Complex Interaction of Hb Q-Thailand with α and β Thalassemia in a Hakka Family

Lin Zheng
Fujian Maternity and Child Health Hospital

Hailong Huang
Fujian Key Laboratory for Prenatal Diagnosis and Birth Defect, Fujian Maternity and Child Health Hospital Affiliated Hospital of Fujian Medical University

Xiaoqing Wu
Fujian Key Laboratory for Prenatal Diagnosis and Birth Defect, Fujian Maternity and Child Health Hospital Affiliated Hospital of Fujian Medical University

Qingmei Shen
Fujian Key Laboratory for Prenatal Diagnosis and Birth Defect, Fujian Maternity and Child Health Hospital Affiliated Hospital of Fujian Medical University

Meihuan Chen
Fujian Key Laboratory for Prenatal Diagnosis and Birth Defect, Fujian Maternity and Child Health Hospital Affiliated Hospital of Fujian Medical University

Meiying Wang
Fujian Key Laboratory for Prenatal Diagnosis and Birth Defect, Fujian Maternity and Child Health Hospital Affiliated Hospital of Fujian Medical University

Linjuan Su
Fujian Key Laboratory for Prenatal Diagnosis and Birth Defect, Fujian Maternity and Child Health Hospital Affiliated Hospital of Fujian Medical University

Liangpu Xu (xiliangpu@fjmu.edu.cn)
https://orcid.org/0000-0001-8815-2735

Research

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Abstract

Background HbQ-Thailand is an α-globin chain variant that results from a point mutation at codon 74 of the α1-globin gene on chromosome 16p. It commonly appears with a leftward single α-globin gene deletion (-α 4.2). There have been few reports regarding the interaction between HbQ-Thailand and other globin gene disorders. Here we found and diagnosed it in the Hakka population of the Fujian Province, China. The study provides an important reference for the clinic diagnose and genetic counseling of thalassemia and hemoglobin diseases.

Methods Fresh peripheral blood samples were collected from the proband and her family members testing hematological parameters, hemoglobin components, thalassemia gene, and hemoglobin variants.

Results The proband (II1) and her sister (II5) manifested in the obvious microcytic hypochromic anaemia. The CE electropherogram of II1 showed an abnormal band in the migration time at 185 s, which was confirmed as HbQ-Thailand. Another exception band appeared at 250 s of migration time and was proved to be HbE by sequence analysis method. The CE electropherogram of I1 and II3 showed an anomalous band HbE. The mother of the proband (I2) and the III4 and III5 of the family members showed a HbQ-Thailand. The gene results showed that the father (I1) also carried α- and β-thalassemia genes. His genotype was – SEA and β codons26 ; – SEA was inherited to II1, II 3, II5, III 1, and III2, and β codons26 was inherited to II1 and II3. The mother (I2) carries the -α 4.2 gene, which was inherited to II1, II5, III4, and III5.

Conclusion It was complex to diagnose when the thalassemia combined with several abnormal haemoglobin disorders, and we may use various methods to mutual confirmation. Here we found and diagnosed a rare hemoglobin disease in the Hakka population of the Fujian Province. The study provides an important reference for the clinic diagnose and genetic counseling.

Introduction

Hemoglobinopathies are a group of inherited autosomal recessive disorders, including the alteration of hemoglobin quantity (thalassemias) and quality (Hb variants)[1]. They are widely distributed in Mediterranean countries, the Middle East, Africa, and Southeast Asia, including southern China[2–4]. HbQ-Thailand [α74(EF3)Asp→His(α1),GAC□CAC, HBA1:C.223G□C], also known as Hb G-Taichung, Mahidol, Kurashiki-I, and Asabara[5], is an α-globin chain variant that results from a point mutation at codon 74 of the α1-globin gene on chromosome 16p. It commonly appears with a leftward single α-globin gene deletion ($-\alpha^{4.2}$). The heterozygotes of HbQ-Thailand may show minor clinical symptoms or may be asymptomatic, whereas the double heterozygosity of it and thalassemia may lead to several clinical diseases[6, 7]. However, there have been few reports regarding the interaction between HbQ-Thailand and other globin gene disorders. Furthermore, the association among these disorders has important implications in clinical manifestation, laboratory diagnosis, and genetic counseling.

The Hakka people are intriguing Han Chinese populations that primarily reside in southern China, including Guangdong, Fujian, Jiangxi, and Taiwan. However, their cultural background, lifestyle, and customs are different from those of the southern Hans, and they have unique localisms[8]. Here we found a rare family of Hakka people who carry the genes of thalassemia/HbE (HBB:c.79G□A) and HbQ-Thailand, which is an under described condition at present.

Results

The proband, a 35-year-old Chinese female from Longyan City of Fujian Province, displayed anemia with the following hematological parameters: RBC 6.27×10^{12}/L, Hb 93 g/L, MCV 45.9 fl, MCH 148 pg, and MCHC 323 g/L. The automated capillary electrophoresis (CE) (capillarys 2; Sebia, France) showed three dominant peaks, which revealed Hb variants and thalassemia. Most notable observation was that CE revealed a lack of adult hemoglobin, which is the main component in normal adult hemoglobin. Therefore, the proband and her twelve family members were referred to our laboratory for further investigation after obtaining informed consent. They are all Hakkas from Longyan City of Fujian Province. The pedigree of this family is shown in Fig. 1.

The 13 family members had no history of blood transfusion. Fresh peripheral blood samples were collected from all family members using a automated blood cell analyzer for hematological parameters analysis (Bc-5390; Mindray, China), and the results are shown in Table 1. The proband (□1) and her sister (□5) manifested in the obvious microcytic hypochromic anaemia. Analysis of hemoglobin components and variants was performed using a CE hemoglobin analyzer (capillary2™; Sebia, France). The results are shown in Table 1, and the electropherogram is shown in Fig. 2. The CE electropherogram of □1 [Fig. 2 (a)] did not show a normal HbA band, however, there was an abnormal band in the migration time at 185 s, which was confirmed as HbQ-Thailand[9]; the corresponding $HbQA_2$ appeared in the migration time of 260 s. Another exception band appeared at 250 s of migration time and was proved to be HbE[10] inherited from his father. The CE electropherogram of □1 [Fig. 2 (b)] shows an anomalous band HbE in the Z4 area. The peak type of family member □3 is similar to □1. The HbQ-Thailand of the proband originated from his mother (□2), and her (□2) CE electropherogram [Fig. 2 (c)] shows a HbQ-Thailand appearing in the Z7 region and HbQA2 in the Z1 region.The □4 and □5 of family members are similar to the peak patterns. The sister (□5) also has no normal HbA band (the same as the proband), and in her CE electropherogram [Fig. 2 (d)] in addition to HbQ-Thailand and $HbQA_2$; HbQF peak appeared in the migration time 215 s, along with a fast swimming bands of HbH and Hb-Bart's. Figure 2 (e) is the CE electropherogram of the husband of the proband, and the result indicates that there is no presence of abnormal hemoglobin.

Table 1

The hematological and molecular data of the family members under study.

Family members	sex-age (years)	Hb (g/L)	RBC (10^{12}/L)	MCV fL	MCH pg	HbA %	HbA_2 %	HbE %	HbQ-T %	HbQ-A2 %	αGenotype	βGenotype
Hb: hemoglobin; RBC: ed blood cell counts; MCV: mean corpuscular volume; MCH: mean corpuscular Hb; Hb Q-T:HbQ-Thailand												
□1	M-61	121	5.14	75.9	23.6	80.0	3.9	16.1	0	0	$--^{SEA}$/α	$\beta^{codons26}/\beta^{A}$
□2	F-57	148	5.39	99.3	27.5	67.6	1.8	0	29.7	0.9	α/-$\alpha^{4.2-Q-Thailand}$	β^{A}/β^{A}
□1	F-35	93	6.27	45.9	14.8	0	0	11.3	85.2	3.3	$--^{SEA}$/-$\alpha^{4.2-Q-Thailand}$	$\beta^{codons26}/\beta^{A}$
□2	M-36	152	5.07	94.3	30.0	97.2	2.8	0	0	0	α/α	β^{A}/β^{A}
□3	M-34	130	5.71	72.6	22.7	80.6	4.0	15.4	0	0	$--^{SEA}$/α	$\beta^{codons26}/\beta^{A}$
□4	F-33	132	5.12	85.3	30.1	97.0	3.0	0	0	0	α/α	β^{A}/β^{A}
□5	F-29	85	4.26	77.2	20.0	0	0	0	81.1	0.6	$--^{SEA}$/-$\alpha^{4.2-Q-Thailand}$	β^{A}/β^{A}
□6	M-35	158	5.08	93.1	30.8	97.4	2.6	0	0	0	α/α	β^{A}/β^{A}
□1	M-11	118	6.17	61.9	19.1	97.6	2.4	0	0	0	$--^{SEA}$/α	β^{A}/β^{A}
□2	F-9	121	6.07	65.8	20.3	97.7	2.3	0	0	0	$--^{SEA}$/α	β^{A}/β^{A}
□3	M-5	119	4.33	92.6	27.5	97.1	2.9	0	0	0	α/α	β^{A}/β^{A}
□4	F-12	128	5.05	88.9	27.3	68.5	1.9	0	28.8	0.8	α/-$\alpha^{4.2-Q-Thailand}$	β^{A}/β^{A}
□5	M-6	112	4.51	87.1	24.8	68.2	2.0	0	29.0	0.8	α/-$\alpha^{4.2-Q-Thailand}$	β^{A}/β^{A}

The α-thalassemia deletions ($--^{SEA}$/, -$\alpha^{4.2}$/, and -$\alpha^{3.7}$/) commonly found in Chinese populations were typed by gap-PCR. The three non deletional mutations($\alpha^{cs}\alpha$/,$\alpha^{Qs}\alpha$/, and $\alpha\alpha^{ws}$/) and 17 species of β-mutant genes were performed by RDB assay in this subject.The results showed that the father (□1) also carried α- and β-thalassemia genes. His genotype was $--^{SEA}$ and $\beta^{codons26}$; $--^{SEA}$ was inherited to □1, □ 3, □5, □ 1, and □2, and $\beta^{codons26}$ was inherited to □1 and □3. The mother (□2) carries the -$\alpha^{4.2}$ gene, which was inherited to □1, □5, □4, and □5. The hemoglobin variant was identified by sequence analysis method. □1 and □5 were HbQ-Thailand homozygotes; □2, □4 and □5 were HbQ-Thailand heterozygotes, and the sequencing results are shown in Fig. 3. The figures (a) was the father of the proband (□1) sequencing diagram, in the α1-globin gene, the 74th base of G, is the normal sequence. The sequence diagram (b) was the result of the mother of the proband (□2), showing the 74th base of G > C of the α1-globin gene, and the peak type was shown as heterozygote. The sequence diagram (c) was the result of the proband (□1), showing the 74th

base of G > C of the α1- globin gene. It was shown as homozygote. We have summarized the genotypes of all family members with the results of hemoglobin analysis, the genetic tests, and generation sequencing in Table 1.

Discussion

HbQ-Thailand is a hemoglobin variant caused by a point mutation in the α-chain of hemoglobin, commonly associated with the left side deletion of the $-\alpha^{4.2}$ [9]. This is verified by the presence of the HbQ-Thailand in the 5 family members carrying the deletion of $-\alpha^{4.2}$. HbQ-Thailand is generally asymptomatic, or the presence of the deletion of $-\alpha^{4.2}$ may cause microcytic hypochromic anaemia [11]. Family members Ⅱ2, Ⅱ4, and Ⅱ5 carry HbQ-Thailand variant, accompanied with the deletion of $-\alpha^{4.2}$. Ⅱ5 has slight anemia, whereas Ⅱ2 and Ⅱ4 are not characterized by anemia, with hematological parameters in the normal range. The proband (Ⅱ1) and her sister (Ⅱ5) were the coinheritance of HbQ-Thailand with $-\alpha^{4.2}$ and $--^{SEA}$ deletion, they cannot synthesize normal alpha globin, owing to the absence of a normal alpha gene. They presented microcytic hypochromic anemia. Because of the deletion of 3 α- globin gene ($--^{SEA}/-\alpha^{4.2}$) and the HbQ-Thailand point mutation, only a small number of mutated alpha-globin chains were synthesized, creating a relative surplus of β-globin category, forming HbH (β4) and the HB-Bart's (γ4); thus, the CE electropherogram shows two fast electrophoresis bands. In contrast, the proband (Ⅱ1) is also carrying the $\beta^{codons26}$ point mutation. In this individual, the protein α-chain synthesis decreased and simultaneously, the β-chain synthesis decreased; hence, her anemia severity was less than that of the younger sister (Ⅱ5). Thus, the combination of α- and β- thalassemia, leads to more mildly anemia than a α- thalassemia.

In general, HbQ-Thailand ($\alpha^{Q}_2\beta_2$) is shown as a special peak presented at the Z7 region in the CE electropherogram, while $HbQA_2$ ($\alpha^{Q}_2\delta_2$) is shown as a special peak presented at the zone1 region [Figure 2 (c)]. If the HbA and the zone7 band appear simultaneously, the individual is likely to be heterozygous for HbQ-Thailand, while the homozygous is missing the HbA. In this family, the CE electropherogram of Ⅱ2, Ⅱ4, and Ⅱ5 showed two peaks, HbA and HbQ-Thailand, which were manifestations of heterozygous mutations.The CE electropherogram of Ⅱ1 and Ⅱ5 did not show HbA peak, and only a high and sharp peak in a migration time of 185 s. Due to the absence of HbA and HbA_2 peaks, it was difficult to determine the specific zone corresponding to the anomaly in the original electropherogram; hence, the two specimens were mixed with the quality control products for testing to determine the zone position [12]; the obtained results were then combined with the results of genetic testing, to determine the genotype of $--^{SEA}/-\alpha^{4.2-Q-Thailand}$. The absence of normal alpha globin chains in their erythrocytes result in the absence of HbA and HbA_2. All specimens were tested by sequencing to verify the existence of mutations in HbQ-Thailand, of which Ⅱ2, Ⅱ4, and Ⅱ5 showed double peaks. Ⅱ1 and Ⅱ5 samples had single peaks. It verified the results of the CE electropherogram. It is noteworthy that when the alpha chain is mutated, the mutated alpha chain binds to the δ chain, forming a $HbQA_2$ ($\alpha^{Q}_2\delta_2$), which may cause false reduction of HbA2 ($\alpha_2\delta_2$), resulting in an α- thalassemia misdiagnosis or β- thalassemia missed diagnosis. Therefore, in the CE

electropherogram, HbA_2 and $HbQA_2$ should be superimposed for the evaluation of the thalassemia. In general, in α- thalassemia patients with decreased α-chain synthesis, hemoglobin analysis showed a decline in HbA_2 ($\alpha_2\delta_2$). β-thalassemia may lead to a increasing in the level of Hb A_2($\alpha_2\delta_2$), because of the reduction of β-chain and the increasing of δ-chain and γ-chain accordingly. However, when β- thalassemia was combined with α- thalassemia and/or α -chain variant, the compound of α-chain and β-chain was reduced concurrently, which could lead to a normal HbA_2 ratio. It may cause potential pitfalls for thalassemia. So, when screening of the thalassemia by hemoglobin analysis, we must pay attention, even if it is a normal HbA_2, we cannot completely eliminate the possibility of thalassemia. The results of hemoglobin analysis should be evaluated with the results of assessment of erythrocyte parameters and the clinical characteristics of the patients, and a systematic diagnosis of thalassemia and hemoglobin diseases should be performed using gene testing and sequencing.

All the members of the family who participated in the study were from Longyan, Fujian Province (□2 was from Changting County, □4 was from Liancheng County, and the rest were from Yongding County); all the participants were Hakkas. Hakkas in Guangdong, Fujian, Jiangxi, and Taiwan, being the southern ancient Han immigrant groups, regardless of factors such as language, folklore, and lifestyle, appear particularly mysterious. Here we found and diagnosed a rare hemoglobin disease in the Hakka population of the Fujian Province. This study provides an important reference for the clinic diagnose and genetic counseling of thalassemia and hemoglobin diseases in Hakka communities.

Conclusion

It was complex to diagnose when the thalassemia combined with several abnormal haemoglobin disorders, and we may use various methods to mutual confirmation. Here we found and diagnosed a rare hemoglobin disease in the Hakka population of the Fujian Province. The study provides an important reference for the clinic diagnose and genetic counseling.

Materials And Methods

Fresh peripheral blood samples were collected from the proband and her family members using a automated blood cell analyzer for hematological parameters analysis (Bc-5390; Mindray, China). Analysis of hemoglobin components and variants was performed using a CE hemoglobin analyzer (capillary2™; Sebia, France). The α-thalassemia deletions (--^{SEA}/, -$\alpha^{4.2}$/, and -$\alpha^{3.7}$/) commonly found in Chinese populations were typed by gap-PCR. The three non deletional mutations($\alpha^{cs}\alpha$/,$\alpha^{Qs}\alpha$/, and $\alpha\alpha^{ws}$/) and 17 species of β-mutant genes were performed by RDB assay in this subject. And the hemoglobin variants were identified by sequence analysis method.

Abbreviations

Hb

hemoglobin; RBC:red blood cell; MCV:Mean Corpuscular Volume; MCH:Mean Corpuscular Hemoglobin; MCHC:Mean Corpuscular Hemoglobin Concentration; CE:capillary electrophoresis; RDB:reverse dot blot;

Declarations

Acknowledgements

Not applicable.

Authors' contributions

Lin Zheng, Hailong Huang and Xiaoqing Wu prepared the main manuscript; Lin Zheng, Qingmei Shen and Meihuan Chen prepared the experiment. All authors have read and approved the final article.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The present study was approved by the Protection of Human Ethics Committee of Fujian Maternity and Child Health Hospital, affiliated hospital of Fujian Medical University (NO.2016-101).

Consent for publication

Written informed consents were obtained from the patients for publication of this manuscript.

Competing interests

The authors declare that they have no competing interests.

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Figures

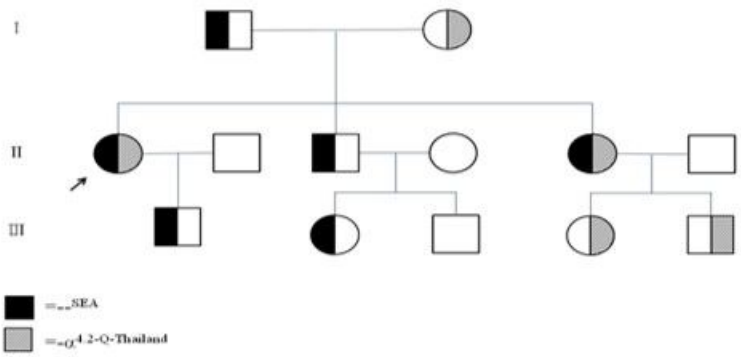

Figure1. Pedigree of the family

Figure 1

Pedigree of the family

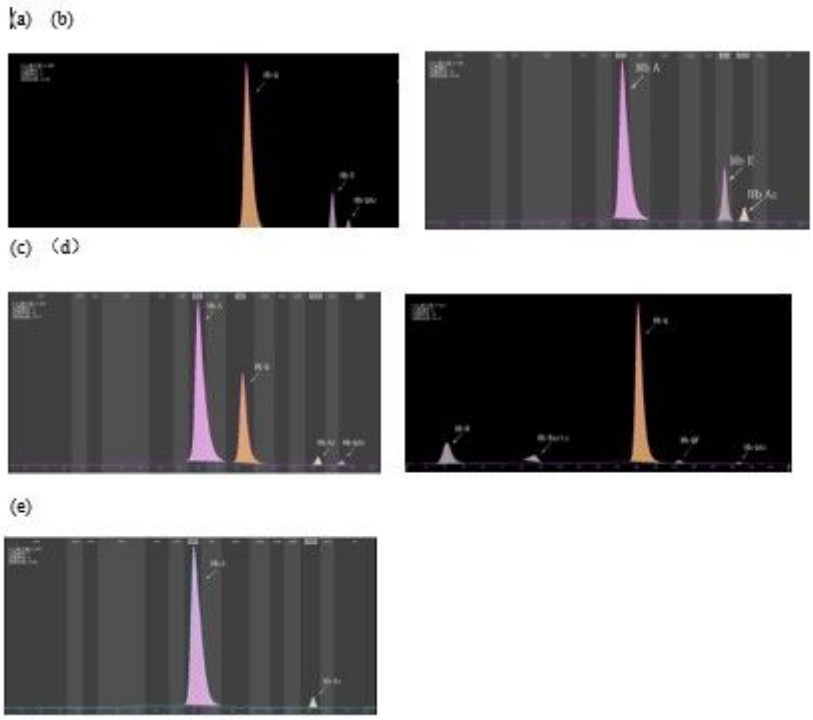

Figure 2

Hemoglobin analysis of the proband and family members using CE. (a) The proband; (b) the father; (c) the mother; (d) the sister; (e) the husband. HbA, HbQ-Thailand, HbA2, HbQA2, HbQF, HbE, HbH, and HB-Bart's are indicated by arrows.

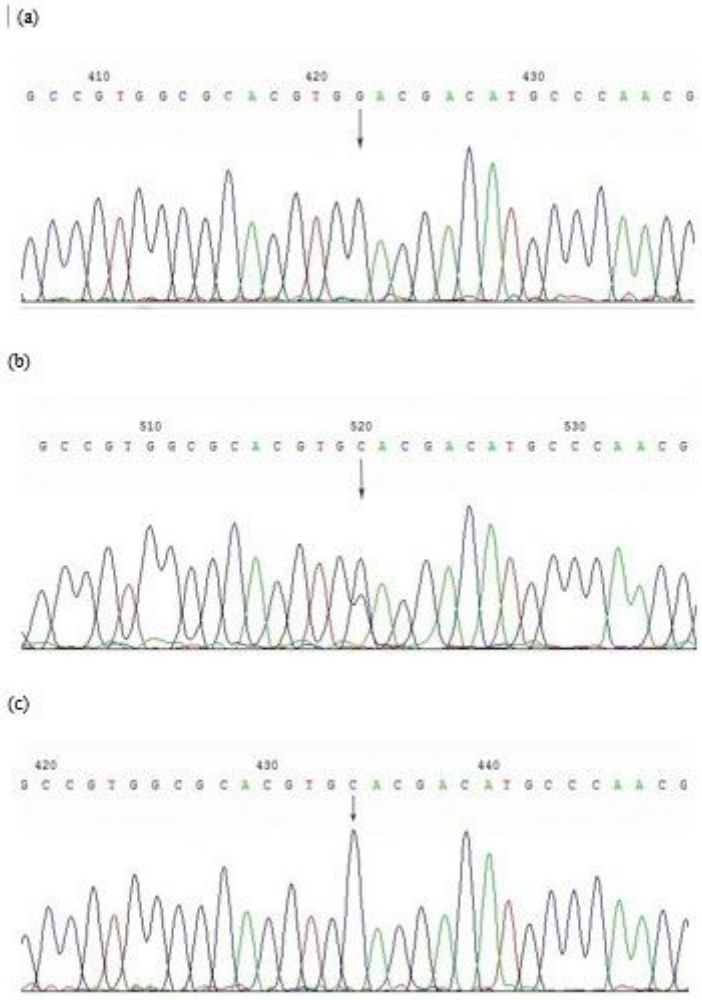

Figure 3

DNA sequence analysis of the amplified α1-globin gene from the proband and her family members. (a) The father; (b) the mother; (c) the proband. The downward arrow indicated the G□C substitution at codon 74 of the α1-globin gene.