Received: 6 May 2023 | Revised: 18 August 2023 | Accepted: 7 September 2023

DOI: 10.1002/mgg3.2285

Molecular Genetics & Genomic Medicine Open Access WILEY

CLINICAL REPORT

Clinical application of targeted long read sequencing in prenatal beta-thalassemia testing and genetic counseling

Hui-Lin Chin[1,2] | **Miles C. Benton**[3] | **Lin Yang**[3] | **Kok Siong Poon**[4] | **Karen M. L. Tan**[4] | **Saumya S. Jamuar**[5] | **Roger Foo**[6] | **Hai Yang Law**[7] | **Denise Li-Meng Goh**[1,2] | **Samuel S. Chong**[2,4,8] | **Paola Florez de Sessions**[3]

[1]Division of Genetics and Metabolism, Department of Paediatrics, Khoo Teck Puat-National University Children's Medical Institute, National University Hospital, Singapore, Singapore

[2]Department of Paediatrics, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore

[3]Oxford Nanopore Technologies, Singapore, Singapore

[4]Department of Laboratory Medicine, National University Hospital, Singapore, Singapore

[5]Genetics Service, Department of Paediatrics, KK Women's and Children's Hospital, Singapore, Singapore

[6]Cardiovascular Research Institute, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore

[7]DNA Diagnostic and Research Laboratory, KK Women's and Children's Hospital, Singapore, Singapore

[8]Department of Obstetrics and Gynaecology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore

Correspondence
Hui-Lin Chin, Division of Genetics and Metabolism, Department of Paediatrics, Khoo Teck Puat-National University Children's Medical Institute, National University Hospital, 5 Lower Kent Ridge Road, Singapore 119074, Singapore.
Email: hui-lin_chin@nuhs.edu.sg

Abstract

Background: Beta thalassemia, related to *HBB* mutation and associated with elevated hemoglobin A2 (HbA2), is an important genetic hemoglobinopathy with high incidences of disease and carrier rates in Singapore. Carrier screening is essential to facilitate prenatal counseling and testing. However, when individuals with elevated HbA2 do not have an identifiable *HBB* disease-associated variant, there is ambiguity on risk to their offspring.

Methods: We describe a case report of a proband with elevated HbA2, no identifiable *HBB* disease-associated variant, whose partner was a beta thalassemia carrier. Through clinical *HBB* gene sequencing, multiplex ligation-dependent probe amplification (MLPA) analysis, as well as targeted Nanopore long read sequencing of selected genes, we performed a complete analysis of *HBB* including the promoter region, 5′UTR and coding gene sequence, as well as evaluation for potential modifier variants and other rare structural variants.

Results: This process identified that the proband was heterozygous for *KLF1*:c.544T>C (p.Phe182Leu), a potential functional polymorphism previously known to be associated with benign elevated HbA2 levels. The presence of disease variants in the *HBB* locus was excluded.

Conclusion: This finding provided clarity and enabled family planning for the proband and her family.

KEYWORDS

Beta-thalassemia, Carrier testing, Elevated HbA2, Long read sequencing, Modifier variants

1 | INTRODUCTION

Beta thalassemia is a disease where a mutation in *HBB* (OMIM #141900) results in the production of abnormal hemoglobin, usually inherited in an autosomal recessive manner. The places with the highest reported incidences are Cyprus, Sardinia, and countries in Southeast Asia (Origa, 2021). The prevalence of beta thalassemia in Singapore is 0.9% (Tan et al., 2014). Beta thalassemia major is a severe condition where an affected individual has severe anemia due to decreased or absent β-globin synthesis leading to ineffective erythropoiesis as well as chronic hemolytic anemia. Affected individuals are frequently blood transfusion dependent and are at risk of iron overload which can result in cardiac, endocrine and liver complications. Treatment is by regular blood transfusions, iron chelation therapy or hematopoietic stem cell transplant (Origa, 2021). In spite of treatment, this disease comes with significant morbidity and can seriously impact the quality of life of an affected individual and their family (Lam et al., 2021).

Individuals with heterozygous disease-associated variants in *HBB* are known as beta thalassemia minor or carrier. In Singapore, the frequency of genetic carrier status is estimated to be 9% for both alpha and beta thalassemia (Kham et al., 2004). Carriers are healthy but exhibit thalassemia traits which may include mild microcytic anemia. They are not transfusion dependent and thus do not have iron overload. Their main risk is related to procreation; if two *HBB* carrier individuals procreate, the couple has a 25% risk of conceiving an offspring with biallelic variants manifesting the beta thalassemia major or an intermedia phenotype (Origa, 2021).

Screening and genetic counseling for thalassemia is essential, especially in countries where this disease is prevalent like Singapore (Lee et al., 2019). It enables identification of carriers, prenatal identification of fetuses with thalassemia major, use of preimplantation genetic testing to avoid an offspring with thalassemia major, and allows couples to exert family planning-related choices. In Singapore, screening is performed in several settings, one of which is at the antenatal clinic. In the latter, hemoglobin electrophoresis is performed on pregnant women who have microcytic anemia or if their partners are known to be thalassemia carriers (Lee et al., 2019). Elevated hemoglobin A2 (HbA2) (>3.5%) is used as the cut-off to detect beta thalassemia carriers (Old, 2016). Genotyping via full *HBB* Sanger sequencing and multiplex ligation-dependent probe amplification (MLPA) analysis of the beta globin gene cluster is offered if the hemoglobin electrophoresis is suggestive of beta thalassemia trait, major or intermedia. Genetic counseling and prenatal testing are offered if both partners are found to be thalassemia carriers.

HbA2 levels, however, are also known to be subject to modifiers such as iron deficiency and other genetic factors (Colaco & Nadkarni, 2021; Cyrus et al., 2021). Approximately 0.9% of ethnic Chinese individuals who display elevated HbA2 levels do not carry any identifiable disease-associated variant on *HBB* genetic analysis (Jiang et al., 2018). This presents a diagnostic conundrum and challenges with genetic counseling, especially in the prenatal setting.

Of late, long read sequencing offers promise for increased clinical application toward understanding of genetic conundrums (Mitsuhashi & Matsumoto, 2020). Compared to second-generation sequencing which produces genomic reads of up to 600 bases, long read sequencing generates reads lengths in excess of 10 kb. These longer reads obviate the need for assembly against a reference sequence and allow for de novo assembly, detection of structural variants, evaluation of hard to sequence regions, and improves mapping certainty (Amarasinghe et al., 2020; Mitsuhashi & Matsumoto, 2020).

We describe a female proband found with microcytosis and elevated HbA2 during prenatal thalassemia screening, and first line clinical *HBB* gene analysis returned negative for disease-associated variants. The objective of study was to evaluate for potentially missed *HBB* variants on first line analysis or other findings that could explain the proband's raised HbA2 levels.

2 | METHODOLOGY

2.1 | Editorial Policies and Ethical Considerations

The proband and control participating in this study gave written, informed consent as part of a clinical protocol approved by the SingHealth Centralised Institutional Review Board and NHG Domain Specific Review Board (DSRB) ethics committee.

2.2 | *HBB* sequencing and MLPA

Sequencing analysis was performed on DNA extracted from whole blood for the proband. *HBB* was sequenced from −101 to end of the primary transcript including the entire intron 2 using Sanger sequencing (DNA Diagnostic & Research Laboratory, KK Women and Children's Hospital). Sanger sequencing at a different clinical lab (Centogene, Germany) covered the promoter regions, exons,

5′UTR regions and intron 1 with partial coverage of intron 2. MLPA was completed by both laboratories.

2.3 | Targeted long read sequencing

Targeted long read sequencing was performed on DNA extracted from whole blood from the proband and a control matched for sex, age, and ethnicity. Oxford Nanopore library preparation was performed with ligation sequencing (LSK110) chemistry Long read sequencing was performed on the GridION Mk1 with R9.4.1 MinION flow cells, running MinKNOW 22.03.2. Adaptive sampling is a pore level enrichment method that does not require amplification, only a BED format file of genomic targets. A panel of 100 genes previously associated with thalassemia or thalassemia-like phenotypes were targeted and are provided in Supplemental File 1, including *HBB, HBA1, HBA2*, and *HBD*. The BED file includes genomic coordinates inclusive of 30 kb padding on either side of the genes of interest (a total of 0.34% of the genome was targeted). The human reference genome used was GRCh38 (specifically GCA_000001405.15). Raw fast5 data were basecalled with Guppy 6.2.11 using the super accuracy model (SUP) with modified bases called using Remora, and alignment with minimap2 against GRCh38 (version as above). All bioinformatic processing and analysis were carried out using the available ONT EPI2ME Labs workflows. For SNV, indel and structural variant identification the wf-human-variation workflow v0.2.2 (https://github.com/epi2me-labs/wf-human-variation) was used. Additionally, copy number variant (CNV) analysis was performed using the wf-cnv workflow v0.0.1 (https://github.com/epi2me-labs/wf-cnv) An overview of the complete workflow is provided in Supplementary Figure S1.

3 | CASE REPORT

A 30-year-old female proband and her husband were referred to the Genetics clinic for prenatal counseling in view of the familial risk of thalassemia. Both are ethnic Chinese from South East Asia and non-consanguineous. At the time, the proband was at 19 + 2 weeks gestation of an otherwise uncomplicated first pregnancy. She had no significant past medical history and was not on chronic medications. The proband had no significant family history of hematological disorders. She was found on antenatal screening full blood count to have microcytosis. Her hemoglobin was 11.7 g/dL (normal range 11.4–14.7), mean corpuscular volume was 78.6 fL (normal range 83–95.5), mean corpuscular hemoglobin 26.6 pg (normal range 26.5–31.5), and mean corpuscular hemoglobin concentration 33.1 g/dL (normal range 30.8–34.2). Her Mentzer index was 16.7. Subsequent hemoglobin electrophoresis showed that she had an elevated HbA2 fraction (5.2%) (normal range 1.7–3.2), normal HbF (0.9%) (normal range 0–1.0), and HbH inclusion bodies were not detected. Iron studies excluded the possibility of iron deficiency. Based on these findings she was thought to be a beta thalassemia carrier.

Her husband is healthy and known to be a beta thalassemia carrier with a family history of beta thalassemia trait. On hemoglobin electrophoresis, he had an elevated HbA2 fraction (5.4%) (normal range 1.7–3.2), normal HbF (1.0%) (normal range 0–1.0), and HbH inclusion bodies were not detected. Thalassemia genotyping showed that he is heterozygous for *HBB* (NM_000518.5):c.-78A>G, a known prevalent pathogenic variant in the ethnic Chinese population (Li et al., 2020).

Proband genotyping with *HBB* Sanger sequencing and MLPA analysis returned negative. Based on the clinical impression, even though the proband's variant was not identified, the couple were counseled on the possible 25% risk of an offspring with beta thalassemia major. However, prenatal testing to identify a fetus with beta thalassemia major was not possible without knowledge of both parental variants. With this, the couple decided to carry on with the pregnancy.

The proband delivered a healthy male Infant at term gestation. At 6 months of age, he was evaluated and found to also have microcytosis and elevated HbA2 fraction. He had Hb 12 g/dL (normal range 10.1–12.9), MCV 61 fL (normal range 73–109), HbA2 7.1% (normal range 2.1–3.3), and HbF 12.4% (normal range <1.4). Iron studies were normal. These indices were suggestive of beta thalassemia carrier status. By the age of 2 years, this child remained healthy with normal growth and development. The family declined genotyping and further investigation for him.

Several years after their initial visit to the Genetics clinic, the couple returned seeking further analysis to identify the maternal thalassemia variant. To facilitate this, we first concurrently re-reviewed the previous sequencing data and pursued repeat clinical *HBB* analysis. We consequently performed research-based targeted long read sequencing of selected hematology genes, hypothesizing that this method could enable exclusion of rare structural variants, deep intronic or upstream promoter variants in *HBB* and concurrently evaluate for other modifier variants that could shed light on the proband's phenotype which would in turn facilitate genetic counseling for the family.

4 | RESULTS

A thorough re-analysis of the prior sequencing and MLPA data revealed no disease-associated variants. Repeat

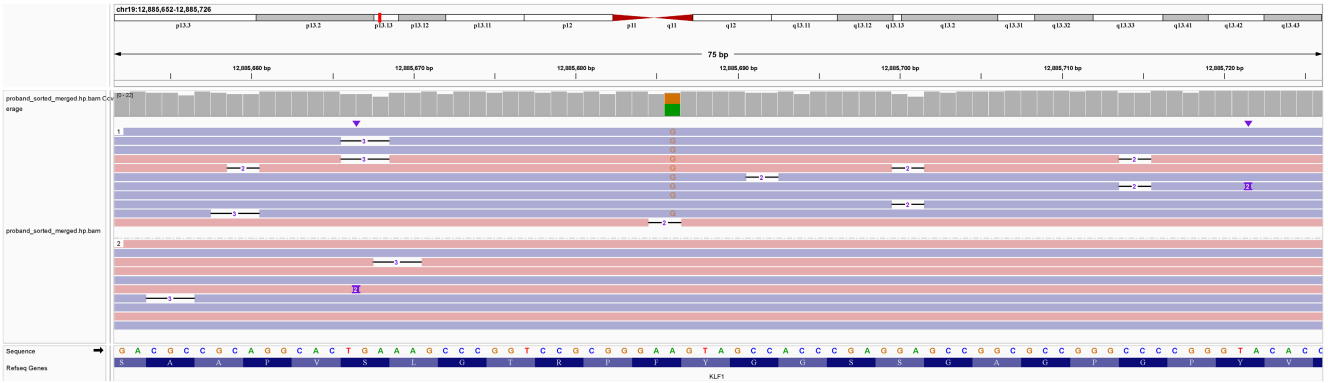

FIGURE 1 IGV view of *the KLF1:c.544T>C (p.Phe182Leu)* variant identified in the proband using targeted Nanopore long read sequencing.

sequencing and MLPA analysis of *HBB* at a different laboratory also returned negative.

Long read sequencing analysis characterized the β-globin gene cluster and excluded any disease-associated single nucleotide variants, copy number variants (Supplemental Figure 2), and structural variants for beta thalassemia. Analysis of other thalassemia-associated genes identified that the proband was heterozygous for *KLF1(*NM_006563.5):c.544T>C, p.(Phe182Leu) (Figure 1). This variant, while classified as Benign for congenital dyserythropoietic anemia type IV (Clinvar RCV000320797.3), has been previously associated with elevated HbA2 levels in individuals without thalassemia and could be considered a potential functional polymorphism (Borgio et al., 2018, p. 1; Liu et al., 2014, p. 1; Zaker-Kandjani et al., 2015). Its global minor allele frequency is 0.04952 (Clinvar RCV000320797.3), suggesting around 5% of the population carry this variant and may potentially have elevated HbA2 on screening.

In addition to the above, we observed that we were able to obtain genome-wide CNV profiles from the low coverage whole genome that is a by-product of adaptive sampling. This also allowed us to exclude significant CNVs in the proband (Supplemental Figure S2). A summary of other rare variants identified is included in Supplemental Table S1. Other variation potentially contributing to an elevated HbA2 level such as α-globin triplication were not identified.

5 | DISCUSSION

HbA2 is a tetramer of two α- and two δ-globin chains whose levels are typically elevated in beta thalassemia carriers. Elevated HbA2 (>3.5%) is an established test used to screen for beta thalassemia (Steinberg & Adams, 1991). HbA2 levels in beta thalassemia carriers can be lowered by certain modifiers, including iron deficiency and co-inheritance of beta thalassemia with alpha or delta thalassemia (Colaco & Nadkarni, 2021). Conversely, in recent years, β-globin gene expression modifier variants in *KLF1*, *HBG2, HBBP1, OR51B6* have been reported to be associated with elevated HbA2 levels (Cyrus et al., 2021).

KLF1 (OMIM #600599) is an erythroid-specific transcription factor that is crucial in erythropoiesis and hemoglobin switching (Caria et al., 2022). It is known that *KLF1* influences β-globin expression; *Klf1*-deficient mice showed silencing in β-globin expression (Perkins et al., 1995). Reduced *KLF1* expression results in reduced *HBB* expression and therefore increased competitive interaction with *HBD* at the β-globin locus control region. This increases the δ-globin gene expression and consequent increase in HbA2 (Caria et al., 2022). Supporting this observation, exon 2 nonsense, frameshift and missense variants, some within the zinc-finger domains in *KLF1* have been associated with elevated HbA2 levels (Perseu et al., 2011). Studies also showed that 35.9% of a Sardinian cohort, 7.6% of an Indian cohort and 2.7% of an Italian cohort with borderline elevated HbA2 levels were heterozygotes for *KLF1* variation (Colaco & Nadkarni, 2021; Hariharan et al., 2019; Perseu et al., 2011).

In our proband, Nanopore long read sequencing enabled evaluation for and exclusion of rare structural variants, deep intronic or upstream promoter variants in *HBB*, with concurrent evaluation for potential modifier variants that could influence the proband phenotype. Long read sequencing additionally allowed for accurate examination of loci with high homology such as between *HBB* and *HBD*, as well as between *HBA1* and *HBA2*, which are typically challenging with short read technologies. The thorough evaluation of *HBB* and exclusion of disease-associated variation at both the α- and β-globin gene clusters, as well as identification of a

potential functional polymorphism in a β-globin expression regulator gene in this proband gave confidence for counseling the proband and her husband on their unlikely risk of an offspring with beta thalassemia major. Her identified *KLF1* variant has previously been associated with elevated HbA2 levels and likely influenced her elevated HbA2 levels in the absence of *HBB* variation. The authors acknowledge that this observation could be improved by studying larger populations and findings supported by functional studies evaluating how *KLF1* variation influences HbA2 levels. There may be other genetic determinants or modifiers outside of the genomic regions targeted and not investigated for that could contribute to the proband phenotype as well.

This case highlights the complexity of thalassemia screening. 1.5% of the world's population carries a thalassemia gene mutation (Williams & Weatherall, 2012) and the American College of Obstetrics and Gynecology (ACOG) recognizes that patients with African, Mediterranean, and Southeast Asian ancestry are at increased risk for hemoglobinopathies, including sickle cell and thalassemia ("ACOG Practice Bulletin No. 78", 2007), recommending prenatal or preconception screening. Current practice screens individuals for elevated HbA2 obtained via hemoglobin electrophoresis as a marker for beta thalassemia carrier status (Steinberg & Adams, 1991). Some individuals with elevated HbA2 levels do not have β-globin disease-variants and would therefore get a false positive screen result. For example in ethnic Chinese, this frequency is estimated at 0.9% (Jiang et al., 2018), The possibility of both genetic and non-genetic modifiers influencing hemoglobin electrophoresis findings needs to be considered when conducting population wide thalassemia carrier screening as these significantly influence genetic counseling and family planning-related decisions. This case also demonstrates the utility of Nanopore long read sequencing and its potential for future integration into clinical practice (Snell et al., 2023).

In summary, we demonstrate the use of long read sequencing in conjunction with clinical sequencing methods in evaluating a proband with elevated HbA2 levels and identification of a potential functional polymorphism influencing the phenotype which facilitated prenatal genetic counseling.

AUTHOR CONTRIBUTIONS

Hui-Lin Chin, Miles C. Benton, Lin Yang, Paola Florez de Sessions: Conceptualization, Methodology, Writing – original draft. **Kok Siong Poon, Karen M. L. Tan**: Methodology, Writing – review and editing. **Saumya S. Jamuar, Roger Foo, Hai Yang Law, Denise Li-Meng Goh, Samuel S. Chong**: Conceptualization, Methodology, Writing – review and editing.

ACKNOWLEDGMENTS

The authors would like to acknowledge Dr Benedict Yan, Department of Laboratory Medicine, National University Hospital, Singapore and Dr Swee Lay Thein, National Heart, Lung and Blood Institute, National Institutes of Health, USA for their advice and contributions to this case.

CONFLICT OF INTEREST STATEMENT

P.F.d.S., L.Y., and M.C.B., are full-time employees of, and share/phantom shareholders in, Oxford Nanopore Technologies and Oxford Nanopore provided certain flow cells and services in support of the research. Oxford Nanopore Technologies products are intended for molecular biology applications and are not intended for diagnostic purposes.

DATA AVAILABILITY STATEMENT

ClinVar accession RCV000320797.3; The data that support the findings of this study are available from the corresponding author upon request.

ORCID

Hui-Lin Chin https://orcid.org/0000-0001-7431-6794
Kok Siong Poon https://orcid.org/0000-0002-0873-2682

REFERENCES

ACOG Committee on Obstetrics. (2007). ACOG practice bulletin No. 78 Hemoglobinopathies in pregnancy. *Obstetrics & Gynecology*, *109*(1), 229–238. https://doi.org/10.1097/00006250-200701000-00055

Amarasinghe, S. L., Su, S., Dong, X., Zappia, L., Ritchie, M. E., & Gouil, Q. (2020). Opportunities and challenges in long-read sequencing data analysis. *Genome Biology*, *21*(1), 30. https://doi.org/10.1186/s13059-020-1935-5

Borgio, J. F., AbdulAzeez, S., Al-Muslami, A. M., Naserullah, Z. A., Al-Jarrash, S., Al-Suliman, A. M., Al-Madan, M. S., & Al-Ali, A. K. (2018). KLF1 gene and borderline hemoglobin A2 in Saudi population. *Archives of Medical Science: AMS*, *14*(1), 230–236. https://doi.org/10.5114/aoms.2018.72245

Caria, C. A., Faà, V., & Ristaldi, M. S. (2022). Krüppel-like factor 1: A pivotal gene regulator in erythropoiesis. *Cell*, *11*(19), 3069. https://doi.org/10.3390/cells11193069

Colaco, S., & Nadkarni, A. (2021). Borderline HbA2 levels: Dilemma in diagnosis of beta-thalassemia carriers. *Mutation Research. Reviews in Mutation Research*, *788*, 108387. https://doi.org/10.1016/j.mrrev.2021.108387

Cyrus, C., Vatte, C., Chathoth, S., Sayed, A. A., Borgio, J. F., Alrubaish, M. A., Alfalah, R., Alsaikhan, J., & Al Ali, A. K. (2021). Haemoglobin switching modulator SNPs rs5006884 is associated with increased HbA2 in *β*-thalassaemia carriers. *Archives of Medical Science*, *17*(4), 1064–1074. https://doi.org/10.5114/aoms.2019.86705

Hariharan, P., Colah, R., Ghosh, K., & Nadkarni, A. (2019). Differential role of Kruppel like factor 1 (KLF1) gene in red blood cell disorders. *Genomics*, *111*(6), 1771–1776. https://doi.org/10.1016/j.ygeno.2018.11.032

Jiang, F., Chen, G.-L., Li, J., Zhou, J.-Y., Liao, C., & Li, D.-Z. (2018). Analysis of the genotypes in a Chinese population with increased Hb A2 and low hematological indices. *Hemoglobin*, *42*(3), 154–158. https://doi.org/10.1080/03630269.2018.1509869

Kham, S. K., Quah, T. C., Loong, A. M., Tan, P. L., Fraser, A., Chong, S. S., & Yeoh, A. E. (2004). A molecular epidemiologic study of thalassemia using newborns' cord blood in a multiracial Asian population in Singapore: Results and recommendations for a population screening program. *Journal of Pediatric Hematology/Oncology*, *26*(12), 817–819.

Lam, J. C. M., Lee, S. Y., Koh, P. L., Fong, S. Z., Abdul-Kadir, N. I., Lim, C. Y., Zhang, X., Bhattacharyya, R., Soh, S. Y., Chan, M. Y., Tan, A. M., Kuperan, P., & Ang, A. L. (2021). Clinical and health-related quality of life outcomes of transfusion-dependent thalassaemia patients in Singapore. *Blood Cells, Molecules, and Diseases*, *88*, 102547. https://doi.org/10.1016/j.bcmd.2021.102547

Lee, S. Y., Yap, E. S., Lee, E. Y., Goh, J. H., Liu, T. C., & Yip, C. (2019). Evaluation of thalassaemia screening tests in the antenatal and non-antenatal populations in Singapore. *Annals of the Academy of Medicine, Singapore*, *48*(1), 5–15.

Li, J., Zhou, Z., Sun, H.-X., Ouyang, W., Dong, G., Liu, T., Ge, L., Zhang, X., Liu, C., & Gu, Y. (2020). Transcriptome analyses of *β*-thalassemia −28(a>G) mutation using isogenic cell models generated by CRISPR/Cas9 and asymmetric single-stranded oligodeoxynucleotides (assODNs). *Frontiers in Genetics*, *11*, 577053. https://doi.org/10.3389/fgene.2020.577053

Liu, D., Zhang, X., Yu, L., Cai, R., Ma, X., Zheng, C., Zhou, Y., Liu, Q., Wei, X., Lin, L., Yan, T., Huang, J., Mohandas, N., An, X., & Xu, X. (2014). KLF1 mutations are relatively more common in a thalassemia endemic region and ameliorate the severity of *β*-thalassemia. *Blood*, *124*(5), 803–811. https://doi.org/10.1182/blood-2014-03-561779

Mitsuhashi, S., & Matsumoto, N. (2020). Long-read sequencing for rare human genetic diseases. *Journal of Human Genetics*, *65*(1), 11–19. https://doi.org/10.1038/s10038-019-0671-8

Old, J. (2016). *Prevention and diagnosis of haemoglobinpathies*. Thalassemia international federation TIF Publication No. 21.

Online Mendelian Inheritance in Man, OMIM®. Johns Hopkins University. MIM Number: {141900}: {02/13/2023}. World Wide Web URL: https://omim.org/

Online Mendelian Inheritance in Man, OMIM®. Johns Hopkins University. MIM Number: {600599}: {02/11/2014}. World Wide Web URL: https://omim.org/

Origa, R. (2021). Beta-thalassemia. In M. P. Adam, D. B. Everman, G. M. Mirzaa, et al. (Eds.), *GeneReviews® [Internet]*. University of Washington, Seattle; 1993–2022. https://www.ncbi.nlm.nih.gov/books/NBK1426/

Perkins, A. C., Sharpe, A. H., & Orkin, S. H. (1995). Lethal beta-thalassaemia in mice lacking the erythroid CACCC-transcription factor EKLF. *Nature*, *375*(6529), 318–322. https://doi.org/10.1038/375318a0

Perseu, L., Satta, S., Moi, P., Demartis, F. R., Manunza, L., Sollaino, M. C., Barella, S., Cao, A., & Galanello, R. (2011). KLF1 gene mutations cause borderline HbA(2). *Blood*, *118*(16), 4454–4458. https://doi.org/10.1182/blood-2011-04-345736

Snell, L. B., Alcolea-Medina, A., Charalampous, T., Alder, C., Williams, T. G. S., Flaviani, F., Batra, R., Bakrania, P., Thangarajah, R., Neil, S. J. D., van Nispen Tot Pannerden, C., Botgros, A., Aarons, E., Douthwaite, S. T., Edgeworth, J. D., & Nebbia, G. (2023). Real-time whole genome sequencing to guide patient-tailored therapy of severe acute respiratory syndrome coronavirus 2 infection. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*, *76*(6), 1125–1128. https://doi.org/10.1093/cid/ciac864

Steinberg, M. H., & Adams, J. G. (1991). Hemoglobin A2: Origin, evolution, and aftermath. *Blood*, *78*(9), 2165–2177.

Tan, E. S., Koh, C., Law, H. Y., Tan, G. P., Lai, A. H. M., & Ng, I. S. L. (2014). Haemoglobin E-beta Thalassaemia in Singapore. *Annals of the Academy of Medicine, Singapore*, *43*(6), 331–333.

Williams, T. N., & Weatherall, D. J. (2012). World distribution, population genetics, and health burden of the hemoglobinopathies. *Cold Spring Harbor Perspectives in Medicine*, *2*(9), a011692. https://doi.org/10.1101/cshperspect.a011692

Zaker-Kandjani, B., Namdar-Aligoodarzi, P., Azarkeivan, A., Najmabadi, H., & Banan, M. (2015). Mutation screening of the Krüppel-like factor 1 gene using single-strand conformational polymorphism in a cohort of Iranian *β*-thalassemia patients. *Hemoglobin*, *39*(1), 24–29. https://doi.org/10.3109/03630269.2014.991023

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Chin, H.-L., Benton, M. C., Yang, L., Poon, K. S., Tan, K. M. L., Jamuar, S. S., Foo, R., Law, H. Y., Goh, D.-M., Chong, S. S., & de Sessions, P. F. (2024). Clinical application of targeted long read sequencing in prenatal beta-thalassemia testing and genetic counseling. *Molecular Genetics & Genomic Medicine*, *12*, e2285. https://doi.org/10.1002/mgg3.2285