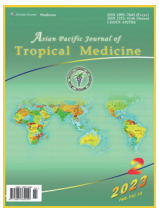

Systematic Review

Asian Pacific Journal of Tropical Medicine

apjtm.org

doi: 10.4103/1995-7645.370147

Impact Factor: 3.041

Application of next-generation sequencing in thalassemia screening: A systematic review and meta-analysis

Xingyi Fang[1,2,3], Yi Gong[2,3,4], Yanlin Ma[1,2,3], Yuanhua Huang[1,2,3]✉

[1]*Hainan Provincial Key Laboratory for Human Reproductive Medicine and Genetic Research, Reproductive Medical Center, International Technology Cooperation Base "China-Myanmar Joint Research Center for Prevention and Treatment of Regional Major Disease" by the Ministry of Science and Technology of China, the First Affiliated Hospital of Hainan Medical University, Hainan Medical University, Haikou 570102, China*

[2]*Key Laboratory of Tropical Translational Medicine of Ministry of Education, Hainan Medical University, Haikou 570102, China*

[3]*Hainan Provincial Clinical Research Center for Thalassemia, the First Affiliated Hospital of Hainan Medical University, Hainan Medical University, Haikou 570102, China*

[4]*Department of Obstetrics and Gynecology, Southern Medical University Nanfang Hospital, Guangzhou 510515, China*

ABSTRACT

Objective: To evaluate the value of next-generation sequencing (NGS) in the prevention and management of thalassemia.

Methods: A systematic search was performed in eight databases including China Biomedical Literature Database, Chinese National Knowledge Infrastructure, Chinese Scientific Journals Database, Wanfang database, PubMed, EMBASE, Web of Science, and Cochrane Library from the inception to 1 June 2022. Stata 17.0 and Review Manager 5.4 were used for the meta-analysis.

Results: Nine studies containing 14 794 participants were included in the meta-analysis. Compared with the routine genetic testing (including Gap-PCR and reverse dot blot), NGS had higher detection rates in screening thalassemia (*RR* 1.22, 95% *CI* 1.13-1.31, $P<0.01$), particularly for the α-thalassaemia mutation carriers (*RR* 1.24, 95% *CI* 1.07-1.44, $P<0.01$). However, no significant difference was found in the screening of β-thalassemia (*RR* 1.10, 95% *CI* 0.99-1.23, $P>0.05$).

Conclusions: Compared with routine genetic testing, NGS had a higher detection rate in general, particularly in the detection of α-thalassemia.

KEYWORDS: Thalassaemia; Next-generation sequencing; Meta-analysis

1. Introduction

Thalassemia is an inherited single-gene genetic disease, which is described as a set of hemoglobinopathies in which one or more globin peptide chain synthesis disorders are caused by a globin gene deficiency[1]. Thalassemia is a severe health threat that has been reported in 229 countries. A total of 5.2% of the global population, 7% of pregnant women, and 1% of married couples are at risk for thalassemia[2]. Southern China has a high thalassemia prevalence, with α-thalassemia rates ranging from 8% to 15% and β-thalassemia rates ranging from 2% to 11%[3]. Thalassemia is a significant public health issue that is passed from parents to their children, resulting increased health and treatment costs for the family[4]. Countries embrace premarital screening to identify high-risk couples and assist them to produce healthy babies via genetic counseling. The screening a simple and cost-effective way for avoiding the birth of infants with thalassemia[5].

As a reliable and quick way of detecting monogenic illness,

Significance

Next-generation sequencing (NGS) may be more precise than traditional genetic testing. However, it remains unclear how NGS varies from routine genetic testing (Gap-PCR and RDB-PCR) in terms of thalassemia screening outcomes. Unlike other studies, our analysis not only compares the difference between NGS and routine genetic testing in the detection rate of thalassemia, but also investigates their accuracy in different types of thalassemia.

✉To whom correspondence may be addressed. E-mail: huangyh4180@hainmc.edu.cn

How to cite this article: Fang X, Gong Y, Ma Y, Huang Y. Application of next-generation sequencing in thalassemia screening: A systematic review and meta-analysis. Asian Pac J Trop Med 2023; 16(2): 51-57.

Article history: Received 13 October 2022 Revision 14 January 2023
Accepted 21 February 2023 Available online 27 February 2023

next-generation sequencing (NGS) may provide a vast amount of data that can be used to establish gene sequences and assess potential hereditary risks. NGS is a reliable molecular diagnostic tool for α-and β-globin mutation identification since it uses high throughput sequencing for the entire length gene sequence of α- and β-globin gene[6]. Since 2016, NGS has been used extensively in Southern China for molecular screening of thalassemia and prenatal diagnosis. As a first-tier DNA screening method, NGS may be more precise than traditional thalassemia screening, according to preliminary findings. In addition to increasing the sensitivity of carrier detection, this has led to the discovery of novel variations[7]. However, it remains unclear how next-generation sequencing varies from traditional genetic testing (Gap-PCR and RDB-PCR) in terms of thalassemia screening outcomes. In this systematic review and meta-analysis, we attempted to evaluate current available evidences to determine the utility of next-generation sequencing for the prevention and management of thalassemia.

2. Materials and methods

This systematic review was done in compliance with the recommended reporting standards for systematic review and meta-analysis studies. In the International Prospective Register of Systematic Reviews database, the protocol for the systematic review was recorded with the code CRD42022340982.

2.1. Literature search

Eight databases including China Biomedical Literature Database (CBM), Chinese National Knowledge Infrastructure (CNKI), Chinese Scientific Journals Database (VIP), Wanfang database (WF), PubMed, EMBASE, Web of Science (WOS), and Cochrane Library were searched from the inception to 1 June 2022 using Medical Subject Headings and free-text terms. The search words included "Thalassemia", "High Throughput Nucleotide Sequencing", "Next Generation Sequencing", "Next-Generation Sequencing", "Sequencing, High-Throughput Nucleotid", "Sequencing, Next-Generation", and "Deep Sequencing". The complete search strategy of PubMed and CBM is shown in Supplementary Table 1 and Supplementary Table 2.

2.2. Inclusion criteria and study selection

Inclusion criteria including (1) Patients: people screened for thalassemia; (2) Intervention: in the experimental group, NGS technology was used in addition to routine genetic testing, as was done in the control group; (3) Comparison: the people of the control group were given the routine genetic testing [the routine genetic testing included Gap-PCR and reverse dot blot (RDB)]; (4) Outcomes: (a) thalassemia carriers, (b) α-thalassemia carriers, (c) β-thalassemia carriers, (d) composite α and β-thalassemia carriers; (5) Study types: case-control studies or retrospective studies were included. This followings were excluded: (1) repeated published literature; (2) review, case reports and animal experiments; (3) data in the literature is inconclusive and muddled.

2.3. Quality assessment

The data extraction and quality evaluation were conducted by two reviewers in turn. In the event of a dispute, it must be addressed by discussion or consultation with the participation of a third party. The validity of the research included in this analysis was evaluated using the critical appraisal skills program techniques. In order to answer the study question, the data was analyzed and critically assessed in a descriptive manner.

2.4. Statistical analysis

Stata 17.0 and Review Manager 5.4 software were used to analyze the data. The I^2 statistic and the χ^2 test will be used to analyze statistical heterogeneity. If $P \geq 0.05$, $I^2 \leq 50\%$, it is homogeneity of several comparable studies, and a fixed effect model is used for assessment; if $P<0.05$, $I^2>50\%$, it is heterogeneity of numerous studies, and the random effect model will be used for further meta-analysis. Simultaneously, a sensitivity analysis is used to assess the findings' robustness. Additionally, funnel plots will be used to analyze possible reporting bias, and the Egger' test will be used to determine funnel plot asymmetry. The ratio (*RR*) was employed as the combined statistic in this study.

3. Results

3.1. Data collection and analysis

A total of 914 relevant papers were searching in the eight databases. There are 582 papers left after manually deleting the duplicate documents by scanning the title and abstract. The complete text is then read, and the literature is analyzed qualitatively and quantitatively. Finally, 9 articles (one of which was written in English)[8-16] were included (Figure 1). Detailed information of the nine studied were presented in Table 1.

3.2. Overall detection rate of thalassemia

The researches evaluated the effects of NGS on the overall detection rate of thalassemia[8-16]. The heterogeneity among studies was large (I^2=94%, $P<0.001$). We further produced labbe (Figure 2A) and galbraith plots (Figure 2B), as well as performed sensitivity

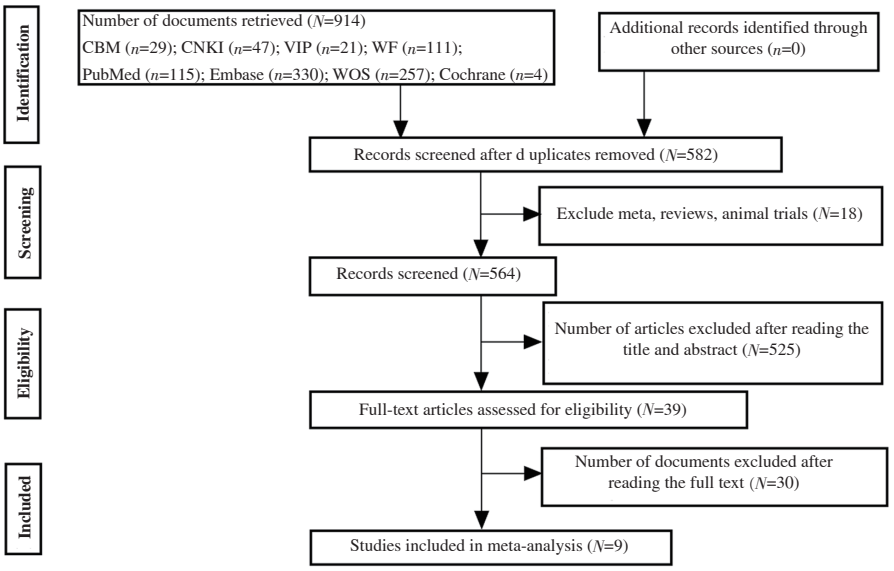

Figure 1. Flowchart for selection of studies.

Table 1. Characteristics of the studies.

First author and year	Reference	Location of study	Number of samples	Next-generation sequencing				Routine genetic testing				Quality assessment
				α-thalassemia	β-thalassemia	αβ-thalassemia	Total	α-thalassemia	β-thalassemia	αβ-thalassemia	Total	
Lun M, *et al.*, 2021	[8]	Shandong, China	50	20	28	-	48	17	23	-	40	High
Luo GX, *et al.*, 2020	[9]	Guangdong, China	219	-	-	-	217	-	-	-	168	High
Ye JX, *et al.*, 2019	[10]	Guangdong, China	1036	74	20	6	100	28	11	2	41	High
Wang K, *et al.*, 2019	[11]	Guangdong, China	2858	691	254	-	945	506	246	-	752	High
Yang YH, *et al.*, 2018	[12]	Guizhou, China	7866	583	254	31	868	437	-	-	704	High
He SZ, *et al.*, 2018	[13]	Guangdong, China	249	-	-	-	247	-	-	-	221	High
He J, *et al.*, 2017	[14]	Yunnan, China	951	290	99	82	471	73	79	57	209	High
Yang Q, *et al.*, 2017	[15]	Guangdong, China	197	-	-	-	28	-	-	-	22	High
Song CL, *et al.*, 2016	[16]	Guangdong, China	1368	313	210	-	523	303	196	-	499	High

analysis (Figure 2C), which showed that when 3 study[10,14,16] was omitted, I^2 dropped from 94% to 75% and a random-effect model was conducted to analyze the data. The conclusion was *RR* 1.22, 95% *CI* 1.13-1.31 (Figure 3). The outcome indicated the detection rate in NGS was 1.22 times higher than routine genetic testing, and the miss rate of routine genetic testing is 22%. The difference was statistically significant (Z=5.02, P<0.01). Using the Funnel and Begg's test to measure publication bias, it was revealed that none of the included papers had publication bias (P=0.324, Figure 2D).

3.3. Detection rate of α–thalassemia

Six studies evaluated the effects of NGS on the detection rate of α-thalassemia[8,10–12,14,16]. The heterogeneity among studies was large (I^2=95%, P<0.01). We further produced labbe (Figure 4A) and galbraith plots (Figure 4B), as well as performed sensitivity analysis (Figure 4C), which showed that when 2 study[10,14] was omitted, I^2 dropped from 95% to 73% and a random-effect model was conducted to analyze the data. The conclusion was: *RR* 1.24, 95% *CI* 1.07-1.44 (Figure 5). The outcome indicated the detection rate of α-thalassemia in NGS was 1.24 times higher than routine genetic testing, and the miss rate of routine genetic testing is 24%. The difference was statistically significant (Z=2.82, P=0.005). Using the Funnel and Begg's test to measure publication bias, it was revealed that none of the included papers had publication bias (P=0.677, Figure 4D).

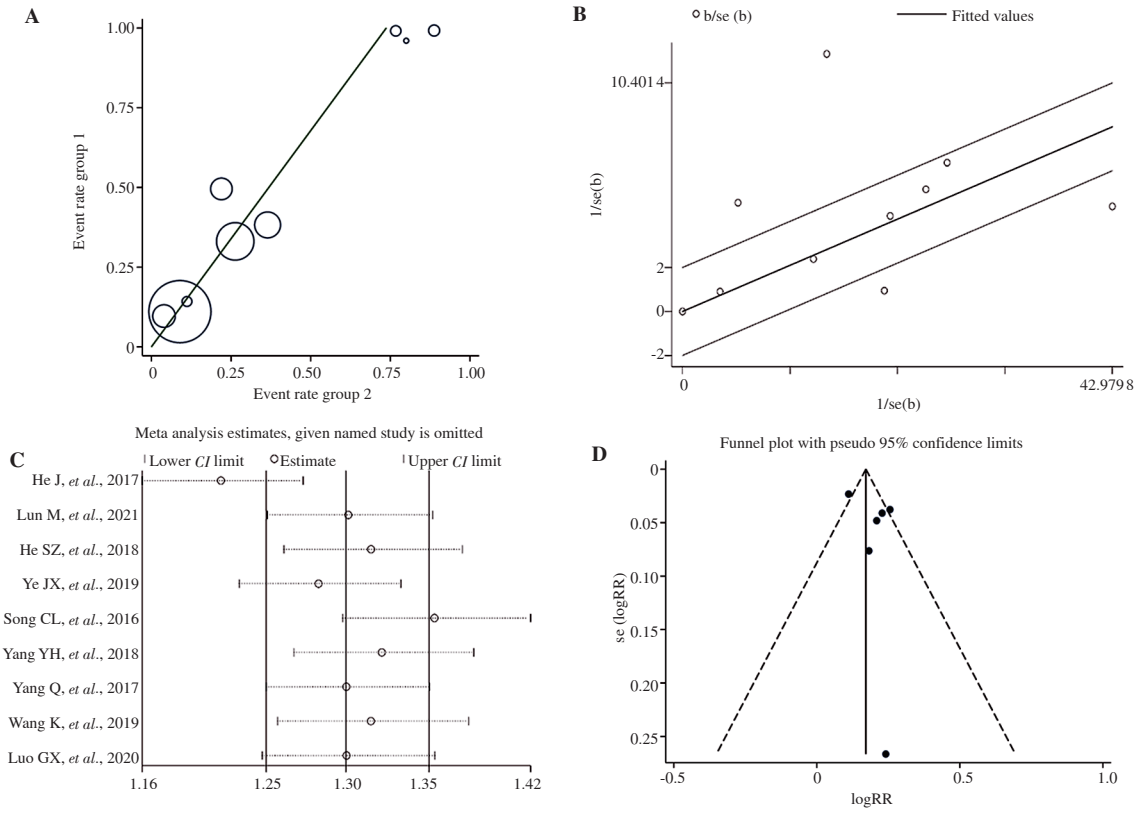

Figure 2. Meta-analysis of overall detection rate of thalassemia between next-generation sequencing and routine genetic testing group. (A) Labbe graph, (B) Galbr test, (C) Sensitivity analysis, and (D) Funnel plot.

	Next-generation sequencing		Routine genetic testing			Risk ratio	Risk ratio
Study or subgroup	Events	Total	Events	Total	Weigh	M-H, Random, 95% *CI*	M-H, Random, 95% *CI*
He SZ *et al*., 2018	247	249	221	249	24.2%	1.12 (1.07, 1.17)	
Lun M *et al*., 2021	48	50	40	50	13.2%	1.20 (1.03, 1.39)	
Luo GX *et al*., 2020	217	219	168	219	21.2%	1.29 (1.20, 1.39)	
Wang K *et al*., 2019	945	2 858	752	2 858	20.5%	1.26 (1.16, 1.36)	
Yang Q *et al*., 2017	28	197	22	197	2.0%	1.27 (0.75, 2.15)	
Yang YH *et al*., 2018	868	7 866	704	7 866	18.9%	1.23 (1.12, 1.35)	
Total (95% *CI*)		11 439		11 439	100.0%	1.22 (1.13, 1.31)	
Total events	2 353		1 907				

Heterogeneity: Tau^2=0.01; Chi^2=19.89, *df*=5 (*P*=0.001); I^2=75%
Test for overall effect: *Z*=5.02 (*P*<0.000 01)

Figure 3. Forest plot of overall detection rate of thalassemia between next-generation sequencing and routine genetic testing group.

3.4. Detection rate of β-thalassemia

Five articles evaluated the effects of NGS on the detection rate of β-thalassemia[8,10,11,14,16]. There was no heterogeneity among studies (I^2<50%, *P*=0.47), and a fixed-effect model was conducted to analyze the data. The conclusion was: *RR* 1.10, 95% *CI* 0.99-1.23 (Figure 6). The outcome indicated the detection rate of β-thalassemia in next-generation sequencing was 1.10 times higher than routine genetic testing, but the difference was not statistically significant (*Z*=1.75, *P*=0.08). From a statistical standpoint, there was no significant difference between NGS and routine genetic testing in the screening of β-thalassemia.

Begg's test revealed probable publication bias among the included studies (*P*=0.015, Figure 7A). We further performed trim-and-fill analysis using the metatrim module in Stata. Before metatrim, the pooled ES for the detection rate of β-thalassemia was 1.10 (95% *CI* 0.99-1.23, *P*=0.47). After metatrim, two more papers were included in the meta-analysis, and the pooled ES for the β-thalassemia detection rate was 1.07 (95% *CI* 0.96-1.18, *P*=0.22). Both the results before and after metatrim are steady and not statistically significant, indicating that publication bias in the current research is negligible (Figure 7B).

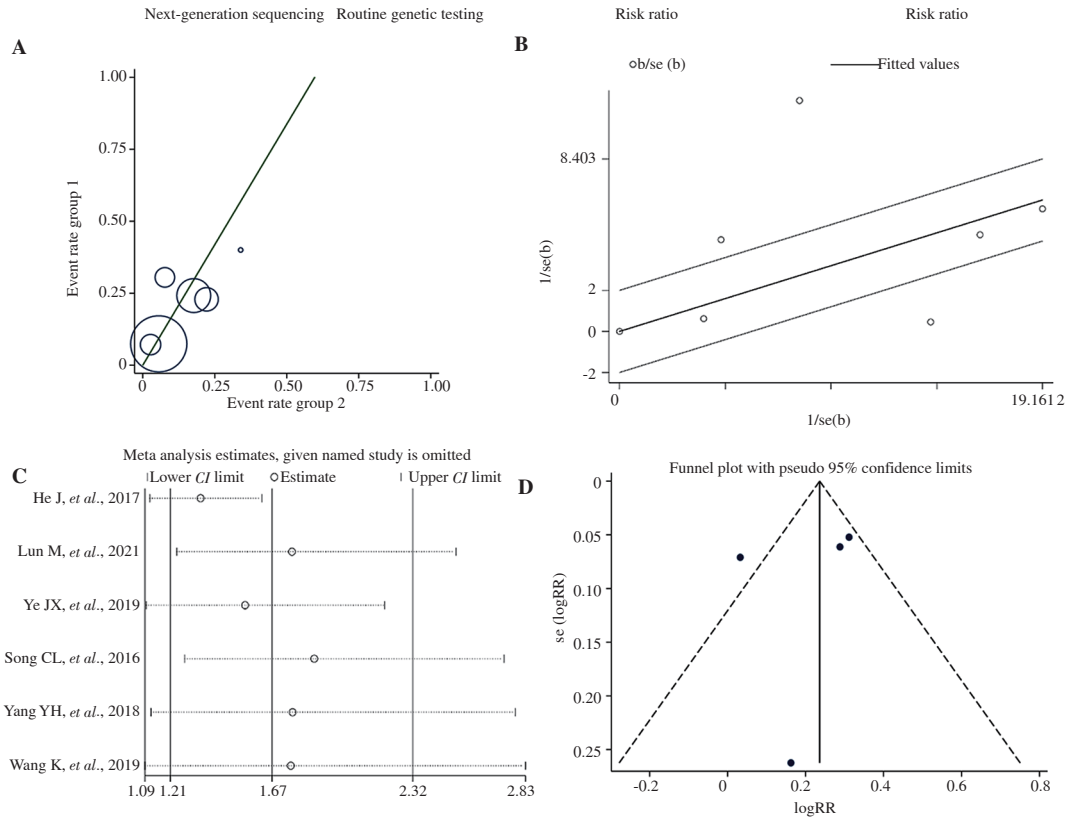

Figure 4. Meta-analysis of detection rate of α-thalassemia between next-generation sequencing and routine genetic testing group. (A) Labbe graph, (B) Galbr test, (C) Sensitivity analysis, and (D) Funnel plot.

Study or subgroup	Events	Total	Events	Total	Weigh	M-H, Random, 95% *CI*
Lun M *et al*., 2021	20	50	17	50	6.9%	1.18 (0.70, 1.97)
Song CL *et al*., 2016	313	1 368	303	1 368	13.2%	1.03 (0.90, 1.19)
Yang YH *et al*., 2018	583	7 866	437	7 866	31.1%	1.33 (1.18, 1.50)
Wang K *et al*., 2019	691	2 858	506	2 858	33.0%	1.37 (1.23, 1.51)
Total (95% *CI*)		12 142		12 142	100.0%	1.24 (1.07, 1.44)
Total events	1 607		1 263			

Heterogeneity: Tau^2=0.01; Chi^2=11.15, *df*=3 (*P*=0.01); I^2=73%
Test for overall effect: *Z*=2.82 (*P*=0.005)

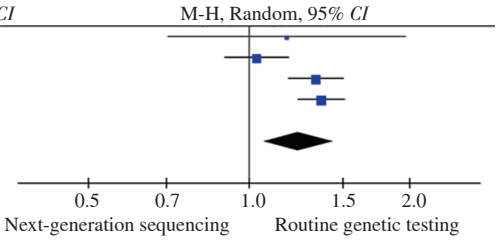

Figure 5. Forest plot of detection rate of α-thalassemia between next-generation sequencing and routine genetic testing group.

	Next-generation sequencing		Routine genetic testing		Risk ratio
Study or subgroup	Events	Total	Events	Total	Weigh	M-H, Fixed, 95% *CI*
He J, *et al*., 2017	99	951	79	951	14.2%	1.25 (0.95, 1.66)
Lun M, *et al*., 2021	28	50	23	50	4.1%	1.22 (0.83, 1.79)
Song CL, *et al*., 2016	210	1 368	196	1 368	35.3%	1.07 (0.90, 1.28)
Wang K, *et al*., 2019	254	2 858	246	2 858	44.3%	1.03 (0.87, 1.22)
Ye JX, *et al*., 2019	20	1 036	11	1 036	2.0%	1.10 (0.88, 3.78)
Total (95% *CI*)		6 263		6 263	100.0%	1.10 (0.99, 1.23)
Total events	611		555			

Heterogeneity: Tau^2=3.53; Chi^2=4, *df*=3 (*P*=0.47); I^2=0%
Test for overall effect: *Z*=1.75 (*P*=0.08)

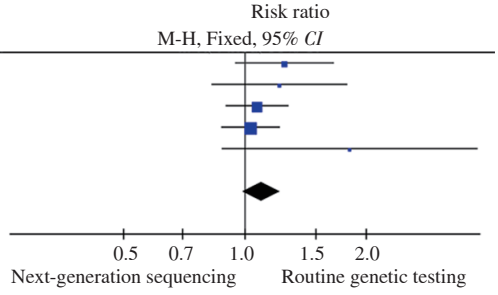

Figure 6. Forest plot of detection rate of β-thalassemia between next-generation sequencing and routine genetic testing group.

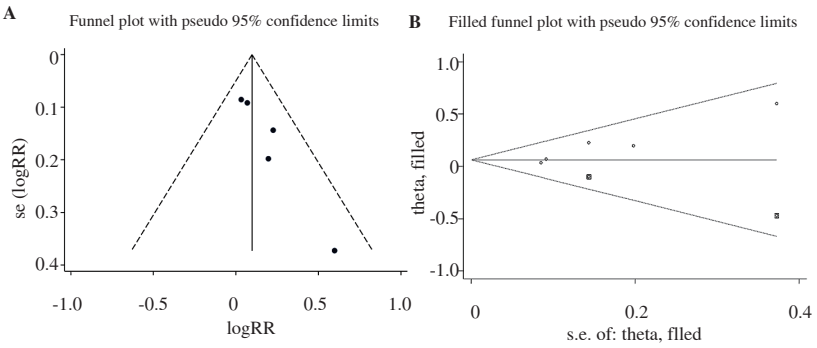

Figure 7. Meta-analysis of detection rate of β-thalassemia between next-generation sequencing and routine genetic testing group. (A) Funnel plot, (B) Corrected funnel plot based on fill-and-trim method.

4. Discussion

Depending on whether globin gene is mutated, thalassemia is categorised as α-thalassemia or β-thalassemia[17]. α-thalassemia happens when the *HBA1* gene, the *HBA2* gene, or their regulatory regions in the α-globin gene cluster are deleted or changed in some way[18]. β-thalassemia globin gene abnormalities include point mutations and deletions, with point mutations being the most prevalent. Over 200 mutations in β-globin genes have now been found[19]. About 100 000 babies are born with thalassemia each year worldwide[20], so it is essential to implement premarital screening interventions.

A classic regular process for thalassemia carrier screening detects individuals with phenotypic features associated with thalassemia utilizing hematological and biochemical tests, followed by molecular genetic testing to establish definite diagnoses in these individuals[21]. Gap-PCR and RDB were used to investigate prevalent thalassemia mutations. Gap-PCR was employed to screen for the four α-globin gene deletions, while the RDB assay was used for the three frequent non-deletional α-thalassemia mutations and the 17 known Chinese β-thalassemia variants[22]. Currently, different tests are needed for α- and β-thalassemia, which makes the tests difficult, time-consuming, and inconvenient. In recent years, however, significant advances in sequencing technology and computational methodologies have led to the development of next-generation sequencing technologies that have substantially reduced the time and expense involved with complete genome analysis[23]. The use of NGS in several clinical labs has aided the diagnosis of new genetic variants and uncommon anomalies[24].

A total of 9 articles involving 14 794 people were included in this analysis. From the results obtained, two main characters can be concluded: (1) In comparison with the routine genetic testing, the NGS group presented to have higher detection rate in general, particularly in the detection of α-thalassemia. (2) In the detection of β-thalassemia, there was no significant difference between NGS and routine genetic testing.

5. Limitations

The current study had some limitations. Due to the diversity of the genetic testing methods involved in thalassemia, only Gap-PCR and RDB were selected as the routine genetic testing group, which may not be able to comprehensively cover and assess all genetic testing techniques. The number of included studies was relatively small and only 1 of the 9 articles were published in English.

Despite the fact that several studies have shown the excellent accuracy of NGS in finding carriers, this is not readily replicable in different populations, and the cost remains prohibitive, particularly for endemic low-income nations. Also, another strategy was to utilize a combination of NGS and Gap-PCR to find the common deletions that account for 80% of the genetic causes of alpha-thalassemia. In a research using 15 807 samples, Ahlem et al. reported that combining NGS and Gap-PCR could discover 40 genomic variations, including 11 uncommon and new variants, but combining RDB and Gap-PCR could detect only three deletions and 20 kinds of mutations[25]. In addition, using NGS-Gap-PCR, Mei *et al*. detected 65 thalassemia-carriers and related mutations that are undetectable by conventional methods, containing the frequent -50 G>A mutation and a number of uncommon mutations, such as the poly A (A→G) AATAAA→AATGAA, Hb Phnom Penh, and initiation codon (-T) mutations, amongst 18 309 neonates[26]. These data indicate that the combination of NGS and Gap-PCR may efficiently discover novel mutations and minimize the incidence of misdiagnosis. Combined Gap-PCR and NGS approach is a cost-effective screening method for thalassaemia carrier screening, notably for α-thalassaemia mutant carriers[27].

6. Conclusions

In conclusion, research evidence supported NGS is superior to routine genetic testing methods. Compared with routine genetic testing, the NGS group presented to have higher detection rate in general, particularly in the detection of α-thalassemia. But in the detection of β-thalassemia, there was no significant difference between NGS and routine genetic testing.

Conflict of interest statement

The authors declare that they have no conflict of interest.

Data availability

The published paper includes the data used to support the study's findings.

Funding

This work was supported by the Hainan Provincial Natural Science Foundation (No. ZDKJ2021037); the China Postdoctoral Science Foundation (No. 2021M691466); and National Natural Science Foundation of China (No. 8220061871).

Authors' contributions

Conception and design of the study by all the authors; acquisition of data by XYF and YG; analysis and interpretation of data by all the authors; drafting and revision of the manuscript by all the authors; and approval of the final version by all the authors.

References

[1] Muncie HL Jr., Campbell J. Alpha and beta thalassemia. *Am Fam Physician* 2009; **80**(4): 339-344.

[2] Tarım H, Öz F. Thalassemia major and associated psychosocial problems: A narrative review. *Iran J Public Health* 2022; **51**(1): 12-18.

[3] Yang Y, Zhang J. Research progress on thalassemia in southern China. *Chinese J Exp Hematol* 2017; **25**(1): 276-280.

[4] Wu Y, Han L, Chen X, He J, Fan X, Dai J, et al. Effects of thalassemia on pregnancy outcomes of women with gestational diabetes mellitus. *J Obstet Gynaecol Res* 2022; **48**(5): 1132-1140.

[5] Prathyusha K, Venkataswamy M, Goud KS, Ramanjaneyulu K, Saikrupa K. Thalassemia: A blood disorder, its cause, prevention and management. *Res J Pharm Dosage Forms Technol* 2019; **11**(3): 975-4377 .

[6] Zhang J, Xie M, Peng Z, Zhou X, Zhao T, Jin C, et al. Five novel globin gene mutations identified in five Chinese families by next-generation sequencing. *Mol Genet Genomic Med* 2021; **9**(12): e1835.

[7] Munkongdee T, Chen P, Winichagoon P, Fucharoen S, Paiboonsukwong K. Update in laboratory diagnosis of thalassemia. *Front Mol Biosci* 2020; **7:** 74.

[8] Lun M. Application of next generation sequencing in genetic screening of neonatal thalassemia. *China Health Vision* 2021; **14**: 3.

[9] Lou GX, Li L, Zhou Y, Li L. Application of high throughput sequencing technology in genetic screening of neonatal thalassemia. *Clin Lab J* 2020; **9**(2): 137-138.

[10]Ye JX. *Application value of high-throughput sequencing technology in screening for thalassemia in pregnant women.* Guangdong: Shantou University; 2019.

[11]Wang K, Dang Y, Li Z, Pu Y, He S. Clinical significance of screening thalassemia with high throughput sequencing. *J Exp Hematol* 2019; **27**(4): 1220-1226.

[12]Yang YH. *Epidemiological analysis of thalassemia in Guizhou province by next-generation sequencing.* Guizhou: Zunyi Medical University; 2018.

[13]He SZ, Pu Y, Su H, Cao J, Mo L. Results of genetic screening of thalassemia by NGS and routine screening. *Med J Nat Defend Forces Southwest China* 2018; **28**(5): 404-406.

[14]He J, Song W, Yang J, Lu S, Yuan Y, Guo 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.

[15]Yang Q. *Evaluation of high-throughput molecular diagnostic techniques in thalassemia screenning.* Guangdong: Guangdong Medical University; 2017.

[16]Song CL, Liu Z, Chen S, Liu D, Zhou C. The value of next generation sequencing for large-scale genetic testing of thalassemia. *China Med Devices* 2016; **31**(S1): 15.

[17]Akca T, Ozdemir GN, Aycicek A, Ozkaya G. Long-term results of splenectomy in transfusion-dependent thalassemia. *J Pediatr Hematol Oncol* 2022. doi: 10.1097/MPH.0000000000002468.

[18]Long J, Sun L, Gong F, Zhang C, Mao A, Lu Y, et al. Third-generation sequencing: A novel tool detects complex variants in the α-thalassemia gene. *Gene* 2022; **822**: 146332.

[19]Wang M, Zhang X, Zhao Y, Lu Z, Xiao M. Prevalence and genetic analysis of thalassemia in childbearing age population of Hainan, The Free Trade Island in Southern China. *J Clin Lab Ana* 2022; **36**(3): e24260.

[20]Nabavian M, Cheraghi F, Shamsaei F, Tapak L, Tamaddoni A. The psychosocial challenges of mothers of children with thalassemia: A qualitative study. *J Educ Health Promot* 2022; **11**: 37.

[21]Shang X, Peng Z, Ye Y, Asan, Zhang X, Chen Y, et al. Rapid targeted next-generation sequencing platform for molecular screening and clinical genotyping in subjects with hemoglobinopathies. *EBioMedicine* 2017; **23**: 150-159.

[22]Chen DM, Ma S, Tang XL, Yang JY, Yang ZL. Diagnosis of the accurate genotype of HK$\alpha\alpha$ carriers in patients with thalassemia using multiplex ligation-dependent probe amplification combined with nested polymerase chain reaction. *Chin Med J* 2020; **133**(10): 1175-1181.

[23]Liang C, Chen XY, Gao X, Chen HJ, Jin YX, Zhou Y, et al. Spectrum of thalassemia mutations in fetuses of Han and Li ethinicities in Hainan province, China. *Asian Pac J Trop Med* 2019; **12**(12): 537-544.

[24]Chen P, Yu X, Huang H, Zeng W, He X, Liu M, et al. Evaluation of Ion Torrent next-generation sequencing for thalassemia diagnosis. *J Int Med Res* 2020; **48**(12): 300060520967778.

[25]Achour A, Koopmann TT, Baas F, Harteveld CL. The evolving role of next-generation sequencing in screening and diagnosis of hemoglobinopathies. *Front Physiol* 2021; **12**(2): 686689.

[26]Tan M, Bai Y, Zhang X, Sun J, Huang C, Tian R, et al. Early genetic screening uncovered a high prevalence of thalassemia among 18309 neonates in Guizhou, China. *Clin Genet* 2021; **99**(5): 704-712.

[27]Zhao J, Li J, Lai Q, Yu Y. Combined use of gap-PCR and next-generation sequencing improves thalassaemia carrier screening among premarital adults in China. *J Clin Pathol* 2020; **73**(8): 488-492.

Publisher's note