Research Article

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Hemoglobinopathy: Hematologic and Molecular Characteristics in Shanghai, Eastern China

Yefei Wang[1]*, Beiying Wu[2], Wenquan Xia[1], Ning Chen[1] and Yiqun Hu[1]

[1]*Faculty of Medical Laboratory Science, Ruijin Hospital, School of Medicine, Shanghai Jiao Tong University Shanghai, China*

[2]*Department of Clinical Laboratory, Ruijin Hospital, School of Medicine, Shanghai Jiao Tong University Shanghai, China*

***Corresponding author:** Yefei Wang, Faculty of Medical Laboratory Science, Ruijin Hospital, School of Medicine, Shanghai Jiao Tong University, 197 Ruijin Er road, shanghai 200025, China

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ABSTRACT

Hemoglobinopathies are common inherited diseases in southern China. The aim of the present study is to analyze the hematologic and molecular features of hemoglobinopathies in Shanghai, in order to provide a reference data for screening hemoglobinopathies. A total of 1029 samples were studied using High Performance Liquid Chromatography (HPLC) on the Bio-Rad Variant II HPLC system. GAP-PCR and reverse dot blot (RDB) were used to detect globin gene mutation or deletion. DNA sequencings for Alpha Globin gene (HBA1/A2) and Beta Globin gene (HBB) were simultaneously performed. We found that among 1029 samples, beta-thalassemia was the predominant type of hemoglobinopathy (39.16%). Six (0.58%) cases of beta-thalassemia major and 397 (38.58%) cases of beta-thalassemia carriers were identified. Among those beta-thalassemia samples, a total of 19 mutations were found, among which the β^{654}/β^{N} mutation (168/1029,16.33%) was the main type, followed by the $\beta^{41\text{-}42}/\beta^{N}$ mutation (118/1029,11.47%). There were 136 (13.22%) cases of alpha-thalassemia (silent carrier & minor) and 39 (3.79%) cases of HbH disease. A total of 10 gene mutations were found in the alpha-thalassemia samples, among which the main genotype of deletion α-thalassemia was --SEA /αα(119/1029,11.56%), while non-deletion α-thalassemia was uncommon in our report(2/1129,0.19%) both of which was $\alpha^{WS}\alpha/\alpha\alpha$. The main genotype of HbH was --SEA /-$\alpha^{3.7}$ (28/1029, 2.72%). In 13 (1.26%) cases of αβ- thalassemia, with a total of 9 genotypes were found, among which the more common genotypes were -$\alpha^{3.7}$/ β^{654}, --SEA/$\beta^{41\text{-}42}$ and --SEA/β^{17}. The ten main structural hemoglobin variants of 14 (1.36%) cases were Hb E, HbG-Taipei, Hb Q-Thailand, Hb Youngstown, Hb Guangzhou-Hangzhou, Hb M-Boston, Hb G-Siriraj, Hb J-Baltimore, Hb J-Sicilia and Hb Tamano. In addition, a single synonymous mutation (HBB c.9T>C, His>His) was found in 2 cases. Our results provide a detailed prevalence, hematologic and molecular characterization of hemoglobinopathies in Shanghai. We hope these findings will help to increase the awareness of this disease and provide useful epidemiological information for screening.

Keywords: Hemoglobinopathies; High Performance Liquid Chromatography; Globin Gene

Introduction

Hemoglobinopathy is a type of genetic defect that results in abnormal structure or amount in one of the globin chains of the hemoglobin molecule, including thalassemias and structural hemoglobin variants (abnormal hemoglobins). The thalassemias are an autosomal recessively inherited group of disorders of hemoglobin synthesis characterized by the absence or reduction in output of one or more of the globin chains of hemoglobin. The structural variants result from substitution of one or more amino acids in any of the globin chains of the hemoglobin molecule, usually in α or β globin chain. The substitution of amino acids in most of the Hb variants so far doesn't result in an abnormal stability and dysfunction of the hemoglobin molecule, and they are usually clinically silent or nonsignificant. Significant symptoms such as anemia may exist if it is present along with thalassemia or other disorders. The key element in the diagnosis of hemoglobinopathies is laboratory findings.

More than one thousand hemoglobin variants have been identified regarding changes in the globin chains [1]. Hemoglobinopathies

have a wide geographical distribution [2]. In China, obvious ethnic and regional differences in hemoglobinopathies exist. There is a high incidence of α- or β-thalassemias in southern China, especially in Guangdong, Guangxi and Hainan provinces [3]. More than 80 structural Hb variants have been reported so far, among which Hb E is the most common one in Yunnan, the province with the highest morbidity of abnormal hemoglobin. Hb G/ D is more common in northern China while Hb E / J is more common in southern China [3]. As an international metropolitan city, population migration is also more common in Shanghai, which leads to the migration of diseases. However, there have been few epidemiological studies of hemoglobinopathies in Shanghai. We analyzed the Hb variants screened by HPLC, comparing with their properties in the globin gene detection to investigate the hematologic and molecular characteristics in local population, and to provide epidemiological data related to structural Hb variants.

Material and Methods

Subjects

A Total 1029 cases were investigated in our laboratory during the study period from Nov.2014 to Dec.2018. This population included patients with clinical suspicion of hemoglobinopathies and those individuals' presenting hemolysis who were screened for the presence of hemoglobinopathies in Ruijin Hospital affiliated to Shanghai Jiao Tong University School of Medicine. The following combination of tests were used in hemoglobinopathy screening. (Figure 1).

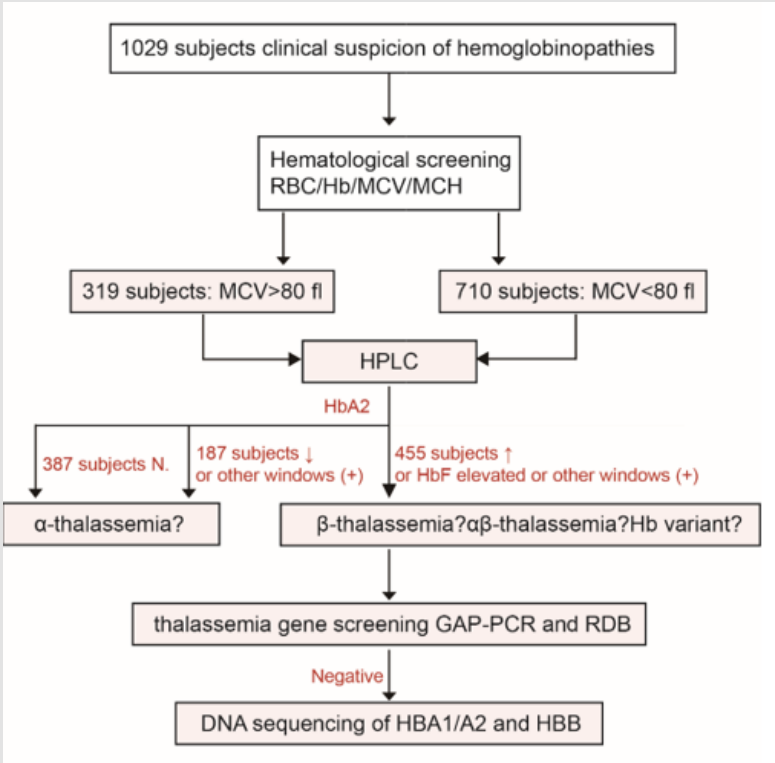

Figure 1: Combination of tests potentially required to evaluate carrier status in screening hemoglobinopathies N: $HbA_2$2.5%~3.5%,:HbA_2<2.5%, ↑: HbA_2>3.5%.

Methods

Hematologic Routine Tests: Complete blood count and red blood cell indices (Hb, MCV, MCH, MCHC) were measured by the automated cell analyzer (SYSMEX XE-2100). Wright-stained peripheral blood smears were examined for red blood cell morphology.

Hemoglobin Analysis: 2ml of whole venous blood were collected in a vacuum blood collection tube containing EDTA as an anti-coagulent. HbA_2, HbF, and other hemoglobin variants were analyzed using HPLC method used for chromatographic separation of human hemoglobin on the Variant Hemoglobin Testing System (Variant II Beta Thalassemia Short Program, Bio-Rad Laboratories). No preparation was required unless the sample was collected from severe anemic patients or there was less than 500 μL of sample in the tube. In such case, sample was manually prediluted by mixing 1.0mL wash/diluents with 5 μL of whole blood sample. HbA_2/F calibrators and normal and abnormal controls were analyzed at the beginning of each run. Reports and chromatograms generated were studied and interpret-

ed by observing HbA_2 and F concentration for beta thalassemia and retention time and area percentage of other peaks and windows for structural variants. Each chromatogram shows peaks of HbA0, A2, and HbF along with C, D and S window, and two minor peaks, P2 and P3.

Globin Gene Analysis: Molecular analyses for common alpha deletions and common beta mutations were performed by using GAP-PCR and reverse dot blot (RDB). Full DNA sequencing of Alpha Globin (HBA_1/A_2) and Beta Globin (HBB) genes was simultaneously performed in individuals with an abnormal HPLC results but a negative thalassemia gene screening.

Statistical Method

Data entry and analysis were performed using the Statistical Package for Social Sciences (SPSS21.0). P<0.05 was considered statistically different.

Results

Hematological Findings

Means of RBC Parameters and HbA_2: Comparison of all groups is shown in Table 1. In our studies, 439 subjects had HbA_2 levels > 3.5%, in which 423 (96.4%) cases were identified as β-thalassemia carriers. αβ- thalassemia and structural Hb variants by globin gene analysis (HbA_2 range 3.6%~72.7%). 40 subjects had an increased area at retention times of 0.125±0.021min and 0.406±0.091min, among which 39 (97.5%) subjects were identified to have HbH disease by globin gene analysis (HbA_2 range 0%~ 2.1%) (Figure 2). A significant (p<0.05) difference in HbA_2 was found in each comparison group, except for those between the gene negative group and the α- thalassemia silent carrier and minor groups(p=0.421>0.05, and between theβ- thalassemia and theαβ- thalassemia group (p=0.572>0.05. A significant (p<0.05) difference of RBC was found in the gene negative group compared with all other groups. A significant (p<0.05) difference of MCV was found in the gene negative group compared with the α-, β-,αβ- thalassemia or Hb variants groups (Table 1) (Figure 3).

Table 1: Hematological findings of all groups (Means ± SD).

Parameters	Gene (-)	α- thalassemia (silent carrier & minor)	α- thalassemia (HbH disease)	β- thalassemia	αβ- thalassemia	Hb variants
HbA_2(%)	2.87±0.60	2.68±0.32	1.41±0.56	5.36±0.78	4.98±1.71	10.99±18.92 (1.6~72.7)
RBC($\times10^{12}$/L)	3.96±1.00	5.29±1.04	4.72±0.71	5.05±1.08	5.77±1.25	3.30±1.46
Hb(g/L)	103.16±29.50	113.12±20.63	85.51±8.60	100.87±17.53	117.77±28.70	89.19±36.00
MCV(fl)	82.91±12.76	69.61±7.62	63.62±8.68	65.02±5.87	65.32±9.48	91.67±19.64
MCH(pg)	26.57±5.52	21.63±2.71	18.33±1.85	20.25±1.99	20.52±3.01	30.43±11.51
MCHC(g/L)	317.96±26.21	310.58±11.04	289.62±17.42	311.93±10.91	314.39±11.40	323.75±51.37

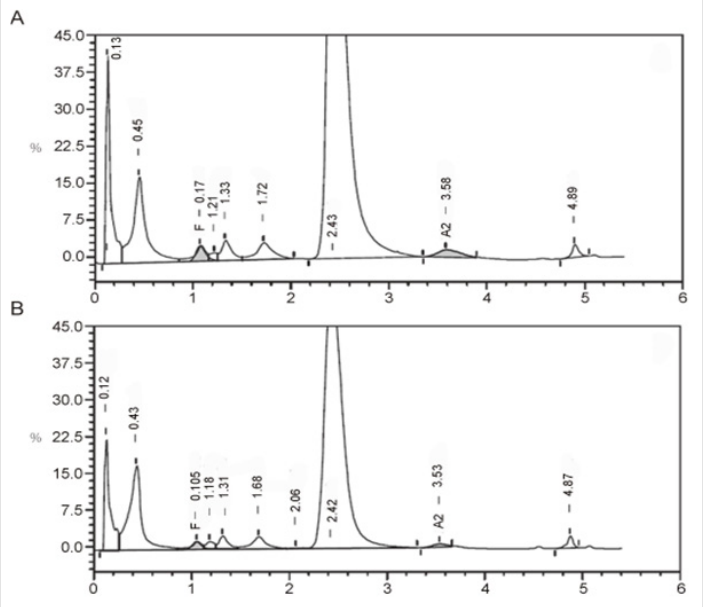

Figure 2: Chromatogram of HbH disease.

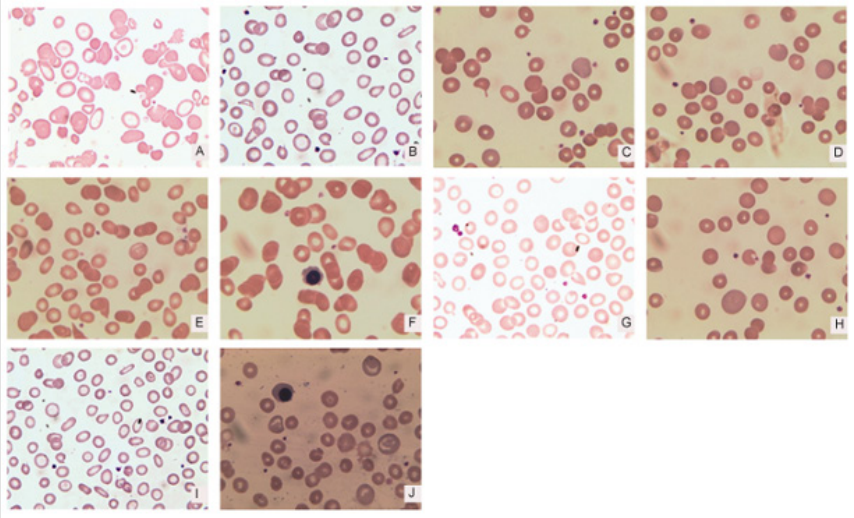

Figure 3: RBC morphology of structural hemoglobin variants
A. Marked anisocytosis, enlarged central pallor with target cells and irregular RBCs.
B. Anisocytosis, hypochromic, microcytic cells with target cells and polychromasia.
C. Anisocytosis with elliptocytes and polychromasia.
D. Anisocytosis with polychromasia.
E. Anisocytosis with polychromasia.
F. Mild anisocytosis with polychromasia and normoblasts.
G. Anisocytosis with hypochromic and RBC fragments.
H. Mild anisocytosis with macrocytes and polychromasia.
I. Mild anisocytosis with hypochromic, microcytic cells, elliptocytes and polychromasia.
J. Anisocytosis with macrocytes, polychromasia and normoblasts.

RBC Parameters and Morphology of Structural Hemoglobin Variants: Comparison of all hemoglobin variants is shown in Table 2.

Table 2: RBC parameters and morphology features of 16 structural hemoglobin variants or gene mutation.

Hb variants	RBC ($\times10^{12}$/L)	Hb (g/L)	MCV (fl)	MCH (pg)	MCHC (g/L)	Blood smear	Clinical diagnosis
Hb E compound β-thalassemia	2.4	47	78.3	19.6	250	Figure 3A	thalassemia
Hb E compound α-thalassemia	4.93	92	63.3	18.7	295	Figure 3B	microcytic hypochromic anemia
Hb Youngstown*	3.44	82	89	23.8	268	Figure 3C	anemia
Hb Youngstown*#	2.52	65	94.8	25.8	272	Figure 3D	anemia
Hb G-Taipei*	0.83	32	109.6	38.6	352	Figure 3E	pancytopenia
Hb G-Taipei	2.59	88	97.7	34.2	350	Figure 3F	ALL
Hb Q- Thailand	4.61	124	79.5	26.8	337	Figure 3G	hyperbilirubinemia
Hb Q- Thailand	4.86	129	81.5	26.5	326	N	hematopathy
Hb Tamano*	4.43	144	97.3	32.5	334	N	CGL
Hb G-Siriraj*	3.83	99	79.3	25.9	326	N	anemia
Hb Guangzhou-Hangzhou*	1.59	73	121.4	45.9	378	Figure 3H	anemia
Hb J-Baltimore	4.43	71	63	16	254	Figure 3I	IDA
Hb J-Sicilia	3.6	109	89.8	30.4	338	N	leukopenia & anemia
Hb M-Boston	5.17	148	86.3	28.7	333	N	cyanosis
Single HBB synonymous mutation	3.09	93	97.1	30.2	311	N	anemia
Single HBB synonymous mutation	0.49	31	141.2	63.3	456	Figure 3J	AIHA

Note: *compound HBB c.9T>C synonymous mutation; #compound HBA2 c.382A>T[127Lys> termination codon; ALL: acute lymphocytic leukemia; CGL: chronic granulocytic leukemia; IDA: iron

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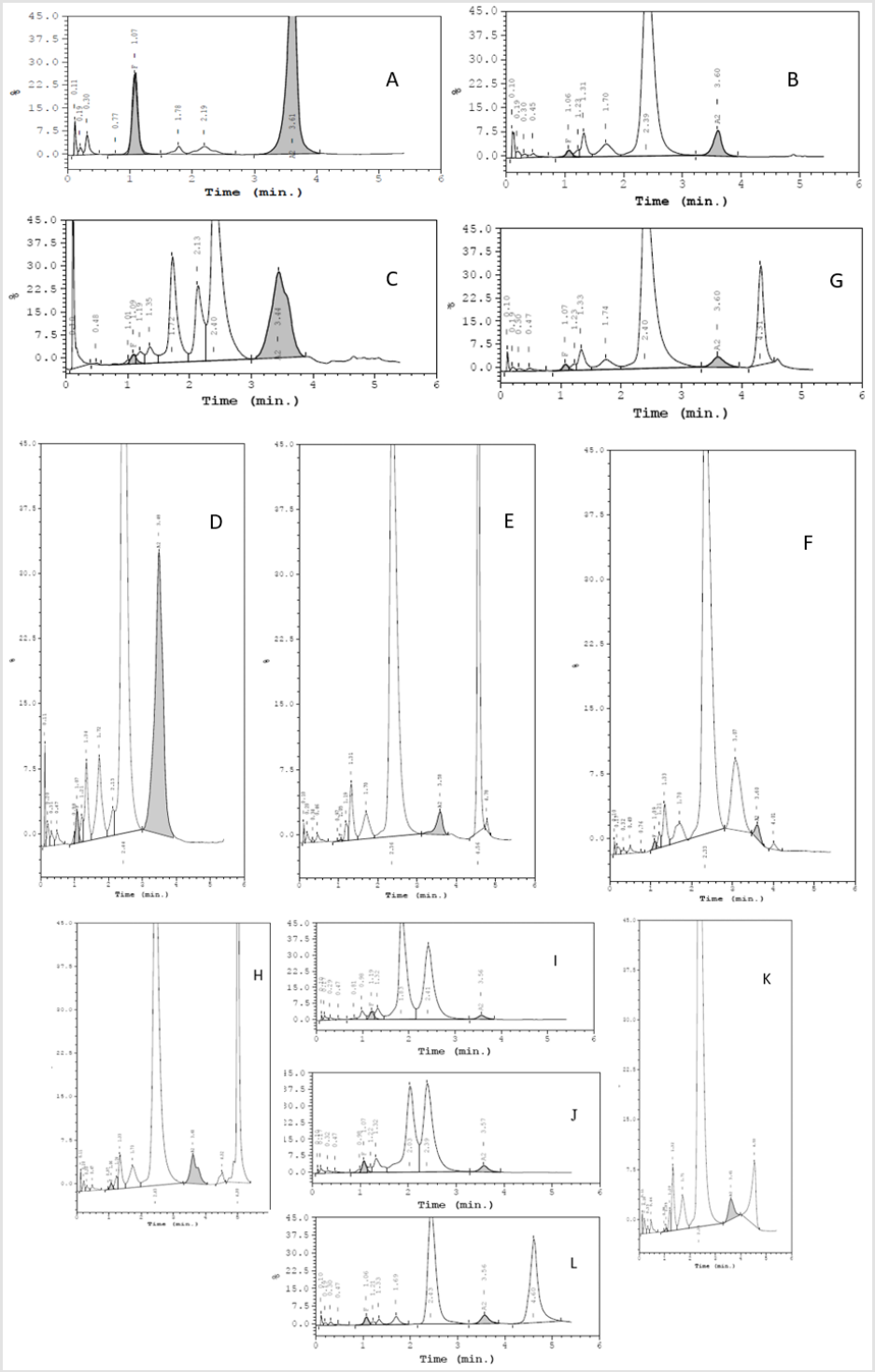

Figure 4: Chromatogram of Hb variants. A2 window: A. Hb E compound β-thalassemia, B .Hb E compound α-thalassemia, C.Hb G-Taipei, D.Hb G-Taipei (compound HBB synonymous mutation); S window: E.Hb Q-Thailand(compound -α4.2/αα.HBB synonymous mutation); G. Hb Guangzhou-Hangzhou (compound HBB synonymous mutation); K.Hb M-Boston; L. Hb Youngstown (compound HBA_2 c.382A>T,α127lys > termination codon (nonsense mutation). HBB synonymous mutation); C window: H.Hb G-Siriraj(compound HBB synonymous mutation) P3 window: I.Hb J-Baltimore(compound HBB synonymous mutation.HBA_2 UTR mutation); J.Hb J-Sicilia(compound HBB synonymous mutation. HBA2 UTR mutation); between A0 window & A2 window: F.Hb Tamano (compound HBB synonymous mutation).

Hemoglobin Analysis of Structural Hemoglobin Variants

Ten structural hemoglobin variants were detected in 14 cases (14/1029,1.36%). Among those 4 cases were shown in A2 window (1 Hb E compound β-thalassemia, 1 Hb E compound α-thalassemia and 2 HbG-Taipei), 6 cases were shown in S window (2 cases of Hb Q-Thailand, 2 cases of Hb Youngstown, 1 case of Hb Guangzhou-Hangzhou, and 1 case of Hb M-Boston), 1 case was shown in C window which is Hb G-Siriraj, 2 cases were shown in P3 window with one Hb J-Baltimore and one Hb J-Sicilia. One case of Hb Tamano was shown between A0 and A2 window (Figure 4). Another 2 cases were shown in C window (Figure 5) which were proved to be a single synonymous mutation by DNA sequencing (HBB c.9T>C, His>His) without abnormal Hb variant. This kind of synonymous mutation was also found in most of the Hb variants above. Presumptive identification of hemoglobin variants was made primarily using retention time (RT) windows and area percent (Table 3).

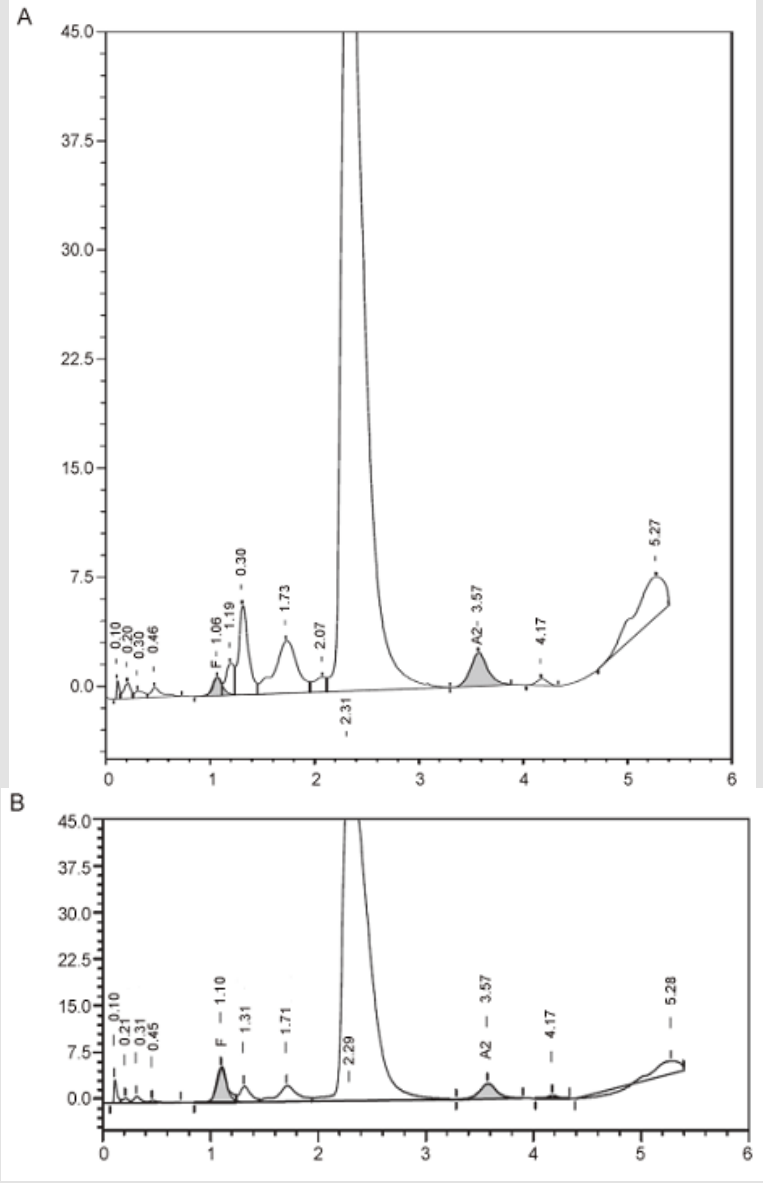

Figure 5: Chromatogram of Single HBB synonymous mutation.

Table3: HPLC results of 16 cases of Hb variants or abnormal genes.

Hb variants (or abnormal genes)	No.	Windows	Retention time(min)	Area percent (%)
Hb E compound β-thalassemia	1	F/A2	1.07/3.61	15.8/72.7
Hb E compound α-thalassemia	1	Unknown /A2	0.10/3.60	2.1/7.9
Hb Youngstown	2	S	4.605*	36.15*
Hb G-Taipei	2	A2	3.46*	30.3*
Hb Q-Thailand	2	S	4.555*	26.15*
Hb Tamano	1	between A0&A2	3.07	13.3
Hb Guangzhou-Hangzhou	1	S	4.31	17.2
Hb G-Siriraj	1	A2/C/ S	3.60/4.99/4.52	5.0/32.3/0.9
Hb J-Baltimore	1	P3	1.83	47.7
Hb J-Sicilia	1	P3	2.03	40.8
Hb M-Boston	1	S	4.53	8.1
Single HBB synonymous mutation	2	C	5.275*	4.6*

Notes: *shown in means.

Globin Gene Analysis

In our studies, a total of 605 subjects were found to be hemoglobinopathies by globin gene analyzing (605/1029, 58.79%), in which β-thalassemia (403/1029, 39.16%) was more common than other hemoglobinopathies. The common mutations in our study population include --SEA/αα(119/1029,11.56%) in α-thalassemia ,and CD41/42(-TTCT) (118/1029,11.47%) or IVS-2-654(C→T)(168/1029, 16.33%) in β-thalassemia (Table 4). The DNA sequencing results of 10 types of hemoglobin variants are shown in Figure 6, in which βglobin gene mutation was more common (9/14, 64.3%), shown in Table 5.

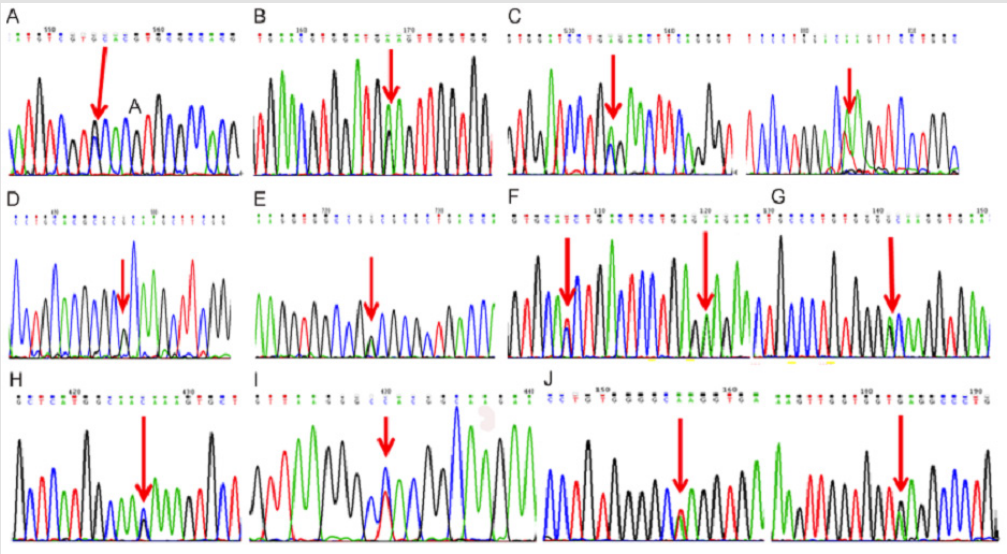

Figure 6: DNA sequence of 10 types of hemoglobin variants.
A. Hb Q-Thailand (HBA1 c.223 GAC>CAC).
B. Hb G-Taipei (HBB c.68GAA>GGA);
C. Hb Youngstown (HBB c.305GAG>GCG) compound HBA2 c.382AAG>TAG (α127 lys > termination codon)
D. Hb Tamano(HBA1 c.269CAC>CGC);
E. Hb Guangzhou-Hangzhou (HBA2c.194GAC>GGC).
F. Hb G-Siriraj (HBB c.22GAG>AAG) compound HBB c.9CAT>CAC.
G. Hb J-Baltimore (HBB c.50GGC>GAC);
H. Hb J-Sicilia (HBB c.198 AAG>AAC).
I. Hb M-Boston (HBA2 c.175CAC>TAC).
J. β17 (HBB c.102AAG>TAG) compound Hb E(HBB c.129 GAG>AAG).

Table 4: Globin genotypes of 1029 cases.

Globin genotype	No.	Percentage(%)
(-)	422	41.01
α-thalassemia	175	17.01
$-\alpha^{3.7}/\alpha\alpha$	10	
$-\alpha^{4.2}/\alpha\alpha$	4	
$\alpha^{WS}\alpha/\alpha\alpha$	2	
--SEA/αα	119	
$-\alpha^{3.7}/-\alpha^{3.7}$	1	
$--SEA/-\alpha^{3.7}$	28	
$--SEA/-\alpha^{4.2}$	4	
$--SEA/\alpha^{CS}\alpha$	5	
$--SEA/\alpha^{QS}\alpha$	1	
--SEA/αα(rare α2 partial deletion)	1	
β-thalassemia	403	39.16
β^{654}/β^{654}	2	
$\beta^{17}/\beta^{IVS-I-5}$	1	
β^{17}/β^{28}	1	
$\beta^{41-42}/\beta^{654}$	1	
β^{41-42}/β^{17}	1	
β^{17}/β^{N}	72	
β^{41-42}/β^{N}	118	
β^{-28}/β^{N}	5	
β^{5}/β^{N}	1	
β^{654}/β^{N}	168	
β^{14-15}/β^{N}	2	
β^{27-28}/β^{N}	13	
β^{43}/β^{N}	2	
β^{71-72}/β^{N}	11	
β^{Int}/β^{N}	1	
β^{IntM}/β^{N}	1	
$\beta^{IVS-I-1}/\beta^{N}$	1	
β~53-T	1	
β^{-}IVS-I-2(T>C)	1	
αβ-thalassemia	13	1.26
$--SEA/\alpha\alpha\ \beta^{41-42}/\beta^{N}$	2	
$-\alpha^{4.2}/\alpha\alpha\ \beta^{654}/\beta^{654}$	1	
$--SEA/\alpha\alpha\ \beta^{17}/\beta^{N}$	2	
$-\alpha^{ws}/\alpha\alpha\ \beta^{17}/\beta^{N}$	1	
$-\alpha^{3.7}/\alpha\alpha\ \beta^{41-42}/\beta^{N}$	1	
$-\alpha^{3.7}/\alpha\alpha\ \beta^{654}/\beta^{N}$	3	
$-\alpha^{4.2}/\alpha\alpha\ \beta^{41-42}/\beta^{N}$	1	
$-\alpha^{CS}/\alpha\alpha\ \beta^{17}/\beta^{N}$	1	
$--SEA/\alpha^{QS}\alpha\ \beta^{654}/\beta^{N}$	1	
Structural Hb variants	14	1.36
Hb E compound β^{17}	1	
Hb E compound $--SEA/\alpha^{CS}\alpha$	1	
Hb Q-Thailand compound $-\alpha^{4.2}/\alpha\alpha$	2	
Hb G-Taipei	2	
Hb Youngstown	2	
Hb Guangzhou-Hangzhou	1	
Hb J-Baltimore	1	
Hb J-Sicilia	1	
Hb G-Siriraj	1	
Hb Tamano	1	
Hb M-Boston	1	
other	2	0.19
Single HBBc.9T>C	2	
Total	1029	100

Table 5: DNA sequencing results of 10 types of hemoglobin variants.

Hb variants	Mutation gene	Substitution	N	Percentage(%)
αglobin gene mutation			5	35.7
Hb Q-Thailand	HBA1 c.223 GAC>CAC	α74ASP>His	2	
Hb Tamano	HBA1 c.269CAC>CGC	α89 His>Arg	1	
Hb Guangzhou-Hangzhou	HBA2c.194GAC>GGC	α64Asp>Gly	1	
Hb M-Boston	HBA2 c.175CAC>TAC	α58His>Tyr	1	
βglobin gene mutation			9	64.3
Hb G-Taipei	HBB c.68GAA>GGA	β22 Glu>Gly	2	
Hb G-Siriraj	HBB c.22GAG>AAG	β7Glu>Lys	1	
Hb Youngstown	HBB c.305GAG>GCG	β101 Glu>Ala	2	
Hb J-Baltimore	HBB c.50GGC>GAC	β16 Gly>Asp	1	
Hb J-Sicilia	HBB c.198 AAG>AAC	β65 lys>Asn	1	
Hb E	HBB c.129 GAG>AAG	β26Glu>Lys	2	
Total			14	100

Discussion

Hemoglobinopathy is a common inherited single gene disorder in southern China. Many studies have been published from southern China on hemoglobinopathies; however most studies focus on epidemiology and screening. While very few studies did extensive analysis on thalassemic and structural Hb variants carriers determined using HPLC reports in Shanghai, an international metropolitan city with frequent population migration that inevitably leads to the migration of diseases. The most common conventional method of screening for any inherited blood disorder is clinical examination followed by CBC, electrophoresis or HPLC [4-7] . Out of these, HPLC method is based on the movement of different hemoglobins in a given gravitational field as they pass through certain adhesive materials. HPLC has the advantage of quantifying HbF and HbA_2 along with detecting other variants in a single screening test [8]. Automated HPLC and β-thalassemia program is an appropriate approach for the screening and presumptive identification of patients as well as carrier of β-thalassemia prior to DNA studies for definitive diagnosis.

As the first laboratory to analyze hemoglobin by using the automated HPLC and β-thalassemia program in Shanghai. 39 (3.79%) HbH disease,403 (39.16%)β-thalassemia, 13 (1.26%)αβ-thalassemia and 14 (1.36%) cases of structural Hb variants had been identified in hemoglobinopathy screening, which were then confirmed to be hemoglobinopathies by globin gene analyzing and confirmation on the the Hbvar database (http://globin.cse.psu.edu/hbvar/menu.html). The automated HPLC method showed a high sensitivity and specificity in β-thalassemia, αβ-thalassemia, HbH disease and structural Hb variants screening, but displayed a low sensitivity in detecting α-thalassemia silent carrier or minor type. A total of 6 (0.58%) cases of β-thalassemia major and 397 (38.58%) cases of β-thalassemia carriers,136 (13.22%) cases of α-thalassemia (silent carrier & minor), 39 (3.79%) cases of HbH disease, 13 (1.26%) cases of αβ- thalassemia were confirmed by gene analyzing. Among them, the common genotypes of deletion α-thalassemia were --SEA /αα, $-\alpha^{3.7}$/ααand $-\alpha^{4.2}$/αα. --SEA /$-\alpha^{3.7}$, --SEA /$-\alpha^{4.2}$, --SEA /α^{CS} αwere of HbH diease. The main genotypes of deletion and non-deletion α-thalassemia were --SEA/αα and $\alpha^{WS}\alpha$/αα. The main genotypes of β-thalassemia wereβ^{654}/β^{N} followed byβ^{41-42}/β^{N} which was different from the results published from southern China [9-14]. Therefore there is a significant geographical difference in the thalassemia gene mutation prevalence which constitutes the diversity of HBA and HBB mutations.

The ten types of structural Hb variants we found were Hb E compound with thalassemia, HbG-Taipei, Hb Q-Thailand, Hb Youngstown, Hb Guangzhou-Hangzhou, Hb M-Boston, Hb G-Siriraj, Hb J-Baltimore, Hb J-Sicilia and Hb Tamano. In comparison, HbE and Hb Q-Thailand are common in southern china. While HbG-Taipei has been reported in both Han and other national minority in Chinese population [15], all other variants are rare. Hb J-Baltimore (β16 Gly→Asp) was first described in1963 in an African-American family. Since then, several cases have been reported in distinct racial groups. Most of those cases were discovered incidentally during the study of other entities, such as thalassemia [16]. Hb J-Sicilia (β65 lys→Asn) was first described in1974 in a young Sicilian woman. This Hb variant doesn't show any difference in function, which can be considered as a homologue of Hb Zambia (β60 lys→Asn [17]. Hb J-Baltimore and Hb J-Sicilia were the first to be reported in Chinese population, both of which have compound homozygous mutation in HbA_2 gene 5'- UTR and a synonymous mutation (c.9T>C) in HBB gene. Whether those mutations cause a change in Hb function needs further research. The heterozygous carrier of Hb J-Baltimore seen in a 37-year-old woman who displayed a moderate microcytic hypochromic anemia with iron deficiency. The case of Hb J-Sicilia was seen in a 61-year-old woman who suffered from leukopenia and slight anemia. In our studies, except for 2 cases of HbE with compound thalassemia and 2 cases of Hb Youngstown display marked hemolysis and anemia and one case of HbM-Boston displays characteristic cyanosis, most of structural Hb variants which were without any clinical effects and were fortuitously found during screening programmes.

The majority of Hb variants fortuitously discovered are of minimal clinical interest. On the contrary, those found during the course of a hematological disorder reveal the aetiological answer for the disease. Often time, unusual clinical presentations may be explained by the presence of several Hb abnormalities and their identification may require further investigations [18]. Many of these variants are of little clinical significance in heterozygous state, but when combined with other variants or disease they may give rise to severe disease. Therefore, in those patients once the Hb variants have been screened by HPLC, genetic analysis is recommended and the clinical characteristics of the disease should be communicated with patients to avoid serious hemolysis complications. Significant ($p<0.05$) differences in HbA_2 were found in each group, except for those between the gene negative group and the α- thalassemia silent carrier and minor group($p>0.05$. Significant($p<0.05$)differences of both RBC and MCV were found in the gene negative group compared withα-, β-,αβ- thalassemia or Hb variants groups. Hence, it is of great value to screen hemoglobinopathies except α- thalassemia silent carrier and minor by detecting RBC, MCV, and HbA_2. For those samples with increased RBC and decreased MCV but normal HbA_2, further gene analysis should be followed to avoid misdiagnosing of α- thalassemia silent carrier and minor.

Conclusion

Our results provide a detailed prevalence, hematologic and molecular characterization of hemoglobinopathies in Shanghai that can definitely help to increase the awareness of hemoglobinopathy screening. β-thalassemia was the predominant type of hemoglobinopathies, with the main mutation being β^{654}/β^{N} followed byβ^{41-42}/βN. The main genotype of deletionα-thalassemia was --SEA /αα, and non-deletion α-thalassemia was uncommon in our report. The main

genotype of HbH was --SEA /-$\alpha^{3.7}$. The more common genotypes of αβ- thalassemia were -$\alpha^{3.7}$/ β^{654}, --SEA/β^{41-42} and --SEA/β^{17}. There are 10 Hb variants in this study focusing on the population in Shanghai. HbE compound with thalassemia and Hb Youngstown presented with marked hemolysis and anemia, HbM-Boston showed characteristic cyanosis, while the other 8 types of Hb variants were clinically insignificant. We identified a rare case of Hb J-Baltimore (β16 Gly→Asp) and a rare case of Hb J-Sicilia (β65 lys→Asn) in Chinese population for the first time, both of which were compounded with a homozygous mutation in HbA_2 gene 5'- UTR and a synonymous mutation(c.9T>C) in HBB gene.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

- Conception and Design: YF. Wang, BY. Wu, YQ. Hu.
- Acquisition of Data: (Provided reagents, acquired and managed patients, provided facilities, etc.): YF. Wang, BY. Wu, WQ. Xia, N. Chen.
- Analysis and Interpretation of Data: (e.g., Statistical analysis, biostatistics, computational analysis): YF. Wang, BY. Wu, WQ. Xia.
- Writing, Review, and/or Revision of the Manuscript: YF. Wang, BY. Wu.
- Administrative, Technical, or Material Support: (i.e., reporting or organizing data, constructing databases): YF. Wang, YQ. Hu.

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References

1. Maria del Pilar Aguinaga, Ming Chan, Tim Clark Davis, et al. (2015) Hemoglobinopathies: Current Practices for Screening, Confirmation and Follow-up. Association of Public Health Laboratories 12: 1-57.
2. Williams TN, Weatherall DJ (2012) World distribution, population genetics, and health burden of the hemoglobinopathies. Cold Spring Harb. Perspect Med 2(9): a011692.
3. HE Jing, YU Xiaoyan, WEN Weixia, et al. (2018) Analysis of abnormal hemoglobin disease of 102 cases in Huizhou area of Guangdong province. Lab Med Clin 15(14): 2049-2054.
4. JaeHwang Jung, Lucas E Matemba, KyeoReh Lee, Paul E Kazyoba, Jonghee Yoon, et al. (2016) Optical characterization of red blood cells from individuals with sickle cell trait and disease in Tanzania using quantitative phase imaging. Scientific Reports 6(1): 31698.
5. JD Losek, TR Hellmich, GM Hoffman (1992) Diagnostic value of anemia, red blood cell morphology, and reticulocyte count for sickle cell disease. Annals of Emergency Medicine21(8): 915-918.
6. A Brants (2011) Hemoglobinopathy and thalassemia detection: traditional methods and a novel method--capillary electrophoresis technology. MLO: Medical Laboratory Observer 43(10): 22-24.
7. RB Colah, R Surve, P Sawant, E D'Souza, K Italia, et al. (2007) HPLC studies in hemoglobinopathies, Indian Journal of Pediatrics 74(7): 657-662.
8. Nigam N, Mishra A, Nair N, et al. (2015) HPLC as a classical tool for screening of β-Thalassemia and Hemoglobinopathies in Kanpur. Rama Univ. J Med Sci (1(1): 6-9.
9. Shi Mingfang, Yang Lan, Yu Xia, et al. (2019) Analysis of the gene detection resuIts of 6545 cases of alpha-thalassemia. Laboratory Medicine 34(1): 8-9.
10. Li-Hua Ye, Hui-Juan Pan, Jun-Yan Hu, Shi-Xin Chen (2019) Analysis of gene mutation types in 920 cases of thalassemia. Journal of experimental hematology 27(2): 545-548.
11. Xu Yunjian, Liao Yingyin, Gao Jun, et al. (2019) Genetic analysis and application of erythrocyte parameters of thalassemia screening in local district. The Journal of Practical Medicine 35(2): 285-293.
12. Zhang Jie, HE Jing, Zeng Xiao-hong, et al. (2016) Genetic heterogeneity of thalassemia in Yunnan province. Journal of Kunming Medical University 37(1): 28-34.
13. Peng-Ju Cao, Liang-Yuan Chen, Li-Li Jiang, Yang Yang, Shao-Ting Chen, et al. (2019) Analysis of gene mutation types of thalassemia in Fuzhou area of China. Journal of experimental hematology 27(3): 893-898.
14. Mengi Li, Song-He Xiang, Yi Ding, Wen-Wen Liu, Yuan-Yuan Xu, et al. (2018) Genotype analysis of patients with thalassemia in Sanya area of Hainan Province in China. Journal of experimental hematology 26(4): 1146-1150.
15. Peng Jie, Gong Wu-xing, Huang Cui-zhen (2011) Research progress of hemoglobin G disease. Medical Recapitulate 17(13): 1948-1950.
16. Manuel A Gargallo, Fernando Ataulfo González, Ana Villegas (2010) Abnormally low HbA1c secondary to hemoglobin J-Baltimore [beta 16(A13) Gly-->Asp]. Family study. Cartas Científica, p. 83-85.
17. G Ricco, P G Pich, U Mazza, G Rossi, F Ajmar, et al.(1974) Hb J Sicilia: beta 65 (E9) Lys-Asn, a beta homologue of Hb Zambia. FEBS LETTERS 39(2): 200-204.
18. Henri Wajcman, Kamran Moradkhani (2011) Abnormal haemoglobins: detection & characterization. Indian J Med Res 134(10): 538-546.

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