways, increasing HbF levels and reducing the need for frequent blood transfusions[26]. Although allogenic hematopoietic cell transplantation provides a possible therapy for thalassemia, it poses various challenges for implementtation, such as the scarcity of compatible donors due to human leukocyte antigen (HLA) matching, the potential for graft rejection in specific situations, and the effect of iron toxicity on the success or failure of hematopoietic stem cell transplantation (HSCT)[27]. A newer approach, lentiglobin gene therapy, can potentially resolve problems such as clonal dominance, graft rejection, and mortality. Nonetheless, certain patients might encounter postponed platelet engraftment[28].

2. Genetic approach

Gene transfer using onco-retroviral vectors

Retroviral vectors for gene addition are promising for treating monogenic disorders. However, when this approach is used for hemoglobinopathies like thalassemia, it presents significant challenges in managing transgene expression, which needs to be: erythroid-specific**,** elevated**,** position-independent and**,** sustained over time.

Numerous studies were conducted prior to the generation of positive preclinical data. Initial efforts were conducted with Oncoviruses. These viruses are part of the extensive *Retroviridae* family and are defined by a genome that encodes the genes gag-pol and env. Onco-retroviral vectors, like those originating from the Moloney murine leukemia virus, effectively deliver therapeutic genes into murine HSCs while not transferring any viral genes. Recombinant onco-retroviruses were the initial viral vectors to deliver the human β-globin gene into mouse HSCs. These experiments led to tissue-specific but low and variable (position-dependent) human β-globin expression in bone marrow chimeras, typically ranging from 0 to 2% of the endogenous mouse β-globin mRNA levels. Research intended to boost expression levels of transferred β-globin genes has concentrated on integrating locus control region (LCR) elements from the human β-globin gene locus into onco-retroviral vectors. The LCR includes cis-acting DNase I hypersensitivity sites (HSs) essential for elevated, sustained, position-independent, and erythroid-specific expression. These HS elements comprise multiple deoxyribonucleic acid (DNA)-binding motifs that aid transcriptional and chromatin remodelling factors in promoting chromatin accessibility. Additionally, these genomic areas enable the attachment of other regulatory components necessary for the elevated expression of the β-globin gene. The integration of the essential components of HS2, HS3, and HS4 from the human β-globin LCR significantly enhanced expression levels in murine erythroleukemia cells, yet did not eliminate the positional variability of expression[17]. Additional erythroid specific transcri-ptional elements were explored within onco-retroviral vectors, comprising the HS40 regulatory region from the human α-locus and various promoters. The ankyrin promoter, associated with a red cell membrane protein, has demonstrated potential in transgenic mice and transduced MEL cells. In mice, the ankyrin promoter has been employed to promote the expression of the human γ-globin gene in double quantity, leading to an average expression level of 8% compared to the native α-globin genes. To prevent transcriptional silencing of the γ-globin promoter in hematopoietic chimeras, mutant γ-globin promoters from individuals with HPFH were also studied. The Greek mutation at position -117 thus seemed to significantly enhance γ-globin expression in MEL cells. Nonetheless, these vectors also did not elevate the γ-globin gene to therapeutic levels. While onco-retrovirus vectors integrate into the genome, a significant number of integrants experience transcriptional silencing, which creates an extra hurdle for the effectiveness of gene therapy with these vectors.

Kalberer and co-workers attempted to avoid gene silencing by preselecting *ex vivo* retrovirally transduced hematopoietic stem cells based onthe expression of the green fluorescent protein (GFP). In this vector, the GFP gene was driven by the phosphorglycerate kinase promoter, while the human β-globin gene was by its promoter and minor elements from the LC[26].

Gene transfer using onco-retroviral vectors also have some limitations

1. This method prevented gene silencing in hematopoietic stem cells within the body and the decline in expression with age. However, issues with lower expression levels and effects due to the positions of different cell types remained.
2. One significant drawback is that onco-retroviral vectors require cells to be infected before or during their division. If this timing is not met, the viral RNA cannot enter the nucleus because of the nuclear membrane. Many hematopoietic stem cells are typically resting, necessitating cytokine induction to prompt division for improved transduction efficiency and expression levels. However, stimulating these quiescent stem cells can negatively affect or even stop their ability to repopulate in the long term.

Gene transfer using lentiviral vectors

With the extensive research on human immuno-deficiency virus-1, it has been realised that lentivirus, engineered to be devoid of any pathogenic elements, can become efficient gene transfer vectors. Lentiviruses are characterised by a complex genome that encodes some accessory proteins besides the canonical retroviral genes gag-pol and env. They share all the typical characteristics of retroviral replication, including receptor-mediated entry, capsid uncoating,

reverse transcription of the viral RNA and integration into the host cell genome[19].They can also transduce non-replicating cells, which confers to these viruses an exceptional value for developing clinically functional gene vectors. Compared to onco-retroviral vectors, the stabilisation of the proviral mRNA genome by the interaction of the accessory protein Rev with its cognate motif Rev-responsive element (RRE) increases their range of application since more prominent genomic elements can be introduced in their genome with limited or no sequence rearrangement. Therefore, lentiviral vectors are thus likely to be selected as vectors of choice for the stable delivery of regulated transgenes in stem cell-based gene therapy.

The use of lentiviral vectors has allowed the introduction of significant genomic elements from the β-globin locus, different promoters, enhancers, and chromatin structure determinants that led to lineage-specific and elevated β-, γ- and α-globin expression *in vivo*. This resulted in ameliorating or correcting anaemia and secondary organ damage in several murine models of haemoglobinopathies, making the recombinant lentiviruses the most effective vector system for gene therapy of these disorders.

α-Thalassemia may serve as a potential target for fetal gene therapy as fetuses affected by this condition often die between the third trimester and shortly after delivery. The possible application of lentiviral vectors for treating α-thalassemia was explored, utilizing a vector that includes the HS2, 3, and 4 regions of the LCR from the human β-globin locus along with the promoter of the human α-globin gene to guide the expression of the human α-globin gene. Utilizing this vector, Han and associates carried out gene delivery in utero during mid-gestation, focusing on embryos impacted by a severe type of α-thalassemia. They demonstrated that the expression of the human α-globin gene in newborn mice was found in the liver, spleen, and peripheral blood[16]. The expression of the human α-globin gene peaked at 3–4 months, reaching 20% in certain recipients. Nevertheless, the expression decreased at 7 months. Colony-forming assays conducted in these mice revealed low levels of transduction and an absence of human α-globin transcript.

Treatment of β-thalassemia and other disorders through lentiviral-mediated gene transfer is studied in murine and primate models. The original studies in mice showed that lentiviral-mediated human β-globin gene transfer could rescue mice affected by β-thalassemia intermedia and β-thalassemia major. The mouse β-globin cluster has two adult β-globin genes, minor-β and major-β globin. Thalassemia mice were generated by deleting the minor-β and major-β globin on one allele, designated as th3/+[33]. These mice have a degree of disease severity (hepatosplenomegaly, anaemia, aberrant erythrocyte morphology) comparable to that of patients affected by thalassemia intermedia.

Gene transfer using lentiviral vectors also has some limitations

There is a need for improved efficiency of gene delivery, insertion of the gene into non-oncogenic sites, and potential negative or positive contributions of the b-thalassemia genotype and potential modifiers to the effectiveness of gene transfer[12].

Gene correction and induced pluripotent stem (IPS) cells: Triplex-forming oligonucleotides and triplex-forming peptide nucleic acids (PNAs) have been shown to stimulate recombination in mammalian cells via site-specific binding and creation of altered helical structures that provoke DNA repair[34]. Cotransfection of PNAs and recombination donor DNA fragments, Chin and co-workers demonstrated that these complexes could promote single base-pair modification at the start of the second intron of the beta-globin gene, which is the site of a common thalassemia-associated mutation. This single base-pair change was detected by restoring proper splicing of transcripts produced from a GFP beta-globin fusion gene. These PNAs' ability to induce recombination depended on dose, sequence, cell-cycle stage, and the presence of a homologous donor DNA molecule.

They also showed that these PNAs effectively stimulated the modification of the endogenous beta-globin locus in human cells, including primary hematopoietic progenitor cells. However, the enhanced recombination did not exhibit frequencies superior to 0.4%[6]. This technology could be a powerful tool in combination with the generation of stem cells. In particular, the introduction of the genes Oct3/4 and Sox2 with either Klf4 and c-Myc or Nanog and Lin28 genes can induce pluripotent stem cells.

Limitations-

- One of the most pressing problems is eliminating transcription factors when they are no longer needed.
- Second, it is necessary to reestablish the correct re-programming so that the iPS cells do not develop into tumours.
- Splice-switching and stop codon read-through**:** Defective β-globin gene expression and β-globin deficiency can be attributed to almost 200 thalassemia mutations.

Only ten mutations account for the majority of global cases, with some of the most common ones affecting the splicing of intron 1 (IVS1-110, IVS1-6, IVS1-5) and intron 2 (IVS2-654, IVS2-745)[17]. These mutations produce incorrectly spliced mRNAs, even though the right splice sites are intact and could function properly. Small nuclear RNA (snRNA) and splice-switching oligonucleotides hold promise, as they can help restore proper splicing, normalising protein synthesis. By using antisense oligonucleotides to block the faulty splice sites, the splicing machinery is encouraged to choose the correct sites instead. Targeting these faulty splice sites with antisense sequences in thalassemia pre-mRNA has proven effective in restoring standard splicing patterns and, consequently, haemoglobin production[35]. This was shown in hematopoietic stem cells and erythroid progenitor cells from a patient affected by IVS2-745/IVS2-1 thalassemia.

CONCLUSION

Genetic information is pivotal in influencing the diagnosis, prognosis, and treatment of thalassemia.

Developments in molecular diagnostics, such as PCR, MLPA, and next-generation sequencing, have facilitated accurate mutation identification, early carrier identification, and enhanced risk stratification. These advancements enhance tailored treatment approaches, ranging from improved transfusion protocols and iron chelation to hematopoietic stem cell transplants and new gene-centric therapies. Identifying genetic modifiers, like co-inherited α-thalassemia and polymorphisms that enhance fetal hemoglobin, provides insight for personalized patient care by forecasting disease severity and treatment response.

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AUTHOR'S CONTRIBUTION

Obeagu EI: conceived the idea, writing the manuscript. **Kwaifa IK:** editing, critical review. **Bagudo IA:** literature survey. **Rabiu U:** editing. **Ayodeji OA:** literature survey. **Bukola IR:** formal analysis, data processing. Final manuscript was checked and approved by all authors.

DATA AVAILABILITY

The empirical data used to support the study's conclusions are available upon request from the corresponding author.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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