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Third-generation sequencing technology in the prevention and control of thalassemia: Applications and advances

Jian Tang[1,2,*], Mengxin Lv[1,2,*], Hongqing Zhang[2,3], Maohua Qin[1,2], Jiacong Yan[4,5], Yizhi Zhang[2,3] and Jianping He[1,2]

Abstract

With the rapid advancement of sequencing technology, third-generation sequencing, particularly single-molecule real-time sequencing and nanopore sequencing, has opened new avenues for the precise prevention and control of genetic diseases. It offers distinct advantages, including long-read lengths, minimal requirement for extensive polymerase chain reaction amplification, and real-time sequencing capability. This narrative review summarizes and critically evaluates recent advances in the application and latest progress of third-generation sequencing in thalassemia. It focuses on the technical advantages and application status of this technology in detecting complex structural variations, large-scale carrier screening, prenatal diagnosis, and preimplantation genetic testing. The review further analyzes the considerable potential of this technology in improving diagnostic accuracy, shortening detection cycles, and reducing technical costs. Additionally, it discusses the challenges currently faced in data interpretation, standardized processes, and clinical translation. Finally, this review provides insights into future development directions, with the aim of providing new technical perspectives and a theoretical basis for optimizing the prevention and control strategies of thalassemia.

[1]Department of Medical Genetics and Prenatal Diagnosis, Kunming Women and Children's Health Hospital, China
[2]Maternal and Child Health Research Center, Kunming Medical University, China
[3]Yunnan Provincial Branch of the National Clinical Research Center for Obstetrics and Gynecology, Kunming Women and Children's Health Hospital, China
[4]Reproductive Medicine Department, First People's Hospital of Yunnan Province, China
[5]NHC Key Laboratory of Preconception Health Birth in Western China, China

*These authors contributed equally to this work and should be considered joint first authors.

Corresponding authors:
Jianping He, Department of Medical Genetics and Prenatal Diagnosis, Kunming Women and Children's Health Hospital, Kunming, Yunnan, China.
Email: 2311378101@qq.com
Yizhi Zhang, Yunnan Provincial Branch of the National Clinical Research Center for Obstetrics and Gynecology, Kunming Women and Children's Health Hospital, Kunming, Yunnan, China.
Email: yizhizhangmrs@163.com

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Keywords
Third-generation sequencing, thalassemia, single-molecule real-time sequencing, nanopore sequencing, genetic disease prevention, prenatal diagnosis

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Introduction

Thalassemia is a highly prevalent monogenic hereditary blood disease worldwide, caused by defects or deletions in globin genes. These defects lead to an imbalance in globin chain synthesis, resulting in hemolytic anemia. This disease follows an autosomal recessive pattern of inheritance.[1–3] Thalassemia exhibits an uneven global distribution and is particularly common in Mediterranean coastal areas, the Middle East, and Southeast Asia, posing a serious threat to the health of local populations.[4,5] This disease shows a high degree of clinical heterogeneity, with severity ranging from asymptomatic carrier states to life-threatening severe hemolytic anemia.[6] The diversity of clinical manifestations primarily depends on the number and type of affected genes. For example, the severity of α-thalassemia is directly associated with the number of α-globin gene deletions,[4] whereas the clinical phenotype of β-thalassemia is strongly influenced by more than 200 types of hemoglobin subunit beta (HBB) gene mutations.[5] Notably, even with the same genotype, clinical manifestations may vary significantly, suggesting the presence of other genetic modifiers, including trans-acting factors located outside the α- and β-globin gene clusters, which can affect clinical severity in β-thalassemia carriers.[6]

Traditional prevention and control strategies are based on carrier screening and prenatal diagnosis, with the aim of preventing the birth of severely affected children by identifying high-risk couples and providing genetic counseling. This approach has achieved significant outcomes in high-prevalence areas in China. For instance, an 11-year retrospective study conducted in Guangdong Province showed that prenatal diagnosis in high-risk pregnancies successfully identified and enabled intervention for a large number of fetuses with moderate to severe thalassemia.[7] However, technologies primarily based on Sanger DNA sequencing (Sanger) and next-generation sequencing (NGS) have limitations in detecting complex structural variations, large-fragment deletions/duplications, and variations located in high GC or homologous regions. Conventional genetic testing methods (such as gap–polymerase chain reaction (PCR) (gap-PCR) and reverse dot blot hybridization) typically identify only common mutation hotspots, which increases the likelihood of missing or misdiagnosing rare variations, complex rearrangements (such as Hong Kong-type αα alleles (HKαα)), or variations located in highly homologous regions (such as the α-globin gene cluster).[8,9] For example, some studies have reported that relying solely on hotspot mutation detection can result in missed diagnoses and that attention should be paid to detecting rare gene variations, including *ααα anti4.2*, *ααα anti3.7*, and *−α2.4*, in prevention and control efforts.[10] Additionally, traditional stepwise screening processes are cumbersome, costly, and require high levels of genetic counseling, thereby presenting significant limitations.[11]

Third-generation sequencing (TGS), with its core advantages of long-read lengths and real-time sequencing, provides a revolutionary approach for directly analyzing complex genomic regions and

variation types associated with thalassemia. Compared with NGS, TGS can produce long-read sequences that span the entire hemoglobin subunit alpha (HBA) 1 (HBA1), HBA2, and HBB genes, thereby enabling the diagnosis of most common and rare thalassemia variants.[12] Its single-molecule real-time (SMRT) sequencing capability allows for accurate identification of complex structural variations that are challenging to detect using traditional methods, including large-fragment deletions, gene duplications, fusion genes, and point mutations located within homologous regions.[13] For example, TGS can directly determine the cis configuration of mutations in two alleles in a single step and accurately identify different genotypes of alleles, such as HKαα, without the need for family-based analysis.[14] In recent years, with improvements in sequencing accuracy and reductions in cost, TGS has gradually transitioned from research to clinical application.[15] Multiple studies have confirmed that TGS demonstrates higher detection rates and diagnostic accuracy in thalassemia carrier screening and genetic diagnosis compared with traditional PCR methods or NGS.[16,17] For instance, in a comparative study conducted in Hunan Province, the mutation detection rate of TGS in individuals with positive hemoglobin tests was 20.49% higher than that of conventional PCR methods.[16] Another back-to-back comparative study conducted in Hainan showed that TGS improved the detection rate by 2.28% compared with NGS and was able to report new types of thalassemia variants locally for the first time.[17] These findings indicate that TGS has the potential to become a more comprehensive and reliable genetic analysis tool, providing key technical support for the precise prevention and control of thalassemia.

Given the rapidly evolving nature of TGS and the current scarcity of sufficiently homogeneous studies to support a systematic review, we conducted a narrative review to synthesize existing evidence on the technology's potential to enhance diagnostic accuracy, shorten detection cycles, and reduce costs. Relevant literature was identified through searches of databases such as PubMed, Web of Science, and China National Knowledge Infrastructure (CNKI). This review also examined key challenges in data interpretation, workflow standardization, and clinical translation as well as outlined future directions. Collectively, these insights aimed to provide a technical and theoretical foundation for optimizing thalassemia prevention and control strategies. This narrative review was conducted following the Scale for the Assessment of Narrative Review Articles (SANRA) framework to ensure methodological rigor and reporting quality.

Sources and selection criteria of articles

We conducted a search of PubMed, Web of Science, and CNKI to identify the most recent peer-reviewed articles published between January 2020 and January 2026, using the following search terms: "Thalassemia," "Third-generation sequencing," "Thalassemia screening," "Thalassemia diagnosis," and "Thalassemia control and prevention." To ensure methodological rigor, we focused on high-level evidence, including systematic reviews, meta-analyses, randomized controlled trials, and large-scale investigations, sourced from prestigious general medicine and critical care publications.

Overview of thalassemia

Thalassemia, also known as Mediterranean anemia, is a group of hemolytic anemia diseases caused by defects or mutations in globin genes, resulting in abnormal synthesis of globin peptide chains.[18] As shown in Figure 1, under normal conditions, human

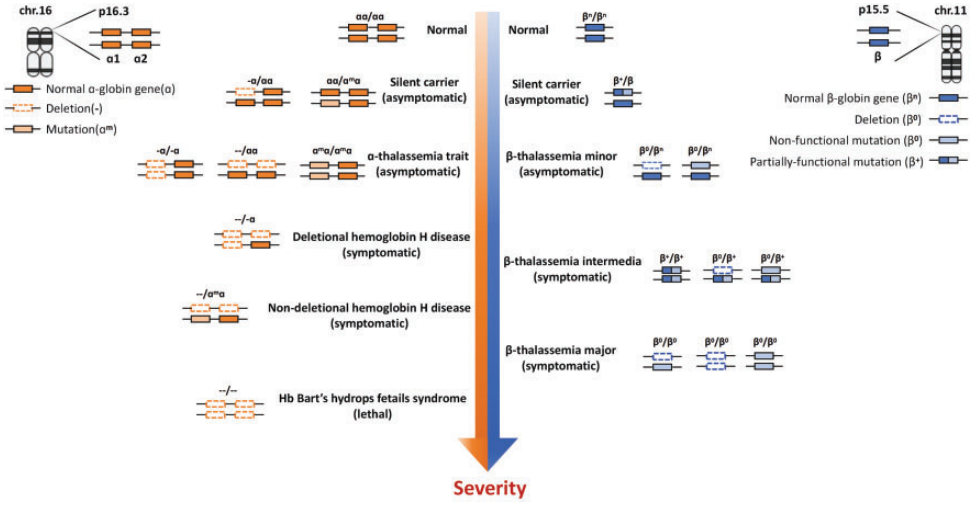

Figure 1. Schematic representation of genotype–phenotype correlations in α- and β-thalassemia, illustrating the relationship between hemoglobin gene dosage, mutation types, and clinical severity. Left panel: α-globin gene cluster on chromosome 16p13.3 (chr16), including normal (αα/αα), silent carrier (−α/αα or α^a/αα), α-thalassemia trait (−α/−α, −α/α^a, or α^a/α^a), symptomatic deletional (−/−α) or nondeletional (−/α^a) hemoglobin H disease, and lethal Hb Bart's hydrops fetalis syndrome (−/−). Right panel: β-globin gene cluster on chromosome 11p15.5 (chr11), including normal (β^N/β^N), silent carrier (β^+/β^N), β-thalassemia minor (β^0/β^N or β^+/β^N), intermediate (β^+/β^+, β^0/β^+), and major (β^0/β^0) phenotypes, with differentiation among deletion (β^0), nonfunctional (β^0), and partially functional (β^+) β-globin mutations. The vertical gradient arrow labeled "Severity" indicates increasing clinical severity from top (asymptomatic) to bottom (lethal). The color-coded legend denotes the following: solid orange, functional α-globin allele (α); dashed orange, α-globin deletion (−); patterned orange, point mutation (α^m); solid blue, functional β-globin allele (β^N); dashed blue, β-globin deletion (β^0); light blue, nonfunctional β mutation (β^0); and blue with white stripe, partially functional β mutation (β^+).

hemoglobin consists of two pairs of different globin chains, α-globin and β-globin chains, which combine with heme to form oxygen-carrying hemoglobin.[19] Defects in globin genes disrupt this balance, thereby affecting the normal synthesis and function of hemoglobin and resulting in a range of pathophysiological changes, which are predominantly classified into α-thalassemia and β-thalassemia.[20] The classification of thalassemia remains complex, as there are significant differences in clinical manifestations and genetic characteristics among different types. Accurate diagnosis and classification are essential for developing appropriate treatment plans and providing genetic counseling.[21,22] The epidemiological characteristics of thalassemia are influenced by a multiple factors, and understanding its distribution patterns is important for developing targeted prevention and control strategies as well as for guiding genetic counseling and screening. Thalassemia is primarily distributed in the Mediterranean region (Italy, Greece, and Cyprus, among others), the Middle East (Saudi Arabia and Iran, among others), and Southeast Asia (Thailand, India, and Malaysia, among others).[23–27] In China, thalassemia exhibits a clear distribution pattern, with higher prevalence in the southern regions and lower prevalence in the north.[28,29] The southern provinces of Guangxi, Guangdong, Hainan, and Yunnan are high-prevalence areas, whereas the

incidence in other inland provinces remain relatively lower. However, the number of cases are gradually increasing with intensified population mobility.[30] No considerable sex or age differences in the distribution of thalassemia have been observed. The probability of males and females being affected is comparable. Patients with mild thalassemia may be identified at any age, often incidentally during health check-ups or visits for other conditions.[31] In contrast, patients with severe or intermediate thalassemia usually exhibit significant clinical symptoms, including growth retardation and anemia, during infancy or childhood, requiring timely diagnosis and management.[32,33]

The clinical manifestations and impact of thalassemia vary depending on disease type, severity, and individual differences.[34] Thalassemia has a substantial impact on patients' physical health and quality of life across multiple domains.[35] As shown in Figure 2, in general, the more severe the condition, the greater the impact on patients' physical health, involving multiple systems and organs.[36] In the hematological system, anemia is the most prominent feature of thalassemia. Under microscopic observation, red blood cell morphology in patients with thalassemia differs significantly from that of normal red blood cells, commonly exhibiting unequal red blood cell

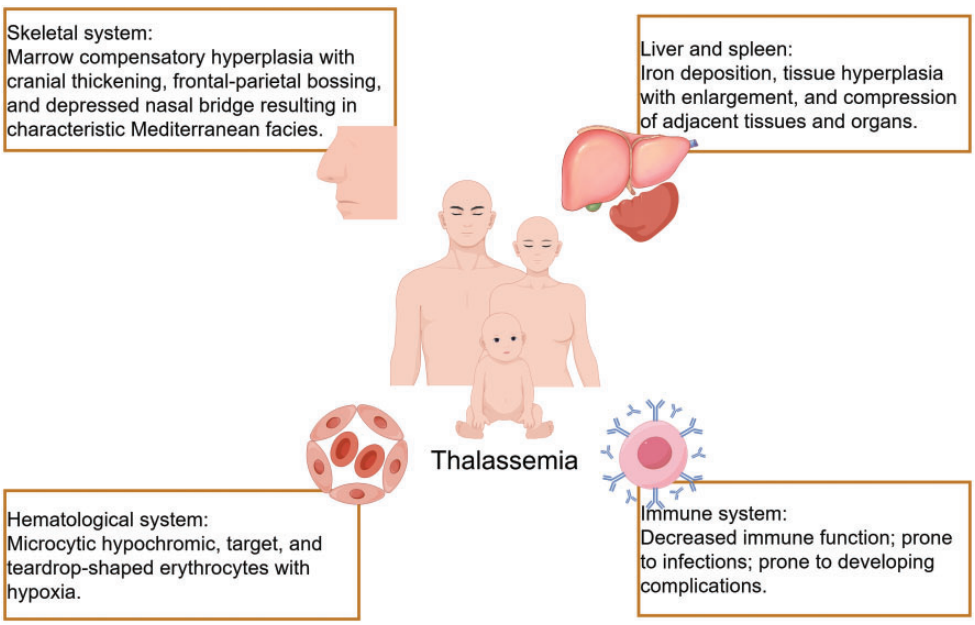

Figure 2. Major clinical symptoms and hazards of thalassemia. Skeletal system: marrow compensatory hyperplasia, leading to cranial thickening (frontal–parietal bossing) and midfacial hypoplasia, resulting in the characteristic "Mediterranean facies." Hematological system: chronic hemolysis and ineffective erythropoiesis, producing microcytic, hypochromic red blood cells with target and teardrop morphology, accompanied by tissue hypoxia. Hepatosplenomegaly: extramedullary hematopoiesis and iron overload, leading to progressive enlargement of the liver and spleen and compression of adjacent structures. Immune system: impaired cellular and humoral immunity, due to splenectomy, iron deposition, and chronic inflammation, increases susceptibility to infections (particularly encapsulated bacteria) and predisposes to immune-mediated complications.

sizes, an increased number of microcytic hypochromic red blood cells, and abnormal forms such as target cells and teardrop cells.[37] In the skeletal system, patients with severe or intermediate thalassemia may develop compensatory bone marrow hyperplasia due to long-term severe anemia, leading to thickening and protrusion of the cranial bones and the characteristic "Mediterranean face."[38] In the context of persistent anemia, long-term extramedullary hematopoiesis can result in the proliferation and enlargement of liver and spleen tissues. This enlargement can compress surrounding tissues and organs, resulting in symptoms such as abdominal distension and pain, and may impair normal organ function, thereby increasing the risk of infections and other complications.[39] Children with severe thalassemia, due to long-term anemia, experience insufficient oxygen and nutrient supply to various tissues and organs, which severely affects growth and development. Long-term blood transfusion therapy can lead to excessive iron accumulation, damaging multiple organs such as the heart, liver, and endocrine glands.[40] In addition, patients and their families face the challenges of long-term disease management, with substantial economic and psychological burdens.[41–43]

Overview of TGS and its technical advantages in thalassemia detection

Core technical principles and platform comparison

TGS represents the recent advancement in genomic sequencing, with SMRT sequencing as its core technology, allowing direct reading of long-fragment nucleic acid sequences.[44] Currently, the two main platforms are the PacBio system, which is based on SMRT sequencing, and the Oxford Nanopore system, which is based on nanopore technology.[45] The core of SMRT sequencing technology is the zero-mode waveguide pore, which enables real-time decoding of sequences by monitoring the light signals generated when fluorescently labeled nucleotides are incorporated into the nascent chain under the catalysis of DNA polymerase.[46] In contrast, nanopore sequencing determines sequences based on characteristic current changes caused by different bases as nucleic acid molecules pass through biological protein nanopores driven by an electric field.[47] There are significant differences in key performance between these two platforms. SMRT sequencing technology is known for its ultra–long-read lengths (up to several tens of kb) and high accuracy, making it particularly suitable for resolving complex genomic structures.[48] In comparison, although nanopore technology also offers advantages in read length and more portable equipment (such as the MinION device), its raw single-base error rate is relatively high; however, its accuracy has significantly improved with increased sequencing depth and algorithm optimization.[49] In terms of throughput, the PacBio Sequel II system offers high-throughput options, whereas the nanopore platform provides flexible choices ranging from portable to high-throughput systems (such as PromethION). Operational costs for both technologies continue to decrease as they mature. In the context of thalassemia prevention and control, SMRT sequencing technology, owing to its high accuracy and long-read lengths, is more suitable for high-precision diagnostic laboratories that require precise identification of complex structural variations and haplotypes. In contrast, the real-time capability and portability of nanopore technology demonstrate unique value in on-site screening or emergency prenatal diagnosis, where rapid preliminary results are essential.[50]

Unique advantages compared with traditional technologies

Compared with traditional technologies represented by PCR and Sanger sequencing, as well as second-generation sequencing characterized by short-read lengths, TGS exhibits multiple unique advantages in thalassemia gene testing. First, its core advantage lies in ultra–long-read lengths. The pathogenic genes associated with thalassemia include the α-globin gene cluster, located within approximately 30 kb on the short arm of chromosome 16 (16p13.3), and the β-globin gene cluster on chromosome 11.[51] Traditional short-read sequencing face challenges in spanning entire gene clusters, making it difficult to directly resolve the precise boundaries of large-fragment deletions, complex rearrangements, tandem repeats, and haplotypes (i.e. cis configurations). In contrast, the long-read lengths of TGS can cover entire gene regions in a single read and directly obtain haplotype information, thereby accurately resolving deletions, such as Southeast Asian–type deletion ($-SEA$) and $-\alpha 3.7$, as well as complex rearrangements, such as HKαα. This capability is crucial for accurately assessing the relationship between genotype and clinical phenotype.[52] Second, TGS enables direct sequencing and simultaneous detection of epigenetic modifications. By avoiding extensive PCR amplification, it completely circumvents allele caused by amplification bias, which is particularly important for detecting regions with high GC content or those with highly homologous sequences (such as HBA1 and HBA2 genes).[53] Additionally, both PacBio SMRT and nanopore sequencing can directly detect DNA base modifications (such as methylation) during sequencing, providing new opportunities for investigating epigenetic regulatory mechanisms underlying thalassemia.[54] Finally, the real-time analysis capability of nanopore technology significantly shortens detection cycles. The time from sample preparation to obtaining preliminary sequencing results can be reduced to a few hours, which is of substantial clinical value for families requiring urgent prenatal diagnosis to manage pregnancies, allowing critical time for clinical decision-making.[55] These advantages collectively make TGS a powerful tool for complementing and improving the existing genetic diagnostic system for thalassemia.

Table 1 [18,56–77] summarizes the advantages and limitations of traditional genetic testing methods, including PCR, gene microarray technology, and TGS in the detection of thalassemia.

As shown in Table 1, the advantages and disadvantages of conventional genetic testing methods, including PCR, multiplex ligation-dependent probe amplification (MLPA), Sanger sequencing, and NGS, are compared with those of TGS.

Applications of TGS in thalassemia carrier screening and genetic diagnosis

Efficient detection of complex and rare variations

TGS, with its long-read lengths and single-molecule sequencing advantages, can accurately identify complex and rare variations that are often missed by traditional methods. Traditional methods, such as gap-PCR and reverse dot blot hybridization, primarily target common hotspot mutations, with limited capability for detecting large-fragment deletions, gene conversion events involving homologous sequences, and rare structural variations.[78] For example, $-SEA$ and Philippine-type deletions are common causes of α-thalassemia; however, traditional PCR methods may fail to effectively detect all subtypes or compound deletions due to primer design limitations.[79] The long-read characteristics of TGS enable it

Table 1. Summary of analytical methods used for thalassemia detection.

Analytical method	Advantages	Disadvantages	References
PCR	Low cost Suitable for large-scale screening High specificity Rapid turnaround time	Qualitative only, lacks quantitative resolution Low sensitivity for low-abundance variants Inability to detect novel or untargeted mutations	Karunanathie et al.[56] Aliyeva et al.[57] Zhou et al.[58] Fu et al.[59]
MLPA	Detects deletion/duplication variants in α- and β-thalassemia genes Simultaneous copy number quantification Moderate throughput	Limited to probe-covered regions (cannot detect deletions outside targeted loci) Poor performance for single-nucleotide variants (SNVs) Requires orthogonal validation for indels	Jomoui et al.[60] Wang et al.[61] Gilad et al.[62] Vijian et al.[63]
Sanger	Gold standard for mutation detection and confirmation Intuitive sequence visualization High accuracy for validating ambiguous results	Low throughput Not feasible for population-scale screening Complex interpretation of heterozygous/mosaic variants	Jamwa et al.[64] Ropero et al.[65] Santos et al.[66] Belmokhtar et al.[67]
NGS	Ultra-high throughput Comprehensive genomic coverage Capable of detecting rare and novel variants Cost-effective at scale	Short read lengths limit resolution of structural variants and precise HBA1/HBA2 differentiation Reduced or uneven coverage in GC-rich and repetitive genomic regions	Mandlik et al.[68] Achour et al.[69] Lee et al.[70] Midha et al.[71] Xu et al.[72] Ling et al.[18]
TGS	Ultra-long read lengths Comprehensive mutation detection Cis/trans discrimination Extreme sensitivity for low-frequency variants Streamlined workflow for structural variant characterization	High capital and operational costs Requires orthogonal validation for rare or low-abundance variants Computationally intensive data analysis High sensitivity to input DNA quality and integrity	Ling et al.[18] Athanasopoulou et al.[73] Oguch et al.[74] Yu et al.[75] Nemashkalo et al.[76] Chen et al.[77]

MLPA: multiplex ligation-dependent probe amplification; NGS: next-generation sequencing; PCR: polymerase chain reaction technology; TGS: third-generation sequencing; HBA: hemoglobin subunit beta.

to span the entire α- and β-globin gene clusters and directly obtain complete haplotype information, thereby accurately identifying the precise breakpoints of these large-fragment deletions and their cis configurations on chromosomes.[80] Furthermore, traditional methods face challenges in distinguishing complex chimeric structures resulting from gene conversion events involving homologous sequences, such as the α-globin gene triplet (e.g. *ααα*$^{anti3.7}$) or complex rearrangements (e.g. HKαα alleles).In contrast, TGS can provide continuous sequences spanning the entire homologous region, clarifying their complex haplotype structures.[81]

In specific research cases, TGS has demonstrated strong capabilities in identifying novel variations and clarifying complex haplotype structures. One study used TGS

to successfully identify a novel 10.3 kb deletion (NC_000016.10:g.172342-182690del) in cases where conventional methods failed to provide a clear diagnosis. This deletion covered the HBA1, hemoglobin subunit theta 1 (HBQ1), and HBA2 genes, contributing to the phenotype of α0-thalassemia.[82] Another study accurately distinguished different subtypes of HKαα alleles (e.g. HKαα/αα and HKαα/-α3.7) using TGS, whereas traditional methods, such as gap-PCR and MLPA, could not accurately determine whether *−α3.7* and *αααanti4.2* were in cis or trans configurations.[83] These findings indicate that TGS not only enhances the comprehensiveness of carrier screening, reducing missed diagnoses due to rare variations, but also significantly improves the accuracy of genetic diagnosis by providing precise haplotype information, thereby offering a reliable molecular basis for subsequent genetic counseling and prenatal diagnosis.[83]

Achieving high-precision haplotype typing and linkage analysis

TGS, through its long-read data, can directly determine the haplotypes of parental chromosomes on which pathogenic mutations are located, which is crucial for assessing the true genetic composition of compound or double heterozygotes. Many patients with thalassemia carry different pathogenic alleles from both parents, forming a compound heterozygous state. Traditional short-read sequencing technologies face challenges to accurately phasing multiple variant loci onto the same chromosome, making it difficult to determine whether these variants are in cis (located on the same chromosome) or trans (located on homologous chromosomes) configurations.[84] The long-read capability of TGS allows coverage of entire gene regions containing multiple variant loci in a single read and directly identify allele sequences on individual DNA molecules, thereby unambiguously determining haplotypes. For example, in a β-thalassemia proband carrying both HBB gene c.313delA and c.126_129delCTTT mutations, TGS can clarify that these mutations are located on two homologous chromosomes (compound heterozygous). This is essential for accurately determining its inheritance patterns and assessing risk to offspring.[85]

This technology has important clinical value in tracing family genetic history, identifying the origin of mutations, and providing reliable haplotype markers for subsequent prenatal diagnosis. By analyzing long-read sequencing data from the proband and family members, a complete family haplotype can be constructed, clearly tracing the transmission of pathogenic alleles.[86] Importantly, in high-risk families requiring prenatal diagnosis, TGS can provide precise haplotype markers for the fetus. By comparing parental haplotypes and those of the proband (if available), fetal inheritance of chromosomes carrying pathogenic mutations can be inferred with high precision, allowing accurate determination of fetal genotype when fetal DNA samples are limited or affected by maternal contamination.[87] This haplotype-based linkage analysis provides greater reliability and information content than approaches relying solely on the detection of mutation loci, particularly in cases involving complex mutation types or highly homologous sequences. Consequently, it provides key evidence for formulating effective genetic intervention strategies.[88]

Advances in TGS in prenatal and preimplantation genetic testing (PGT)

Exploring the potential of noninvasive prenatal single-gene disease testing

TGS, with its long-read characteristics, has opened new technical avenues for

noninvasive prenatal testing of single-gene diseases, particularly thalassemia. Its core principle involves direct analysis of fetal cell-free DNA in maternal plasma, enabling acquisition of parental haplotype information through long-read sequencing, thereby achieving noninvasive genetic diagnosis.[89] Traditional methods rely on short-read sequencing, which makes it difficult to accurately distinguish maternal from fetal alleles, particularly when fetal DNA concentrations are low. In contrast, TGS can span longer genomic regions and directly resolve haplotypes, thereby inferring fetal genotype and providing a noninvasive diagnostic option for high-risk families.[90] A study on β-thalassemia demonstrated that integrating targeted sequencing with a pseudo-tetraploid genotyping method can successfully predict fetal genotype combinations through the analysis of maternal plasma cell-free DNA. This method achieved a total genotype concordance rate of 95.71% and provides new approaches for fetal risk stratification prior to invasive procedures.[91] This represents a shift from proof-of-concept to early clinical application of noninvasive haplotype analysis using TGS.

However, this approach still faces technical challenges in practical applications, including low fetal DNA concentrations and maternal background interference. Fetal cell-free DNA accounts for a small proportion of maternal plasma, particularly in early pregnancy, which poses challenges for accurately capturing fetal-specific signals. Maternal DNA background noise may obscure low-frequency fetal variants, potentially leading to false-negative or false-positive results. Current studies have focused on optimizing bioinformatics algorithms and sequencing strategies to address these challenges. For example, one study proposed a population-based haplotype construction method, inferring parental haplotypes using large-scale retrospective carrier screening datasets and subsequently using parental haplotypes to assist in hidden Markov models to infer fetal haplotypes.[92] This approach reduces reliance on family-based data and improves inference efficiency and accuracy. Additionally, increasing sequencing depth, developing more sensitive variant detection algorithms, and incorporating epigenetic information, such as DNA methylation patterns, to distinguish maternal and fetal DNA are key areas of ongoing development.[93] As sequencing costs decrease and analytical methods improve, these technical challenges are expected to be progressively addressed, enabling broader clinical application of noninvasive prenatal single-gene disease testing.

Optimization of PGT

The application of TGS in PGT has significantly improved embryo screening for single-gene diseases such as thalassemia. Traditional PGT approaches often require separate aneuploidy screening and single-gene disease diagnosis, which can be cumbersome and may lack sufficient information due to limited sample sizes. TGS enables simultaneous detection of aneuploidy and single-gene diseases through long-read sequencing following whole-genome amplification of single blastomeres or trophectoderm cells.[94] Its long-read advantage allows for continuous sequences covering target gene regions to be obtained in a single sequencing run, enabling the detection of point mutations and small insertions/deletions as well as effectively identifying large-fragment deletions, duplications, and other structural variations, which is crucial for thalassemia with high genetic heterogeneity.[95] For example, using PacBio SMRT sequencing, high-depth sequencing of embryo DNA can accurately identify large structural variations such as *HBA1/2*–SEA deletions,

whereas analysis of short tandem repeat sequences can provide confirmatory information for traditional PGT for monogenic disorders (PGT-M) linkage analysis.[96] This "one-stop" testing strategy simplifies workflows, shortens detection cycles, and improves overall efficiency.

Importantly, TGS overcomes diagnostic uncertainties caused by insufficient linkage marker information or recombination events, thereby improving the success rate and reliability of PGT-M diagnoses. In traditional linkage analysis-based PGT-M, the accuracy of diagnosis depends heavily on genetic marker information closely associated with pathogenic mutations. When such marker information is insufficient or when recombination occurs in the embryo, misdiagnosis may occur.[97] In contrast, the long-read capability of TGS enables direct sequencing of haplotypes containing pathogenic mutations and their upstream and downstream large fragments, achieving "haplotype phasing." This allows direct identification whether pathogenic mutations and adjacent genetic variations are located on the same chromosome, thereby enabling accurate determination of whether the embryo has inherited the pathogenic chromosome haplotype without relying on external linkage markers.[98] This method fundamentally avoids uncertainties caused by recombination or insufficient marker information. For example, for couples carrying balanced chromosomal translocations, TGS can not only distinguish whether embryonic chromosomes are balanced but also accurately locate translocation breakpoints, thereby providing opportunities to further distinguish between carrier and noncarrier embryos.[99] This precise haplotype information provides a more reliable basis for selecting embryos that do not carry pathogenic genes, significantly enhancing the diagnostic accuracy and clinical utility of PGT-M, and offering hope for families at risk of genetic diseases to have healthy offspring.

Challenges, integration, and future prospects

Current technical and clinical translation challenges

TGS, particularly SMRT and nanopore sequencing, demonstrates considerable potential in thalassemia gene detection due to its long-read advantages, effectively identifying complex structural variations and rare mutations that are difficult to detect using conventional methods. However, this technology still faces multiple challenges in practical clinical translation. A key technical bottleneck is the relatively high error rate in raw data, which poses severe challenges for subsequent bioinformatics analysis.[100] For instance, the sequencing error rate is relatively high, approximately 10%–15%, particularly in high GC-content regions and repetitive sequences, thereby increasing the likelihood of errors. In the context of thalassemia diagnosis, specialized and optimized bioinformatics analysis processes and error-correction algorithms need to be developed to accurately distinguish sequencing errors from true genetic variants, particularly in regions of high GC content and highly homologous α- and β-globin gene clusters. Although some studies have used population linkage disequilibrium information or improved Levenshtein distance algorithms to enhance the accuracy of data clustering and variant identification, these methods require further validation and optimization on larger-scale thalassemia datasets.[101]

From a clinical translation perspective, cost-effectiveness is one of the key factors hindering the widespread application of TGS. Although sequencing costs are continuously decreasing, the initial equipment

investment and per-test costs are still relatively high compared with the established methods such as PCR–reverse dot blot hybridization or gap-PCR. The complexity of data standardization and report interpretation is another significant barrier. The relationship between thalassemia genotypes and phenotypes is complex, with numerous modifier genes and rare variations. Translating the massive long-read data generated by TGS into gene diagnostic reports that are both understandable and actionable for clinicians as well as establishing unified interpretation standards remain considerable challenges. Furthermore, incorporating TGS into clinical practice guidelines and routine screening pathways requires rigorous clinical validation. Currently, most evidence is derived from retrospective studies or small-scale cohorts, with a lack of prospective, large-scale, multicenter clinical trial data to demonstrate its clear advantages in reducing misdiagnosis and missed diagnosis rates as well as improving patient outcomes. The lack of clinical validation also affects the acceptance of this technology by insurance payers and policymakers.

Integration of multiple technology platforms and automation solutions

To address the limitations of individual technologies and build an efficient, precise, and economical prevention and control model, integrating TGS with other established diagnostic platforms is an inevitable trend. A feasible strategy is to establish a tiered diagnostic system. First, low-cost hematological phenotype screening, including mean corpuscular volume, mean corpuscular hemoglobin, and hemoglobin electrophoresis, can be used for initial screening.[102] For positive screening results, MLPA, PCR–reverse dot blot hybridization, or targeted panel detection using second-generation sequencing can be employed to address the majority of cases.[103,104] For cases in which conventional methods cannot explain the phenotype or when rare/complex variants are suspected, TGS can be used for "ultimate" analysis.[105] This combined strategy can fully leverage the strengths of each method: conventional technologies provide high throughput and low cost, whereas TGS serves as the "gold standard" for resolving difficult cases, thereby optimizing resource allocation and improving overall diagnostic efficiency.

Looking forward, integrated and automated "sample-to-answer" portable nanopore sequencing systems have broad prospects in grassroots screening and rapid on-site diagnosis. The miniaturization and real-time analysis capabilities of nanopore sequencers make them suitable for deployment in community hospitals or screening points in remote areas. Theoretically, technicians only need to add processed samples to the device to obtain reports containing genotype within a few hours, significantly simplifying the operational process and reducing reliance on centralized laboratories and specialized personnel. This approach is highly significant for conducting large-scale population screenings, particularly for mobile populations, in high-prevalence regions such as southern China.[106] However, to realize this vision, further breakthroughs are needed in device stability, reagent room temperature preservation, the intelligence of built-in bioinformatics analysis software, and cost control. Automation solutions not only accelerate the diagnostic process but also reduce human errors, making them a key technological development direction for comprehensive prevention and control of thalassemia.

Future research directions and development trends

With improvements in sequencing chemistry and optimization of bioinformatics algorithms, the accuracy of TGS continues

to increase, and its costs are expected to decrease further with the popularization of technology and market competition. With technological iteration, targeted sequencing strategies and multiplex sample barcoding technologies can reduce sample preparation and sequencing time, thereby lowering per-run sequencing costs. Furthermore, by leveraging the three-tier prevention and control system for birth defects, a coordinated resource-sharing mechanism can be established through a hierarchical service delivery model. In this model, provincial-level centers can centralize sequencing and bioinformatics analysis, whereas primary healthcare institutions can undertake sample collection and preliminary screening. This stratified approach facilitates cost distribution and improves overall operational efficiency. With continued advances in scaled manufacturing, process optimization, and refinement of collaborative frameworks, the economic viability and clinical accessibility of TGS are expected to improve substantially.

Through cloud computing and bioinformatics platforms, researchers and clinicians can remotely access sequencing data and analytical tools, thereby reducing technical barriers and enabling broader institutional participation in TGS applications. Leveraging its long-read capability, TGS enables accurate detection of structural variations, abnormal repeat sequences, and other complex genomic features, making it suitable for screening genetic disorders such as fragile X syndrome, facioscapulohumeral muscular dystrophy (FSHD), and thalassemia. In the future, it may be integrated into newborn screening programs and family-based genetic risk assessment.

It is anticipated that, in the future, TGS will transition from being primarily a diagnostic tool for difficult cases to a first-line diagnostic and screening tool for thalassemia. Its ability to simultaneously detect common and rare variants, accurately resolve cis–trans configurations, and identify structural variations provides comprehensive advantages over existing technologies in preconception screening and prenatal diagnosis.[89]

Future research should focus on several key areas. First, there is an urgent need to establish large-scale population-based long-read genomic databases generated using TGS, particularly comprising data from different regions and ethnic groups in China.[107,108] These databases can provide more accurate allele frequency information for variant pathogenicity interpretation and reveal population-specific genomic structural features, thereby providing a foundation for precise prevention and control. Second, more efficient targeted enrichment strategies should be developed. Given that whole-genome TGS remains prohibitively expensive, targeted long-fragment PCR or probe-based capture targeting globin gene clusters and regulatory regions may enhance sequencing depth and cost-effectiveness, which is essential for routine clinical implementation.[109] Third, the potential role of TGS in monitoring the efficacy of gene therapy warrants exploration. As gene therapies for thalassemia enter clinical practice, it is necessary to analyze the genomic integration sites, vector copy numbers, and potential clonal evolution in treated hematopoietic stem cells. The long-read capability of TGS is well suited for analyzing complex genomic rearrangements and integration events, making it a promising new tool for efficacy assessment and safety monitoring. With sustained investment in these areas, TGS is expected to play an increasingly central role in the lifecycle prevention and control of thalassemia.

Conclusion

The application of TGS in thalassemia prevention represents a shift toward precision medicine, enabling direct observation of

genomic structures and haplotype phases rather than relying on indirect inference. By leveraging long-read capabilities and real-time analysis, TGS effectively resolves complex structural variations and identifies rare variants. It shortens turnaround time, thereby enhancing carrier screening, difficult case diagnosis, and PGT. However, several challenges remain, including high raw error rates, cost considerations, and bioinformatics barriers. Future efforts should focus on technological refinement and algorithm optimization. Establishing clinical-grade standard operating procedures, supported by multiplatform validation, will be essential to expand TGS from specialized diagnostics to large-scale screening. Ultimately, through deep integration with multiomics and clinical data, TGS has the potential to support a more efficient and equitable precision prevention and control system. This approach will not only improve the prevention and treatment of thalassemia but also provide a reference model for achieving early, accurate, and comprehensive management of other genetically heterogeneous monogenic disorders.

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Author contributions

Conceptualization: J.T., M.L., and Y.Z.; methodology: J.T. and M.L.; validation: J.T., M.L., and Y.Z.; writing—original draft preparation: J. T., M.L., and Y.Z.; writing—review and editing: H.Z., M.Q., J.Y., and J.H.; supervision: H.Z., M.Q., J.Y., Y.Z., and J.H. All authors have read and agreed to the published version of the manuscript.

Data availability statement

The data underlying this study are available from the corresponding author upon reasonable request.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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ORCID iD

Jian Tang https://orcid.org/0009-0002-3866-6142

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