Significance of genetic counseling and prenatal diagnosis on α2 codon 30 (-GAG) (HBA2: c.91_93delGAG) mutation

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Research article

Keywords: codon 30 mutation, Hb H disease, genetic counseling, prenatal diagnosis, DNA sequencing

Posted Date: October 16th, 2019

DOI: https://doi.org/10.21203/rs.2.16147/v1

Abstract

Background : Non-deletional hemoglobin (Hb) H disease is the severest form of α- thalassemia (thal) compatible with post-natal life, which is caused by the interaction of an α-globin gene mutation with α 0 -thal. Therefore, it is important to identify rare α-globin gene mutations for the prevention of severe form of non-deletional Hb H disease .

Methods: In all, 61,796 samples were characterized at our center. Common α- and β- thalassemia mutations were detected by routine DNA analysis (gap-PCR and PCR-RDB). The DNA sequencing of α-globin genes was performed to identify the unknown mutation. Statistical analyses were conducted using SPSS 19.0 statistical software. Results: Of the 61,796 samples, eight were identified as α2 codon 30 (-GAG) (HBA2 : c.91_93delGAG) mutation s, and of these, four had coinheritance with - - SEA deletion Patients with the heterozygous α2 codon 30 (-GAG) (HBA2 : c.91_93delGAG) mutation had significantly lower levels of MCV and MCH than healthy individuals ($p < 0.01$), and coinheritance with - - SEA deletion aggravated the α-thal phenotype , associated with severe Hb H disease . Moreover, a significant difference in the clinical severity was found in the Hb H disease patients with the same genotype.

Conclusions: This finding is of great significance for clinicians to provide accurate genetic counseling , particularly prenatal diagnosis and establish a rigorous diagnostic procedure .

Background

α-Thal is caused by an α-globin gene deficiency. In general, the clinical severity in individuals with α-thal ranges from asymptomatic to lethal, and the severity is associated with the number of defective α-globin genes [1, 2]. According to the type of gene change, it can be divided into deletion α-thal and non-deletion α-thal. The former is caused by a large fragment deletion of the α-globin gene and is the main variant type. The latter is caused by a point mutation of the α-globin gene or its regulatory sequence (including single-base substitution and deletion or insertion of one or more nucleotides) [3].

Hb H disease is the severest form of α-thal compatible with post-natal life and occurs when α-globin gene mutations interact to reduce the α-globin chain synthesis to approximately one-quarter of the normal levels [4]. As a result of the defect of three α-globin genes, the synthesis rate of the α-globin chain decreases obviously. The excess β-globin chain is formed tetramer (Hb H inclusion bodies) in erythrocytes and causes chronic hemolytic anemia [5]. Genetically, there are two types of this disease. The most common is the ‘deletional Hb H disease', in which three out of four α-globin genes are removed by deletions. The second type is the 'non-deletional Hb H disease’, which is a combination of deletional and non-deletional defects. Generally, patients with non-deletional Hb H disease present with a more severe phenotype than those with deletional Hb H disease [6]. Some patients may have severe anemia with hepatomegaly and splenomegaly and need periodic transfusions, or even develop the hydrops fetalis syndrome *in utero* [7–10].

In this research, we studied α2 codon 30 (-GAG) (*HBA2:* c.91_93delGAG) mutation and its significance with respect to genetic counseling when it is in combination with α^0-thal.

Methods

Samples and hematological analysis

All the samples analyzed in this study were from individuals who underwent a diagnosis of thalassemia from 2014 to 2018 at The First Women and Children’s Hospital of Huizhou, Huizhou, Guangdong Province, People’s Republic of China. All subjects provided written informed consent. Fresh peripheral blood was collected using EDTA as an anticoagulant, and hematological indices were measured by an automatic analyzer XN–10 (Sysmex; Kobe, Japan). A hemoglobin analysis was determined using a capillary electrophoresis (CE) system (Capillarys2 Flex Piercing; Sebia, Lisses, France).

DNA analysis

Genomic DNA was extracted from peripheral blood leucocytes using a standard phenol chloroform technique. Three common Chinese α-globin gene deletion mutations (- $-^{SEA}$, $-\alpha^{3.7}$, and $-\alpha^{4.2}$) were detected using a gap-polymerase chain reaction (gap-PCR), while three common non-deletional α-thal mutations and 17 common β-thalassemia mutations were detected by the PCR reverse dot-

blot assay. All the DNA samples were collected, which came from individuals who underwent a normal detection by a routine DNA analysis except for a slight anemia and whose spouses were the carriers of α-thal. In addition, the DNA samples were collected because of the obvious discrepancy between phenotypes and genotypes in the individuals with the α-thal trait.

All the collected DNA samples were used in the identification of the unknown mutation by a direct DNA sequencing of the α1- and α2-globin genes.

Statistical analysis

Statistical analyses were conducted using SPSS 19.0 statistical software. The parameters of normal distribution were described as mean ± standard deviation (SD), while of non-normal distribution were represented as median. Wilcoxon rank sum test was applied for continuous values with non-normal distribution. $p < 0.05$ was considered statistically significant.

Results

General findings

The study population included 61,796 subjects who underwent a diagnosis of thalassemia. Among these, we collected 309 DNA samples that conformed to the aforementioned conditions. Of the 309 samples, eight were identified as α2 codon 30 (-GAG) (*HBA2:* c.91_93delGAG) mutations (Fig. 1), including one pedigree, and of these, four had coinheritance with $--^{SEA}$ deletion. The hematological parameters of samples from individuals with the α2 codon 30 (-GAG) (*HBA2:* c.91_93delGAG) mutation are presented in Table 1.

Medical histories

P1 (Table 1) was known to have moderately severe anemia and jaundice, and was transferred to the neonatology department for therapy at the local hospital. Afterwards, he was referred to us for further investigation at the age of three months. A molecular analysis of the α-globin genes revealed the genotype of $\alpha^{codon\ 30\ (-GAG)}\alpha/--^{SEA}$. When he was two and a half years old, he was diagnosed with developmental disorders of speech and language and mild growth retardation. He was asked to undergo a rehabilitation of speech and language and follow-ups. His mother was a carrier of α2 codon 30 (-GAG) (*HBA2:* c.91_93delGAG) mutation (P8, Table 1).

P2 (Table 1) was known to have been pale since birth, and a diagnosis of mild α-thal was made at a regional hospital when he was two months old. At the age of six, he was referred to our pediatrics out-patient clinic because of cough and recurrent fever due to unknown causes, whereafter he was admitted to the hospital for therapy. He had moderately severe anemia, jaundice, but no hepatosplenomegaly. When he was in hospital, his anemia worsened with his hemoglobin level reducing from 79 g/dL to 68 g/dL in only three days. Then, he underwent a further investigation of thalassemia. Using a molecular analysis, the patient was found to have a compound heterozygote of $--^{SEA}$ and α2 codon 30 (-GAG) (*HBA2:* c.91_93delGAG) mutation; thus, he was diagnosed with Hb H disease. Subsequently, he received blood transfusion with his parents' informed consent. With blood transfusion, his anemia improved, and the hemoglobin level increased to 114 g/dL. The next day, he was discharged from the hospital with improvement after treatment. He has to report back for a further follow-up.

P3 (Table 1) was an adult male who had mild hypochromic microcytic anemia. He was known to have been pale since birth, and a diagnosis of Hb H disease was made during his partner's pregnancy examination for thalassemia. His physical and mental development was normal, except for mild anemia. He did not require a blood transfusion.

P4 (Table 1) presented with anemia, and a tentative diagnosis of iron-deficiency anemia was made. She had received few blood transfusions occasionally to correct the symptoms of anemia in the absence of a definitive diagnosis. At the age of 23, she was referred to us, and a definitive diagnosis of Hb H disease was made. She had mild anemia and iron overload with serum ferritin increasing to the level of 350.6 ng/mL.

P5 (Table 1) had mild anemia like the carrier of - -^{SEA}, and P6, P7, and P8 (Table 1) had erythrocyte microcytosis. All the four patients' medical histories were not special.

Heterozygote for α2 codon 30 (-GAG) (HBA2: c.91_93delGAG) mutation

A total of 43 carriers with α-globin gene mutations and 30 healthy individuals were enrolled as control in the present study, while three carriers with the α2 codon 30 (-GAG) (*HBA2:* c.91_93delGAG) mutation were enrolled as experimental group. Among the 43 carriers with α-globin gene mutations, 30 had α^{QS}α mutation; and the rest (n = 13) had the α2 codon 30 (GAG>CAG) (*HBA2:* c.91G>C) mutation (Table 2). Within the experimental group, all the carriers were found had erythrocyte microcytosis, they had significantly lower levels of MCV and MCH than those with the heterozygous α2 codon 30 (GAG>CAG) (*HBA2:* c.91G>C) mutation and healthy individuals ($p < 0.01$). However, there were no statistically significant differences on hematological indices between the carriers with the α2 codon 30 (-GAG) (*HBA2:* c.91_93delGAG) mutation and α^{QS}α mutation ($p > 0.05$).

Compound heterozygous of - -^{SEA} and α2 codon 30 (-GAG) (HBA2: c.91_93delGAG) mutation

A total of 56 α-thalassemic and four patients with α $^{codon\ 30\ (-GAG)}$ α/- -^{SEA} genotype were enrolled as control and experimental group in the present study, respectively. Within the control group, there were 20 patients with α^{QS}α/- -^{SEA} genotype; and 36 patients with - -^{SEA}/αα genotype (Table 3). Within the experimental group, their Hb and MCH levels were significantly lower than that of control group with - -^{SEA}/αα genotype ($p < 0.01$). Between the patients with α $^{codon\ 30\ (-GAG)}$ α/- -^{SEA} genotype and α^{QS}α/- -^{SEA} genotype, the values of hematological indices were compared and found no statistically significant differences ($p > 0.05$).

Discussion

In our study, we described the α2 codon 30 (-GAG) (*HBA2:* c.91_93delGAG) mutation, and that inheriting it led to individuals with erythrocyte microcytosis. Heterozygotes for α2 codon 30 (-GAG) (*HBA2:* c.91_93delGAG) mutation affecting the predominant α2 gene appeared to have hematological features of the α-thal trait (low MCV and MCH), and interaction with α^{+}- or α^{0}-thal aggravated the α-thal phenotype. In our study, the phenotype of compound heterozygous of α2 codon 30 (-GAG) (*HBA2:* c.91_93delGAG) and -α$^{4.2}$ is similar to heterozygous of deletion - -^{SEA}, compatible with the interaction.

It has been reported that an Hb H hydrops fetalis with a coinheritance of ζ-α-thal–1 deletion and α2 codon 30 (-GAG) (*HBA2:* c.91_93delGAG) mutation who died at birth in Hong Kong [11]. In contrast to this report of Hb H hydrops fetalis, three reports from southern China and Hong Kong on Hb H disease patients with a coinheritance of - -^{SEA}α-thal and α2 codon 30 (-GAG) (*HBA2:* c.91_93delGAG) mutation stated that all the patients had survived to adulthood [12–14].

In our four patients with a coinheritance of - -^{SEA} α-thal and the α2 codon 30 (-GAG) (*HBA2:* c.91_93delGAG) defect, two were adults. The intact ζ-globin genes could have led to less chain imbalance in fetal life, less severe anemia, and a milder phenotype. We inferred that the mutation of α2 codon 30 (-GAG) (*HBA2:* c.91_93delGAG) did not affect the splice site consensus sequence in the region, as the last two nucleotides (AG) of the exon and the first six nucleotides in the intervening sequence remained intact, but resulted in an α-globin chain of one less amino acid (α140 as opposed to α141) [11]. The mutation might produce a highly unstable α-globin variant and tend to degrade rapidly after translation, resulting in a reduced level of the α-globin chain. Further, because of its high instability, the α-globin variant was hardly detected by the CE system.

However, in our four patients with Hb H disease, two were children, who had more severe clinical manifestations. One child had developmental disorders of speech and language and mild growth retardation, and the other child received blood transfusion. These severe clinical manifestations are compatible with severe form of non-deletional Hb H diseases. Younger Hb H disease patients may have growth retardation, retardation in brain growth and oedema as described in the literature by Farashi *et al.* [15], which may affect nervous system and development of speech and language. These findings suggested that the α2 codon 30 (-GAG) (*HBA2:* c.91_93delGAG) mutation might not only lead to a reduced level of the α-globin chain but also have other effects altering the red blood cell metabolism, leading to ineffective erythropoiesis, resulting in the severe anemia observed in the two children. In addition, a high level of Hb H is generally associated with clinical severity in Hb H disease, because Hb H tends to decompose into free β-globin chains easily and accumulate to form Hb H inclusion bodies in erythrocytes, thus causing erythrocyte membrane oxidative damage [16, 17]

Every individual with the α2 codon 30 (-GAG) (*HBA2:* c.91_93delGAG) mutation had the similar clinical phenotype to that with the $\alpha^{QS}\alpha$ mutation, while every Hb H disease patient with $\alpha^{\text{codon 30 (-GAG)}}\alpha/--^{SEA}$ genotype had the similar clinical phenotype to that with $\alpha^{QS}\alpha/--^{SEA}$ genotype. In addition, in our study, we found that the clinical severity was different even among patients with the same genotype. This might be attributed to the complex interaction between the genetic and the environmental factors. Therefore, we supposed that the α2 codon 30 (-GAG) (*HBA2:* c.91_93delGAG) mutation was similar to the $\alpha^{QS}\alpha$ mutation [18–20]. Indeed, we audaciously supposed that an Hb H hydrops fetalis with a coinheritance of $--^{SEA}$ α-thal and α2 codon 30 (-GAG) (*HBA2:* c.91_93delGAG) mutation will be found in the future. However, few less current studies of the mutation have defined the impact on individuals with this mutation [11–14, 21]. Further study is required to determine the pathological changes caused by the mutation and shed light on the corresponding genotype–phenotype interaction.

In our study, all the individuals heterozygous of the α2 codon 30 (GAG>CAG) (*HBA2:* c.91G>C) mutation had normal red cell indices, which was consistent with the findings of previous studies [22–24]. Although a defect occurred in the same codon between the α2 codon 30 (-GAG) (*HBA2:* c.91_93delGAG) and the α2 codon 30 (GAG>CAG) (*HBA2:* c.91G>C) mutation, a different clinical severity was found in patients with them. This was probably due to the fact that the latter only results in a change in the molecular structure of hemoglobin, reducing the normal α-globin chain synthesis, but forms dimers or tetramers with the β-globin chains. They have the same function of transporting oxygen.

Farashi*et al.*and Li *et al.* emphasized the importance of detection of some point mutations responsible for more severe form of Hb H disease and the necessity for consideration of prenatal diagnosis in high-risk couples [25, 26]. Carrier-couples identified through screening with a risk of pregnancies and/or children affected by a severe Hb H disease should receive genetic counseling so they can be informed of their reproductive options [27, 28]. However, a routine DNA analysis can only detect several common mutations, but not unknown mutations. It is important to realize that routine screening can lead to the omission of rare or novel mutations [29]. At this moment, DNA sequencing can be used to identify the specific mutation that may be present as it used to be the “gold standard” technique for detection of an unknown mutation.

Conclusions

In conclusion, a significant difference in the clinical severity was found in patients with the same genotype. Indentifying the α2 codon 30 (-GAG) (*HBA2:* c.91_93delGAG) mutation has important implications for accurate genetic counseling, particularly in a prenatal diagnosis. For one individual with erythrocyte microcytosis in which one partner was a carrier of α^0-thal, who should not be ignored, but an investigation for α-thal should be performed. If no common α-thal defects were detected, then a further detection must be performed by DNA sequencing. For parents with a pregnancy in which one partner was a carrier of α^0-thal and the other had the α2 codon 30 (-GAG) (*HBA2:* c.91_93delGAG) mutation, genetic counseling and prenatal diagnosis should be provided.

Declarations

Abbreviations

Hb: hemoglobin; thal: thalassemia; gap-PCR: gap-polymerase chain reaction; PCR-RDB: PCR reverse dot-blot assay; MCV: mean cell volume; MCH: mean cell hemoglobin; CE: capillary electrophoresis; SD: standard deviation

Acknowledgments

We thank the patients and their families for their participation in the study.

Funding

Not applicable.

Data Availability Statement

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

Author contributions

ZG designed the research study and wrote the paper; ZZ and HH performed the research; GZ and KY collected samples of patients and performed the experiment; DC collected the clinical data; ZG, ZZ and HH analyzed the data; JC modified and polished the paper. All the authors reviewed, revised, and approved the final version of the manuscript.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of The First Women and Children's Hospital of Huizhou (permit number: 2019031). The written informed consents were taken from the patients or their parents in the study.

Consent for publication

We confirmed that all patients participate in this study signed written informed consent for publication their own and/or their children's genetic data and relevant information.

Conflict of interest

The authors declare that they have no conflict of interest.

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Tables

Table 1 Hematological data and globin genotpyes of the eight patients carrying the α2 codon 30 (-GAG) (*HBA2*: c.91_93delGAG) mutation.

Patients	Sex	Age (y)	α-genotype	β-genotype	Hb (g/dL)	MCV (fL)	MCH (pg)	Hb A (%)	Hb A_2 (%)	Hb F (%)	Hb H (%)	Hb Bart's (%)
P1	M	0.25	$\alpha^{\text{codon 30 (-GAG)}}\alpha/--^{SEA}$	β^A/β^A	8.1	52.2	16.0	76.6	0.6	1.2	17.6	4.0
P2	M	6	$\alpha^{\text{codon 30 (-GAG)}}\alpha/--^{SEA}$	β^A/β^A	7.9	68.8	18.1	71.2	0.6	-	28.2	-
P3	M	37	$\alpha^{\text{codon 30 (-GAG)}}\alpha/--^{SEA}$	β^A/β^A	10.2	73.6	20.4	81.7	0.7	-	17.6	-
P4	F	23	$\alpha^{\text{codon 30 (-GAG)}}\alpha/--^{SEA}$	β^A/β^A	8.2	80.1	20.5	69.9	0.5	-	29.6	-
P5	F	31	$\alpha^{\text{codon 30 (-GAG)}}\alpha/-\alpha^{4.2}$	β^A/β^A	12.2	66.0	21.3	96.9	2.3	0.8	-	-
P6	F	25	$\alpha^{\text{codon 30 (-GAG)}}\alpha/\alpha\alpha$	β^A/β^A	12.2	75.1	22.8	97.6	2.4	-	-	-
P7	F	27	$\alpha^{\text{codon 30 (-GAG)}}\alpha/\alpha\alpha$	β^A/β^A	12.9	78.9	25.6	97.4	2.6	-	-	-
P8	F	27	$\alpha^{\text{codon 30 (-GAG)}}\alpha/\alpha\alpha$	β^A/β^A	12.9	77.0	26.0	95.9	2.4	1.7	-	-

Table 2 Comparison of hematological indices on four types of α-globin genotpyes.

Type	N	Hb			MCV			MCH		
		Indices	Z	*p*（one-tailed）	Indices	Z	*p*（one-tailed）	Indices	Z	*p*（one-tailed）
$\alpha^{\text{codon 30 (-GAG)}}\alpha/\alpha\alpha$	3	12.67±0.40			77.00±1.90			24.80±1.74		
$\alpha^{QS}\alpha/\alpha\alpha$	30	12.61±1.53	-0.596	0.571[a]	75.50±3.34	-1.034	0.317[a]	23.82±1.08	-1.097	0.288[a]
$\alpha^{\text{codon 30 (GAG>CAG)}}\alpha/\alpha\alpha$	13	13.23±2.00	-0.202	0.900[b]	85.61±3.53	-2.493	0.007[b]	29.34±1.52	-2.627	0.004[b]
αα/αα	30	14.27±1.74	-1.224	0.235[c]	91.17±4.01	-2.818	0.000[c]	30.10±1.71	-2.819	0.000[c]

[a]*p* for $\alpha^{\text{codon 30 (-GAG)}}\alpha/\alpha\alpha$ *versus* $\alpha^{QS}\alpha/\alpha\alpha$; [b]*p* for $\alpha^{\text{codon 30 (-GAG)}}\alpha/\alpha\alpha$ *versus* $\alpha^{\text{codon 30 (GAG>CAG)}}\alpha/\alpha\alpha$; [c]*p* for $\alpha^{\text{codon 30 (-GAG)}}\alpha/\alpha\alpha$ *versus* and αα/αα.

Table 3 Comparison of hematological indices on three types of α-globin genotpyes.

Type	N	Hb			MCV			MCH		
		Indices	Z	*p*（one-tailed）	Indices	Z	*p*（one-tailed）	Indices	Z	*p*（one-tailed）
α $^{\text{codon 30 (-GAG)}}$α/- - $^{\text{SEA}}$	4	8.60±1.07			68.68±11.92			18.75±2.14		
α$^{\text{QS}}$α/- - $^{\text{SEA}}$	20	9.15±1.06	-0.892	0.388[d]	68.25±8.90	-0.620	0.575[d]	19.35±2.66	0.000	1.000[d]
- -$^{\text{SEA}}$/αα	36	12.79±1.41	-3.204	0.000[e]	70.85±2.34	-0.203	0.845[e]	21.96±1.27	-2.957	0.001[e]

[d]*p* for α $^{\text{codon 30 (-GAG)}}$α/- -$^{\text{SEA}}$ *versus* α$^{\text{QS}}$α/- -$^{\text{SEA}}$; [e]*p* for α $^{\text{codon 30 (-GAG)}}$α/- -$^{\text{SEA}}$ *versus* - -$^{\text{SEA}}$/αα.

Figures

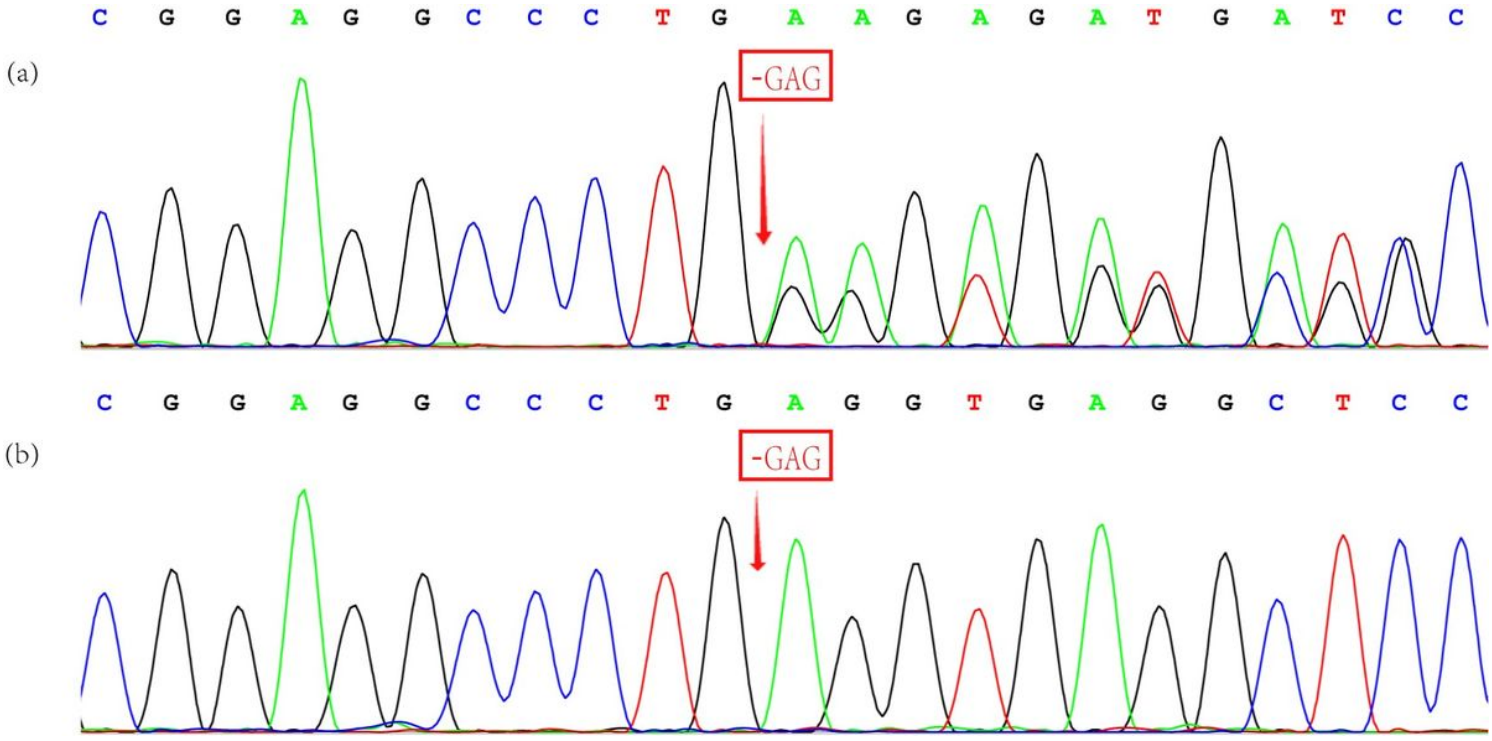

Figure 1

Direct sequence analysis of the α2-globin gene showing the heterozygous α2 codon 30 mutation (-GAG) (HBA2: c.91_93delGAG) (a) and hemizygous mutation (b).