All-in-one solution for single gene disorders with diverse mutational spectrum

Third-Generation Genetic Test - Thalassemia

Background

Thalassemia is a group of inherited hemolytic disorders characterized by reduced or absent production of globin chains, involving a spectrum of globin gene defects (SNVs, InDels, recombination, CNVs, etc.). It significantly impacts regions in Southeast Asia, the Mediterranean, and the Middle East.

Thalassemia carries **a high risk of missed cases** with routine blood tests

Sequential hematological screen MCV + MCH and HbA2		
	Sensitivity	Specificity
α-thalassemia	16.53%	97.24%
β-thalassemia	73.74%	96.10%

Information derived from reference [1]

Due to the complex nature of thalassemia genetic variants, conventional molecular analysis methods cannot provide a comprehensive solution, potentially leading to missed diagnoses, misdiagnoses, or delays in the diagnostic process.

Conventional PCR-based methods, such as PCR-RDB and Gap-PCR, typically focus on a limited range of major or common variants. Although next-generation sequencing (NGS) expands the coverage of variants, its ability to detect structural variants and rare deletions is still constrained by the technical limitations of short-read sequencing.

Comprehensive Analysis of Thalassemia Alleles (CATSA)

shapes the future of thalassemia screening

With single molecule real-time (SMRT) sequencing, a comprehensive solution for thalassemia genetic analysis has been developed — CATSA. This innovative approach simplifies the diagnostic process into a single solution, eliminating the need for multiple tests and significantly reducing diagnostic time.

Patent (China): ZL 202110329821.X

Comprehensive coverage

By taking advantage of long-read SMRT sequencing, CATSA covers thousands of thalassemia and hemoglobinopathy genetic variants from databases. In contrast, other genetic tests often have blind spots for specific variant types and struggle to detect uncommon conditions. Therefore, CATSA is more feasible to identify individuals carrying rare variants in isolated communities or areas of high ethnic diversity, where unexpected mutations or genetic admixtures are frequent.

Coverage of genes and variants by current technologies

Category	Number and Type of Variants	Panel		
α-thalassemia	30 Deletions	Basic	Expanded	Comprehensive
	4 Homologous recombination			
	80 *HBA1* gene mutations			
	139 *HBA2* gene mutations			
β-thalassemia	28 Deletions			
	340 *HBB* gene mutations			
Hemoglobinopathy	324 *HBA1* gene mutations			
	386 *HBA2* gene mutations			
	694 *HBB* gene mutations			
Others	160 *HBD* gene mutations			
	HBA/*HBB* gene low frequency mutations, variant of uncertain significance (VUS) associated with thalassemia			

Technology: Single molecule real-time (SMRT) sequencing
Platform: PacBio Vega, Sequel II, and Sequel IIE system
Sample type: Dried blood spot (DBS), gDNA, blood, buccal swab, and amniotic fluid
Operation hour: 77 hours
(starting from sample preparation to report generation)

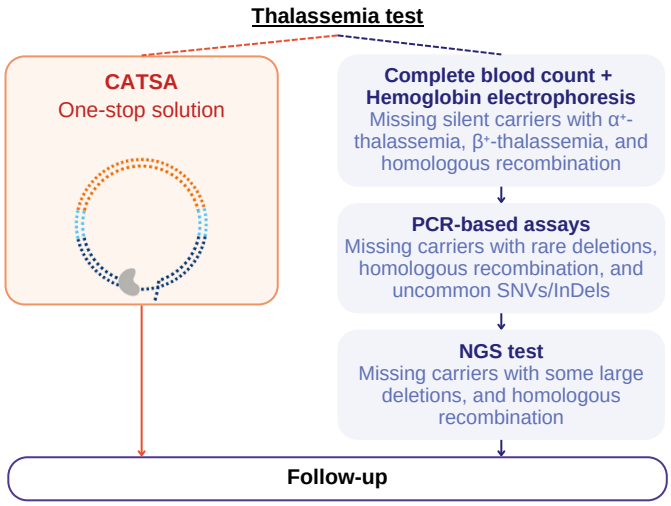
Further genetic insight

Additionally, CATSA can determine the *cis/trans* configuration of variants within the same gene, a capability not achievable with other genetic assays. This allows genetic counselors to accurately assess the zygosity of disease-causing alleles in cases with multiple mutations.

Comprehensive Analysis of Thalassemia Alleles

	PCR-based assays				Sequencing	
	Gap-PCR	PCR-RDB	§Melting curve	+MLPA	NGS	**CATSA**
Deletions	✓	✓	✓	✓	✓	✓
Point Mutations	×	✓	✓	×	✓	✓
Homologous Recombinations	×	×	×	✓	×	✓

\+ Applicable only for large deletion
§ Applicable only for common deletions & point mutations for α-thalassemia as well as common point mutations for β-thalassemia



Performance backed by published data

The new gold standard

In a prospective multicenter study, CATSA demonstrated 100% accuracy in detecting pathogenic thalassemia variants among 1,159 individuals with abnormal hemoglobin parameters, highlighting its reliability. [2] Additionally, in a separate study with 1,122 individuals, CATSA outperformed NGS by identifying and correcting 1.52% discordant results with 100% accuracy, showcasing its comprehensiveness and precision. [3]

Improved pregnancy outcomes

In 278 at-risk amniotic fluid samples, CATSA outperformed PCR-based assays by accurately identifying all genotypes, resolving 4 discordant variants, detecting 11 additional variants, and correcting the predicted phenotype severity in 8 fetuses. Notably, two cases were reclassified from thalassemia trait to intermedia and one from intermedia to trait, providing critical insights that could potentially influence pregnancy outcomes. [4]

Case study: A couple undergoes thalassemia carrier screening before giving birth

1. Post-test genetic counselling: low-risk and regular follow-up

	Mother	Father
Complete blood count	Anemia (Low MCH and MCV)	Normal
PCR test	β-thalassemia carrier ($\alpha\alpha$ /$\alpha\alpha$; $\beta^{CD71/72}/\beta^{N}$)	α-thalassemia carrier ($-\alpha^{3.7}$ /$\alpha\alpha$; β^{N} /β^{N})

2. However, their baby shows severe thalassemia symptoms at 8 months old.

- PCR results show baby genotype is concordant with parental genotypes
- Parents are also different types of thalassemia carrier

Question: Why is the baby exhibiting thalassemia symptoms when the genotype suggests he should not?

3. After having an advanced CATSA test, it is clear that **the incorrect results from the father led to misdiagnosis**.

	Father	Baby
PCR test	$-\alpha^{3.7}$ /$\alpha\alpha$; β^{N} /β^{N}	$-\alpha^{3.7}/\alpha\alpha$; $\beta^{CD71/72}/\beta^{N}$
CATSA	$-\alpha^{3.7}/\alpha\alpha\alpha^{anti3.7}$; β^{N}/β^{N}	$-\alpha^{3.7}/\alpha\alpha\alpha^{anti3.7}$; $\beta^{CD71/72}/\beta^{N}$

This complex case of β-thalassemia heterogeneous ($\beta^{CD71/72}/\beta^{N}$) with α-triplicate ($\alpha\alpha\alpha^{anti3.7}$) enlarge the imbalance of α/β globin ratio, leading to β-thalassemia intermedia.

- The previous tests cannot detect α-triplicate ($\alpha\alpha\alpha^{anti3.7}$)
- This couple has 25% chance of getting β-thalassemia intermedia offspring

References: [1] He J, Song W, Yang J, et al. Next-generation sequencing improves thalassemia carrier screening among premarital adults in a high prevalence population: the Dai nationality, China. *Genet Med*. 2017;19(9):1022-1031. [2] Liang Q, Gu W, Chen P, et al. A More Universal Approach to Comprehensive Analysis of Thalassemia Alleles (CATSA). *J Mol Diagn*. 2021;23(9):1195-1204. [3] Huang R, Liu Y, Xu J, et al. Back-to-Back Comparison of Third-Generation Sequencing and Next-Generation Sequencing in Carrier Screening of Thalassemia. *Arch Pathol Lab Med*. 2024;148(7):797-804. [4] Liang Q, He J, Li Q, et al. Evaluating the Clinical Utility of a Long-Read Sequencing-Based Approach in Prenatal Diagnosis of Thalassemia. *Clin Chem*. 2023;69(3):239-250.
INT_TGSThalav1.11