Whitepaper

Meeting modern demands: How NGS can help overcome thalassemia testing challenges

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Thalassemia is an inherited blood disorder with a worldwide prevalence of nearly 300,000 people diagnosed per year.[1] As the most common hemoglobinopathy, it is a major cause of disability and premature mortality around the world. However, if the disease is diagnosed correctly, treatment can begin promptly, which can help prevent permanent damage caused by excessive iron build-up and provide patients with a better quality of life.

The overall incidence of thalassemia has decreased due to successful prevention programs in areas of high prevalence. However, migration and the resulting shifting demographics have seen the disease occurring in regions not previously endemic.[2] The discovery of more variants makes it challenging for clinicians and laboratories to diagnose and manage appropriately. These regions would benefit from a test that provided comprehensive genetic profiling with an efficient workflow. This whitepaper details how next-generation sequencing (NGS) can be used to help overcome these challenges and provide fast and accurate thalassemia diagnosis.

Thalassemia – a global health challenge

Thalassemia is a blood disorder that affects hemoglobin, the molecule responsible for transporting oxygen. Normal adult hemoglobin (HbA) consists of two α-globin and two β-globin chains, with the genes for α-globin located on chromosome 16 and β-globin on chromosome 11.[3] Mutations in the globin genes can lead to faulty production or absence of protein chains, resulting in thalassemia. Alpha and beta thalassemia are characterized by reduced or absent production of alpha or beta globin chains, which are essential hemoglobin components. The clinical symptoms of these conditions can vary widely depending on the severity of the genetic mutation and the resultant impact on hemoglobin production.

Alpha thalassemia

Mutations in the alpha globin genes cause alpha thalassemia. The severity of the condition depends on how many of the four alpha-globin genes are affected.

The forms of alpha thalassemia and symptoms based on the number of globin genes affected

Number of globin genes affected	Condition	Symptoms
1	Silent carrier	- Typically, asymptomatic - Normal hemoglobin levels and red blood cell indices
2	Alpha thalassemia trait	- Mild microcytic anemia - Often asymptomatic but may experience mild anemia symptoms such as fatigue
3	Hemoglobin H disease	- Moderate to severe microcytic anemia - Fatigue, jaundice, splenomegaly, hepatomegaly - Bone deformities in the face and other parts of the body due to marrow expansion
4	Alpha thalassemia major	- Severe, often fatal condition - Severe anemia, edema, heart failure, intrauterine death

Beta thalassemia

Beta thalassemia results from mutations in the beta globin genes, leading to reduced or absent beta globin chain production. The severity depends on whether one or both genes are affected and the nature of the mutations.

The forms of beta thalassemia and symptoms based on the number of globin genes affected

Number of globin genes affected	Condition	Symptoms
1	Beta thalassemia minor (carrier)	- Mild microcytic anemia - Often asymptomatic but may experience mild anemia symptoms such as fatigue
2 (less severe mutations)	Beta thalassemia intermedia	- Moderate anemia - Fatigue, pallor, jaundice, splenomegaly, bone deformities, growth retardation in children, leg ulcers
2 (more severe mutations)	Beta thalassemia major	- Severe anemia presenting in early childhood - Severe fatigue and weakness, pallor, pronounced jaundice, splenomegaly and hepatomegaly, bone deformities, growth retardation and delayed puberty, iron overload due to frequent blood transfusions, complications such as liver, heart, and endocrine disorders

The economic cost

Aside from impacting quality of life, thalassemia has a significant economic cost across a lifetime of care.

- A study in Israel estimated the cost of 50 years of treatment for beta thalassemia was 1 971 380 USD. However, the cost of preventing beta thalassemia in a single newborn was 63 330 USD.[4]
- In Greece, the estimated annual cost of treating and managing a single patient with beta thalassemia is 32 564 EUR.[5]
- A 2016 study from the UK estimated a cost of 720 201 USD over 50 years of treatment for beta thalassemia.[6]

Shifting population patterns

Mediterranean countries have a long tradition of thalassemia testing and prevention due to the high prevalence of carriers. For example, since starting in 1973, Cyprus' screening policy has significantly reduced the number of children born with the disease.[2] Areas of Southeast Asia also have a high prevalence of carriers, with the largest indigenous group of Malaysia consisting of 33.6% alpha and 12.8% beta carriers.[7] Emigration from countries with high thalassemia prevalence to those with low prevalence is will have a marked impact on the prevalence of the disease.

One of the most poignant examples of changing thalassemia patterns is hemoglobin E-beta-thalassemia. HbE-beta thalassemia is a very common hemoglobinopathy in Southeast Asia, with an incidence approaching 60%.[8] Due to the high prevalence of the condition, thalassemia testing and preventive strategies are well-established in the region.

However, in recent years, HbE-beta thalassemia has emerged thousands of miles away from Southeast Asia. Due to increased migration from Asia, beta thalassemia prevalence is increasing in North America, requiring the healthcare system to adapt.[2, 9]

Therefore, adapting our systems to get the proper tests and assessments is challenging. From initial contact through laboratory to management, thalassemia patients benefit enormously from rapid and accurate diagnosis. Understanding that patterns are changing and preparing for that change is the most critical step we can take to simplify our workflows and improve outcomes.

We need more comprehensive thalassemia testing

Thalassemia patients' survival and quality of life depend on accurate and timely testing. Comprehensive genetic testing for alpha and beta thalassemia can be long and arduous, with various methods needed to reach a clinical diagnosis.

Furthermore, clinical laboratories' workloads are increasing. In the absence of proportional staffing increases, workflow efficiencies must be implemented. As migration rises worldwide, so do genetic diseases in previously non-endemic regions. Variant analysis of thalassemia patients has traditionally relied upon population data. However, this method becomes less reliable when the population changes significantly.

The state of testing

With over 1800 mutations already associated with thalassemia and the emergence of new variants constantly being discovered, thalassemia testing is becoming more complex.[10] Technologies to determine thalassemia variants are often multi-step, complex, and require extensive staff training. Different thalassemia analysis tests are typically used for alpha and beta thalassemia. MLPA, GAP-PCR, and Sanger sequencing are three of the most popular methods used for thalassemia testing. Performing GAP-PCR or Sanger sequencing tests means that each variant must be tested separately and compared to a thalassemia database. Most laboratories have complicated workflows and complex analyses, resulting in long turnaround times.

GAP-PCR and Sanger sequencing have several significant drawbacks that limit their clinical utility in providing fast and efficient patient diagnoses.

Disadvantages of GAP-PCR

- **Limited detection scope:** GAP-PCR is designed to detect specific known deletions or insertions. It cannot identify other types of mutations, such as point mutations, small indels, or complex rearrangements.[11]

- **Target specificity:** Prior mutation knowledge is required as GAP-PCR targets specific regions. If the mutation is unknown or rare, it may be missed by GAP-PCR.[11]

- **Lower throughput:** GAP-PCR is generally a low-throughput technique suitable for analyzing only a few samples simultaneously.[12]

- **Labor-intensive and time-consuming:** GAP-PCR involves multiple steps, including designing primers specific to the mutation, amplification, and analysis.[12]

In recent years, HbE-beta thalassemia has emerged thousands of miles away from its traditionally endemic region, Southeast Asia

Disadvantages of Sanger sequencing

- **Lower throughput:** Sanger sequencing is a low-throughput method typically used for sequencing individual genes or small regions of the genome. It is not practical for sequencing large regions or whole genomes.[10]
- **Limited multiplexing:** Sanger sequencing usually sequences one DNA fragment at a time, limiting its ability to simultaneously analyze multiple genes or regions.[12]
- **High cost for large quantities:** Sanger sequencing is more expensive than other methods, per base, for large-scale testing.[11]
- **Longer turnaround time for large-scale projects:** While Sanger sequencing is accurate and reliable for small-scale projects, the time required to sequence and analyze larger genome regions is long.[11, 12]
- **Difficulty detecting low-frequency variants:** Sanger sequencing is limited in detecting low-frequency variants within a sample.[11]

NGS, the next step in thalassemia testing

Next-generation sequencing offers several significant advantages over traditional methods like GAP-PCR and Sanger sequencing for thalassemia testing:

- **Comprehensive mutation detection:** NGS can identify a wide range of mutations, including point mutations, small insertions and deletions, and large deletions or duplications within the globin genes. This comprehensive approach is critical for accurately diagnosing the various genetic mutations associated with thalassemia, which often include complex and compound heterozygous mutations.[13]
- **Higher sensitivity and specificity:** NGS provides higher sensitivity and specificity than traditional methods. This results in more accurate detection of common and rare mutations, reducing the likelihood of false-negative or false-positive results. This precision is particularly important for conditions like thalassemia, where accurate mutation characterization is essential for diagnosis and treatment planning.[14, 15]
- **Parallel processing:** NGS allows for the simultaneous analysis of multiple genes and multiple patients in a single run. This high-throughput capability significantly reduces the time and cost associated with genetic testing, making it more efficient than the sequential nature of Sanger sequencing and the limited scope of GAP-PCR.[11, 16]
- **Detection of unanticipated variants:** Unlike targeted approaches such as GAP-PCR, which only identify known mutations, NGS can reveal unexpected or novel variants. This ability to detect new mutations expands our understanding of the genetic basis of thalassemia and improves diagnostic outcomes for patients with atypical presentations.[12, 13]

- **Cost-effectiveness over time:** Although the initial setup for NGS can be expensive, the per-sample cost decreases as more samples are processed. In the long run, NGS becomes more cost-effective, especially when dealing with large-scale screenings or complex genetic conditions like thalassemia.[17]

Overall, NGS represents a powerful tool in the genetic diagnosis of thalassemia, offering detailed insights into the genetic landscape of the disease, which can lead to better patient management and personalized treatment strategies.

Conclusion

The advancements in next-generation sequencing present a transformative approach to tackling the complexities of thalassemia testing. Traditional testing methods like GAP-PCR and Sanger sequencing face sensitivity, specificity, and throughput limitations. As migration patterns alter the global demographic landscape, traditional diagnostic NGS emerges as a superior alternative, offering comprehensive mutation detection, higher accuracy, and the ability to process multiple genes and samples simultaneously. Its capability to uncover novel variants and its long-term cost-effectiveness further enhance its value in modern healthcare settings. By integrating NGS into clinical practice, healthcare providers can achieve rapid and precise diagnoses, ultimately improving patient outcomes and optimizing treatment strategies for thalassemia across diverse populations. This shift is crucial for adapting to the evolving prevalence of thalassemia and ensuring effective disease management in previously non-endemic regions.

References

1. GBD 2017 Disease and Injury Incidence and Prevalence Collaborators (2018). Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet (London, England), 392(10159), 1789–1858. https://doi.org/10.1016/S0140-6736(18)32279-7

2. Kattamis, A., Forni, G. L., Aydinok, Y., & Viprakasit, V. (2020). Changing patterns in the epidemiology of β-thalassemia. European Journal of Haematology, 105(6), 692-703. https://doi.org/10.1111/ejh.13512

3. Taher, A. T., Weatherall, D. J., & Cappellini, M. D. (2018). Thalassaemia. Lancet (London, England), 391(10116), 155–167. https://doi.org/10.1016/S0140-6736(17)31822-6

4. Koren, A., Profeta, L., Zalman, L., Palmor, H., Levin, C., Zamir, R. B., Shalev, S., & Blondheim, O. (2014). Prevention of β Thalassemia in Northern Israel - a Cost-Benefit Analysis. Mediterranean journal of hematology and infectious diseases, 6(1), e2014012. https://doi.org/10.4084/MJHID.2014.012

5. Geitona, M., Karagianni, V., Kattamis, A., Voskaridou, E., Drosou, M., Vini, D., & Kalogeropoulou, M. (2014). The Economic Burden of Treating Thalassemia In Greece. Value in health : the journal of the International Society for Pharmacoeconomics and Outcomes Research, 17(7), A526. https://doi.org/10.1016/j.jval.2014.08.1661

6. Weidlich, D., Kefalas, P., & Guest, J. F. (2016). Healthcare costs and outcomes of managing β-thalassemia major over 50 years in the United Kingdom. Transfusion, 56(5), 1038–1045. https://doi.org/10.1111/trf.13513

7. Anne Tan, A. M., Lee, C., Wee, C., Tan, L., Mahali, N. F., George, E., & Chua, H. (2010). High Prevalence of Alpha- and Beta-Thalassemia in the Kadazandusuns in East Malaysia: Challenges in Providing Effective Health Care for an Indigenous Group. Journal of Biomedicine and Biotechnology, 2010. https://doi.org/10.1155/2010/706872

8. Fucharoen, S., & Winichagoon, P. (1992). Thalassemia in SouthEast Asia: problems and strategy for prevention and control. The Southeast Asian journal of tropical medicine and public health, 23(4), 647–655.

9. Hoppe C. C. (2009). Newborn screening for non-sickling hemoglobinopathies. Hematology. American Society of Hematology. Education Program, 19–25. https://doi.org/10.1182/asheducation-2009.1.19

10. Munkongdee, T., Chen, P., Winichagoon, P., Fucharoen, S., & Paiboonsukwong, K. (2020). Update in Laboratory Diagnosis of Thalassemia. Frontiers in molecular biosciences, 7, 74. https://doi.org/10.3389/fmolb.2020.00074

11. Sabath D. E. (2023). The role of molecular diagnostic testing for hemoglobinopathies and thalassemias. International journal of laboratory hematology, 45 Suppl 2, 71–78. https://doi.org/10.1111/ijlh.14089

12. Vijian, D., Wan Ab Rahman, W. S., Ponnuraj, K. T., Zulkafli, Z., & Mohd Noor, N. H. (2021). Molecular Detection of Alpha Thalassemia: A Review of Prevalent Techniques. Medeniyet medical journal, 36(3), 257–269. https://doi.org/10.5222/MMJ.2021.14603

13. Achour, A., Koopmann, T. T., Baas, F., & Harteveld, C. L. (2021). The Evolving Role of Next-Generation Sequencing in Screening and Diagnosis of Hemoglobinopathies. Frontiers in physiology, 12, 686689. https://doi.org/10.3389/fphys.2021.686689

14. Aliyeva, G., Asadov, C., Mammadova, T., Gafarova, S. & Abdulalimov, E. (2019). Thalassemia in the laboratory: pearls, pitfalls, and promises. Clinical Chemistry and Laboratory Medicine (CCLM), 57(2), 165-174. https://doi.org/10.1515/cclm-2018-0647

15. Zhao, J., Li, J., Lai, Q., & Yu, Y. (2020). Combined use of gap-PCR and next-generation sequencing improves thalassaemia carrier screening among premarital adults in China. Journal of clinical pathology, 73(8), 488–492. https://doi.org/10.1136/jclinpath-2019-206339

16. Gupta, N., & Verma, V. K. (2019). Next-Generation Sequencing and Its Application: Empowering in Public Health Beyond Reality. Microbial Technology for the Welfare of Society, 17, 313–341. https://doi.org/10.1007/978-981-13-8844-6_15

17. Suhaimi, S. A., Zulkipli, I. N., Ghani, H., & Abdul-Hamid, M. R. W. (2022). Applications of next generation sequencing in the screening and diagnosis of thalassemia: A mini-review. Frontiers in pediatrics, 10, 1015769. https://doi.org/10.3389/fped.2022.1015769