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Original Article

Frequencies and hematological manifestations of the HKαα allele in southern Chinese population

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Abstract: Introduction: The HKαα (Hong Kong αα) allele containing both the -$\alpha^{3.7}$ and ααα$^{anti4.2}$ is an unusual rearrangement of the α globin gene cluster. Currently, ααα$^{anti4.2}$ fragments cannot be detected through the routine thalassemia diagnostic kit. The genotype of -$\alpha^{3.7}$/αα, HKαα/-$\alpha^{3.7}$ and HKαα/αα is entirely the result of -$\alpha^{3.7}$/αα by the gap-polymerase chain reaction (gap-PCR), which would cause some cases of the HKαα allele to be mistaken as -$\alpha^{3.7}$/αα. Methods: Genetic diagnosis was performed in 17166 cases, using single PCR and two-round nested PCR to detect the HKαα allele in 895 cases. It showed -$\alpha^{3.7}$ and α2 bands by gap-PCR, and reverse dot-blot assays were performed to detect the non-deletional α thalassemia point mutations and β thalassemia point mutations. Results: The HKαα allele was found in 56 samples. The rates of HKαα/αα, HKαα/-$\alpha^{3.7}$ and HKαα/--^{SEA} were 4.92%, 0.67% and 0.67%. The rate of the HKαα allele was 0.33% in a southern Chinese population. In the HKαα/αα thalassemia group, the levels of hemoglobin (Hb), mean cell volume (MCV), and mean cell hemoglobin (MCH) were similar to the -$\alpha^{3.7}$/αα thalassemia group. The hematology of the HKαα/--^{SEA} carriers was similar with the α^0 thalassemia carriers, and Hb is usually over 100 g/l. The two families carrying HKαα/--^{SEA} genotype chose to retain the fetuses in our study. Conclusions: There was a certain proportion of the HKαα allele in southern Chinese population, and the hematologic manifestations were mild. Differential diagnosis of HKαα genotype from -$\alpha^{3.7}$/αα can provide more accurate genetic diagnosis and clinical thalassemia genetic counseling.

Keywords: Two-round nested polymerase chain reaction, α thalassemia, Hong Kong αα, hematology, prenatal diagnosis

Introduction

α-Thalassemia is one of the most common inherited disorders of hemoglobin synthesis, and small cell hypopigmentation anemia is the main feature in humans. the Southeast Asian deletion (--^{SEA}), the rightward deletion (-$\alpha^{3.7}$) and the leftward deletion (-$\alpha^{4.2}$) account for most α-thalassemia in China [1, 2]. The HKαα allele, as a rare type of thalassemia, is α globin gene cluster that contains both the -$\alpha^{3.7}$ and ααα$^{anti4.2}$ fragments by recombination [3]. Currently ααα$^{anti4.2}$ fragments cannot be detected through the routine thalassemia diagnostic kit. When the result shows -$\alpha^{3.7}$/αα by the gap-PCR, the real result may be -$\alpha^{3.7}$/αα, HKαα/-$\alpha^{3.7}$ or HKαα/αα. In our study, we identified the HKαα allele in -$\alpha^{3.7}$ carriers by single PCR and two-round nested PCR. Differential diagnosis of the HKαα allele and -$\alpha^{3.7}$/αα would provide more accurate diagnosis and genetic counseling for patients.

Material and methods

17,166 samples for routine thalassemia gene diagnosis were recruited from 1-2016 to 2-2019, at Fujian Provincial Maternity and Children's Hospital, Fuzhou, Fujian Province, People's Republic of China (PRC). This study was approved by the Clinical Ethics Committee of the Fujian Provincial Maternity and Children's Hospital.

Genomic DNA was extracted from peripheral blood using the QIAGEN DNA Mini Kit (QIAGEN). Gap-PCR for four deletional α-thalassemia alleles was carried out and the reverse dot-blot assays were carried out to detect three non-deletional α-thalassemia point mutations and seventeen β-thalassemia mutations. The HKαα alleles were detected by single PCR and nested PCR [3]. Routine hematological screening was determined with an automated red blood cell counter (Sysmex XS-800i; Sysmex, Tokyo,

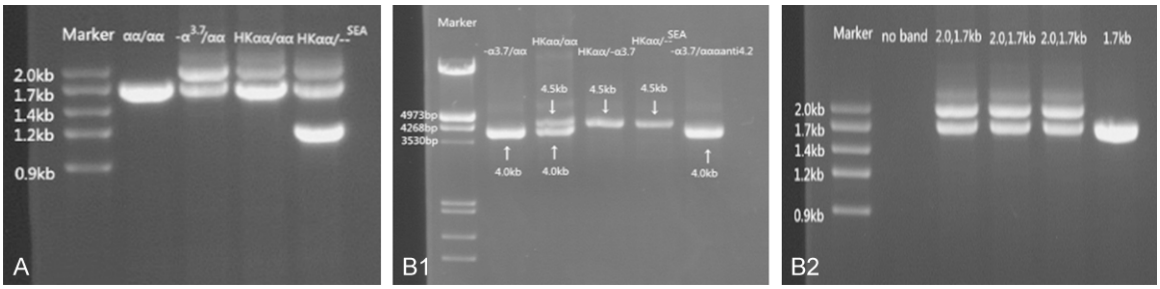

Figure 1. Electrophoretogram of the HKαα allele by PCR assays. A. Results of the gap-PCR of the HKαα allele. B. Results of the two-round nested PCR of the HKαα allele. B1: The first-round PCR. B2: The second-round PCR.

Japan). Hemoglobin electrophoresis was done with a Helena REP automated electrophoresis instrument (VARIANT-Ⅱ, BIO-RAD, USA).

Results

Single PCR was used to detect $\alpha\alpha\alpha^{anti4.2}$ fragments in 889 cases of $-\alpha^{3.7}/\alpha\alpha$ and 6 cases showed the presence of $-\alpha^{3.7}$, $--^{SEA}$ and normal α2 alleles, were positive in 57 samples. Further detection using two-round nested PCR revealed 44 cases of HKαα/αα, 6 cases of HKαα/$-\alpha^{3.7}$, and 6 cases showed the presence of $-\alpha^{3.7}$, $--^{SEA}$ and normal α2 alleles were all the result of HKαα/$--^{SEA}$, and there was 1 case of $-\alpha^{3.7}/\alpha\alpha\alpha^{anti4.2}$. Interestingly, all cases with HKαα/αα genotype and $-\alpha^{3.7}/\alpha\alpha\alpha^{anti4.2}$ genotype showed an α2 band that was thicker than the $-\alpha^{3.7}$ band (**Figure 1**). In 57 cases, 5 cases of CD41/42 (HBB: c.126_129delCTTT) and 1 case of IVS-II-654 (HBB: c.316_197C>T) were detected in HKαα/αα. The hematologic data of the 56 cases carrying the HKαα allele are summarized (**Tables 1**, **2**).

Discussion

The HKαα allele is α globin gene cluster rearrangement containing both the $-\alpha^{3.7}$ and $\alpha\alpha\alpha^{anti4.2}$ crossover junctions. The rate of the HKαα allele in the Guangxi region of China was 0.07% [4], and 2.27% in $-\alpha^{3.7}$ positive samples of Guangdong province [5]. In our study, we found the HKαα allele rate was 0.33% in the population of Fujian Province, and 6.26% in $-\alpha^{3.7}$ positive samples.

In this study, it was interesting to find that in the $-\alpha^{3.7}/\alpha\alpha$ samples, some $-\alpha^{3.7}$ bands were thinner than α2 bands; of these, 44 were identified as the HKαα allele, and one was confirmed to be $-\alpha^{3.7}/\alpha\alpha\alpha^{anti4.2}$ genotype. The reason for the phenomenon may due to the HKαα allele fragments containing one α2 allele and one $-\alpha^{3.7}$ allele through rearrangement, and the wild type α gene contains one α2 allele. Thus, as seen from the electrophoresis diagram, the $-\alpha^{3.7}$ band was thinner than α2 band. The $-\alpha^{3.7}/\alpha\alpha\alpha^{anti4.2}$ was also result of $-\alpha^{3.7}$ bands thinner than α2 bands too. Studies showed that $\alpha\alpha\alpha^{anti4.2}$ was common in the world [6, 7], but it cannot be detected directly by routine thalassemia diagnostic kit. If the results showed $-\alpha^{3.7}/\alpha\alpha$ by gap-PCR, the real results may be -α3.7/αα, HKαα/$-\alpha^{3.7}$ or HKαα/αα. It is suggested that to detect the $\alpha\alpha\alpha^{anti4.2}$ fragments, especially when the $-\alpha^{3.7}$ band was thinner than α2 band by gap-PCR, that two-round nested PCR should be recommended to determine whether it is the HKαα allele.

Since $-\alpha^{3.7}$ has only one α2-α1 fusion gene, and the HKαα allele has a complete α2 gene and an α2-α1 fusion gene, the symptoms of anemia of the HKαα allele carriers may be milder than for the $-\alpha^{3.7}/\alpha\alpha$ carriers. The phenotypes of HKαα/αα carriers and $-\alpha^{3.7}/\alpha\alpha$ carriers were similar in our study, showing small cell hyperpigmented anemia or normal hematologic manifestations. But it is worth mentioning that when one parent has α^0 thalassemia, such as $--^{SEA}$ genotype, and another is carrying $-\alpha^{3.7}/\alpha\alpha$, their children have one-quarter probability of becoming patients with Hb H disease. Hb H disease has significant heterogeneity. Some patients have only mild symptoms of anemia, but some patients may need irregular blood transfusions to maintain life. However, the hematology of the HKαα/$--^{SEA}$ carriers are similar to $--^{SEA}/\alpha\alpha$ carriers, so it is recommend that a fetus that is carrying HKαα/$--^{SEA}$ genotype should be retained instead of inducing labor [4, 5, 8-13]. In our study, the Hb of HKαα/$--^{SEA}$ carriers were all over 100 g/l, and the two families with the HKαα/$--^{SEA}$ genotype chose to retain the fetuses in our study.

Table 1. Genotype and hematological data of cases carrying the HKαα allele

Case	Sex-Age	Hb (g/dL)	MCV (fL)	MCH (pg)	Hb A2 (%)	α Genotype	β Genotype
1*	F-26	125	78.4	29.1	2.7	HKαα/αα	β^N/β^N
2*	F-30	120	83.5	29.6	2.7	HKαα/αα	β^N/β^N
3	F-25	118	76.7	25.6	2.4	HKαα/αα	β^N/β^N
4	M-28	140	83.1	28.8	2.6	HKαα/αα	β^N/β^N
5	M-3	-	-	-	-	HKαα/αα	β^N/β^N
6*	F-24	90	84.4	27.5	2.2	HKαα/αα	β^N/β^N
7*	F-31	104	75.3	24	2.3	HKαα/αα	β^N/β^N
8	M-28	159	84.8	29.8	2.6	HKαα/αα	β^N/β^N
9	F-27	119	80	27.3	2.4	HKαα/αα	β^N/β^N
10*	F-32	113	70.9	24.7	2.5	HKαα/αα	β^N/β^N
11	M-29	147	74.8	27.9	2.7	HKαα/αα	β^N/β^N
12*	F-30	127	84.1	28.5	2.6	HKαα/αα	β^N/β^N
13	M-26	145	79.0	27.0	3.1	HKαα/αα	β^N/β^N
14*	F-30	103	88	29.4	2.6	HKαα/αα	β^N/β^N
15	F-23	125	78.8	26.8	2.3	HKαα/αα	β^N/β^N
16	F-52	91	72.9	19.7	2.2	HKαα/αα	β^N/β^N
17	M-25	146	93	29.8	2.6	HKαα/αα	β^N/β^N
18	M-42	139	82.2	27.9	2.9	HKαα/αα	β^N/β^N
19*	F-30	105	80.8	26.1	2.4	HKαα/αα	β^N/β^N
20*	F-22	108	75.8	25.8	2.5	HKαα/αα	β^N/β^N
21*	F-31	123	81.2	29.6	2.7	HKαα/αα	β^N/β^N
22*	F-27	105	74.4	22.8	2.1	HKαα/αα	β^N/β^N
23*	F-34	131	81.6	28	3	HKαα/αα	β^N/β^N
24*	F-29	133.1	79.4	28.9	2.9	HKαα/αα	β^N/β^N
25*	F-27	116	80.1	27.1	2.6	HKαα/αα	β^N/β^N
26*	F-25	108	89.4	30.2	2.4	HKαα/αα	β^N/β^N
27*	F-26	120	82.0	27.3	2.6	HKαα/αα	β^N/β^N
28*	F-30	107	80.2	27.6	2.7	HKαα/αα	β^N/β^N
29	F-38	75	61.9	18.7	2.4	HKαα/αα	β^N/β^N
30	F-28	114	89.6	31.2	2.6	HKαα/αα	β^N/β^N
31	M-28	141	93.1	29.4	2.6	HKαα/αα	β^N/β^N
32	M-23	147	92.2	29.1	2.4	HKαα/αα	β^N/β^N
33	F-47	93	75.3	22.1	2.5	HKαα/αα	β^N/β^N
34*	F-29	125	78.8	25.3	2.6	HKαα/αα	β^N/β^N
35	F-4	-	-	-	-	HKαα/αα	β^N/β^N
36	F-4	-	-	-	-	HKαα/αα	β^N/β^N
37*	F-26	107	59.4	19.2	5.8	HKαα/αα	$\beta^{Codons\ 41/42}/\beta^N$
38	M-3	-	-	-	-	HKαα/αα	$\beta^{Codons\ 41/42}/\beta^N$
39	F-28	95	72	23.9	5.8	HKαα/αα	$\beta^{Codons\ 41/42}/\beta^N$
40*	F-28	109	62.2	23.2	5.7	HKαα/αα	$\beta^{Codons\ 41/42}/\beta^N$
41*	F-34	79	66.9	21.4	5.4	HKαα/αα	$\beta^{Codons\ 41/42}/\beta^N$
42*	F-43	90	63.2	20.7	5.1	HKαα/αα	$\beta^{IVS\text{-}II\text{-}654}/\beta^N$
43	F-25	126	80.3	27.9	2.3	HKαα/-$\alpha^{3.7}$	β^N/β^N
44	F-25	132	79.6	26.9	2.2	HKαα/-$\alpha^{3.7}$	β^N/β^N
45	M-32	143	75.6	26	2.3	HKαα/-$\alpha^{3.7}$	β^N/β^N
46*	F-26	125	80.4	26.6	3.1	HKαα/-$\alpha^{3.7}$	β^N/β^N
47	M-4	123	80.2	27.3	2.9	HKαα/-$\alpha^{3.7}$	β^N/β^N

48	F-3	125	79.9	28.1	2.8	HKαα/-$\alpha^{3.7}$	β^N/β^N
49	F-42	116	71.9	21.8	2.7	HKαα/--^{SEA}	β^N/β^N
50	F-27	119	66.4	30.1	2.4	HKαα/--^{SEA}	β^N/β^N
51	F-2	108	73	20.7	2.5	HKαα/--^{SEA}	β^N/β^N

Hb: hemoglobin; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; HbA_2: hemoglobin A_2. β^N/β^N: normal β genotype; codons 41/42: HBB:c.126_129delCTTT; IVS-II-654: HBB: c.316-197C4T; -: no data available. *These cases were pregnant at the time of our study.

Table 2. Genotype and hematological data of families carrying the HKαα allele

family	Family members	Age	Hb (g/dL)	MCV (fL)	MCH (pg)	HbA2 (%)	α Genotype	β Genotype
1	Mother*	20	106	68.9	21.9	2.3	--^{SEA}/αα	β^N/β^N
	Father	20	160	85.6	28.5	2.5	HKαα/αα	β^N/β^N
	Fetus (cord blood)	0	132	94.9	30.8	0.0	HKαα/--^{SEA}	β^N/β^N
2	Mother*	30	94	58.5	17.3	1.4	-$\alpha^{4.2}$/--^{SEA}	β^N/β^N
	Father	34	141	87.4	31.3	2.3	HKαα/αα	β^N/β^N
	Son	7	116	58.6	19.7	2.2	HKαα/--^{SEA}	β^N/β^N
	Fetus (amniocyte)	0	-	-	-	-	HKαα/--^{SEA}	β^N/β^N

*These cases were pregnant at the time of our study.

In conclusion, differential diagnosis of -$\alpha^{3.7}$/αα and HKαα genotypes can provide more accurate genetic diagnosis of thalassemia for clinical counseling.

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Disclosure of conflict of interest

None.

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