Rare double heterozygosity for poly A(A〉 G) and CD17(A〉 T) of beta thalassemia intermedia in a Chinese family

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Abstract

Beta thalassemia is a hereditary disorder resulted from mutations in the β globin gene leading to alpha/beta imbalance, ineffective erythropoiesis, and chronic anemia. Three types have been defined, based on the degree of reduced beta-globin chain synthesis and clinical phenotype: major, intermedia and minor (heterozygote carrier state). Beta thalassemia intermedia is characterized by heterogeneity for the wide clinical spectrum of various genotypes and a wide range of presentations. The genotypes of beta thalassemia intermedia are much complicated referring to β^+/β^+,β^+/β^0, Hb E/β^0, β^0/β^0 compounding alpha thalassemia and so on. In this present case, we reported a rare beta thalassemia intermedia genotype of double heterozygosity for poly A (A〉 G) and CD17(A〉 T) indicated of β^+/β^0 in a Chinese family.

Introduction

β thalassemia is an autosomal recessive monogenic disorder characterized by the defect in the synthesis of β-globin chains of hemoglobin tetramer. The disease is due to mutation in β globin locus for which more than 200 alleles have been reported.[1] These mutations either partially or completely terminate the synthesis of β-globin chain which is defined as β^+ and β^0 type mutations respectively. Depending on severity of hematological and clinical conditions, β-thalassemia is classified into three types, namely, β-thalassemia minor (also called as carrier), β-thalassemia intermedia and β-thalassemia major. The clinical severity of β-thalassemia intermedia has ranged from asymptomatic carrier state to severe transfusion-dependent type. Some patients remain asymptomatic for most of their lives with hemoglobin levels ranging between 7 and 10 g/dL, while others present during childhood and require transfusions for normal sustained growth.[2]

In this case report, we describe a rare double heterozygosity for poly A (A〉 G) compounding CD17(A〉 T) resulting in a beta-thalassemia intermedia phenotype in a Chinese family.

Case Report

The proband was a three-year-old female presented to the out-patient of pediatrics with anemia because of dizziness and acratia for more than half a year. The findings of ultrasound indicated of cardio-ventriculus sinister moderate hypertrophy, mild tricuspid regurgitation, hepatomegaly and splenomegaly. On examinations, she had abnormal RBC indices with low Hb (Hb was 80 g/L), low MCV (MCV was 72.6 fl) and low MCH (MCH was 23.9 pg) suspected as microerythrocyte anemia. Furthering screening by high-performance liquid chromatography (HPLC),she had an Hb A_2 of 3.1% and Hb F of 63.1%. Based on the results of the hematologic phenotype screening, this indicated the patient to be susceptive of beta thalassemia intermedia. Following the molecular diagnosis of beta thalassemia by reverse do blot (RDB), only a type of point mutation named CD17(A〉 T) was detected, which was not in accordance with the results of the hematologic phenotype screening. Informed consent was obtained from all the family members including the proband's grandfather, grandmother, father, mother and brother. Peripheral blood was extracted from all the family members. According to the results of red cell indices, Hb A_2 and Hb F, other members except grandmother were considered as β-thalassemia minor (Table 1). Seventeen types of common beta thalassemia point mutations aimed at Chinese were measured by RDB, the proband's mother and brother were both carriers of CD17(A〉 T) mutation. However, results turned out be negative in the proband's father and grandfather for the seventeen common mutations incompatible with that of hematologic phenotype screening (Figure 1). In summary, the proband, grandfather and father were suggested as carriers of rare β-globin gene mutation. Further analysis of 202 types of uncommon β-thalassemia point mutations with PCR-sequencing completed by The Beijing Genomics Institute (BGI), all of the proband, grandfather and father had rare point mutation of poly A (A〉 G) (Figure 2). In the end, the proband was double heterozygosity for poly A (A〉 G) and CD17(A〉 T) conforming to the character of phenotype for beta thalassemia intermedia.

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Discussion and Conclusions

Beta thalassemia is mainly resulted from point mutations of β globin gene. To date, more than 250 mutations that could cause β thalassemia have been reported all over the word. In China, over 40 mutations of β thalassemia have been indentified.[3] The prevalence rate of β thalassemia is 6.43% and 2.54% respectively in Guangxi and Guangdong region of China.[4,5] Seventeen types of β thalassemia mutations involving in CD41-42, IVS-IInt654, CD17, TATAbox-28, CD71-72, CD26(β^E), CD31, CD27/28, IVS-I-1, CD43, TATAbox-32, TATAbox-29, TATAbox-30, CD14-15, Cap +40/43, Int and IVS-I-5 take up 99% of Chinese population. Nevertheless, always there are some rare mutations among beta thalassemia patients, and identification of all rare mutation in each region can help improve screening protocols of the carriers and prevent affected child birth.

The clinical complications of thalassemia intermedia patients present heterogeneous. Some thalassemia intermedia patients are asymptomatic until adult life, whereas others are symptomatic from as

young as 2 years of age. Many patients with thalassemia intermedia receive only occasional or no transfusions, since they are able to maintain hemoglobin levels between 7-9 g/dL.[6] According to the proband's result of clinical manifestations and laboratory tests, she was primarily diagnosed as β thalassemia intermedia based on elevated level of Hb F (63.1%), normal Hb A_2 (3.1%),moderate anemia (Hb:80g/L), hepatomegaly and splenomegaly. However, only CD17(A〉 T) among routine 17 types of β thalassemia mutations was identified via RDB, which was not consistent with the results of thalassemia screening. On the condition of enough informed consent, we examined the blood samples of her grandfather, grandmother, father, mother and brother. In addition to grandmother, the findings showed that they were carries of β thalassemia minor. Following the detection of routine β thalassemia gene mutations, both mother and brother were positive of CD17(A〉 T),the others were negative. We concluded that the proband, grandfather and father might carry with rare β-globin gene mutations. Furthering analysis of uncommon β-thalassemia point mutations with PCR-sequencing, the proband, grandfather and father had rare point mutation of poly A(A〉 G). So, the proband was double heterozygosity for poly A(A〉 G) and CD17(A〉 T). Thalassemia intermedia arises from defective gene function leading to partial suppression of beta-globin protein production. It usually results from a homozygous or a compound heterozygous mutation.[7] The reason for thalassemia intermedia is caused by 3 different mechanisms. The first is the inheritance of a mild or silent beta-chain mutation, which keeps a low level of beta chains, as opposed to its absence in more severe cases making less of an alpha/beta imbalance. The second is the inheritance of determinants associated with increased gamma chain production, which pair with unbound alpha chains. The third is the co-inheritance of alpha-thalassemia, which decreases the number of unpaired chains due to decreased alpha chain synthesis.[8]

CD17(A〉 T) is a type of common mutation which takes possession of 18.9% among β-globin gene in Chinese population. The mechanism of CD17(A〉 T) mutation affecting on β-globin gene translation is that AAG (Lys) changing for TAG (stop) which leads to the advanced termination of β-globin chain synthesis. In a word, the CD17(A〉 T) mutation induces the deletion of β-globin chain resulting in the phenotype of β^0-thalassemia. The mutation of poly A(A〉 G) firstly found in a family from

Table 1. Hematological features of all the family members.

Relation	Age (y)	Gender	HGB (g/L)	MCV (fl)	MCH (pg)	RDW(%)	Hb A2 (%)	Hb F(%)
Proband	3	F	80	72.6	23.9	23.9	3.1	63.1
Grandfather	65	M	135	76.9	25.8	11.8	4.5	0.5
Grandmother	62	F	112	88.9	29.3	10.6	2.9	0.2
Father	28	M	151	79.6	26.4	12.9	4.1	0.9
Mother	27	F	107	63.0	20.6	14.5	5.3	2.2
Brother	0.5	M	100	62.5	20.9	22.9	4.4	25.5

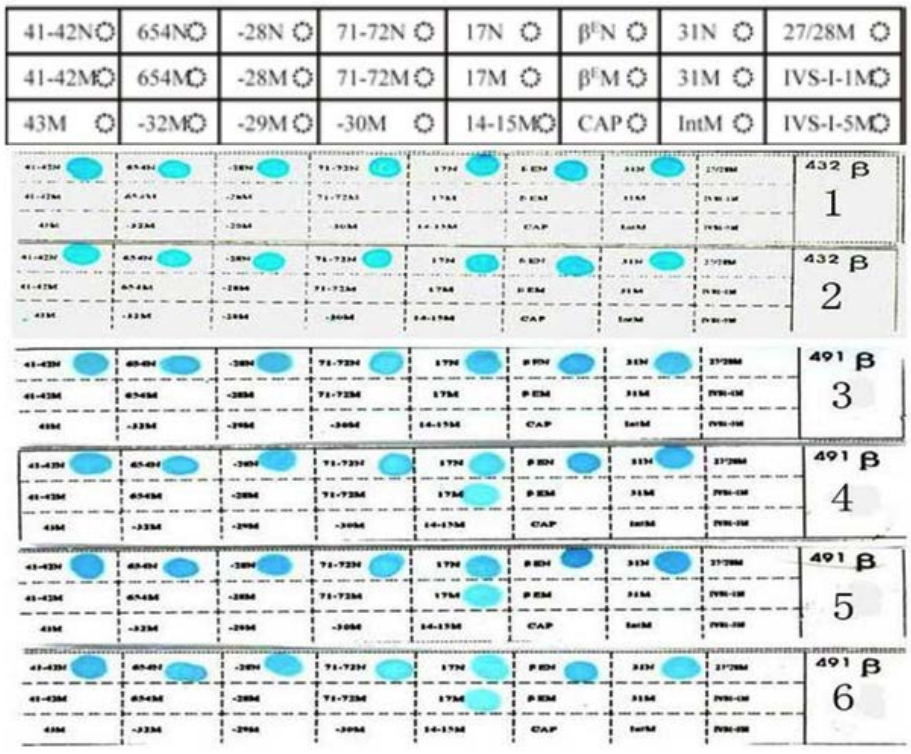

Figure 1. Detection of seventeen common Chinese mutations by reverse dot blot. 1) Grandfather, 2) Grandmother, 3) Father, 4) Mother, 5) Proband, 6) Brother.

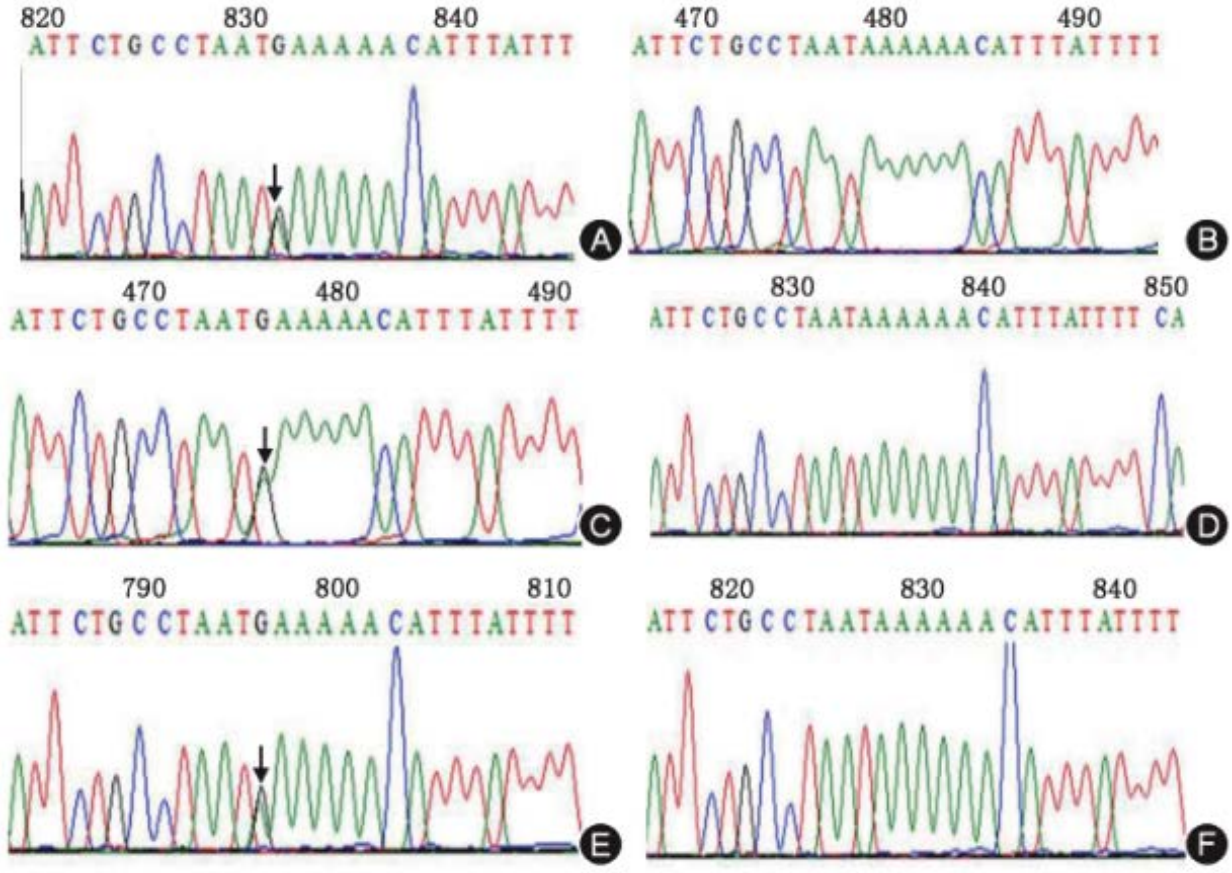

Figure 2. Detection of poly A (A⟩ G) mutation via DNA sequencing. A) Proband, B) Grandmother, C) Grandfather, D) Mother, E) Father, F) Brother.

Yugoslavia is not reported in China.[9] This mutation mostly presents in European including Albania, Macedonian, Bulgarian, Greek *et al.* (http://globin.bx.psu.edu). The mutation of poly A(A⟩ G) proceeds at the polyadenylation site of 3'-UTR named as the codon 111(A⟩ G) influencing the processing and tailing of mRNA and shows the manifestation for β^{+}-thalassemia. By the further molecular diagnosis, the patient is double heterozygous for CD17(A⟩ T) combining with poly A(A⟩ G).We suggest that the molecular basis for β-thalassemia intermedia of this patient is β^{0}/β^{+} which keeps a low level of beta chains making opposite excess of alpha chains.

In conclusion, the hematological screening comprising hemoglobin analysis and red cell indices is imperative to identify thalassemia. If only routine molecular measurements aimed at special race were executed, but the screening for thalassemia hematological phenotype was ignored ,the patients might be missed diagnosis. When the result of thalassemia screening is not consistent with that of routine molecular measurements, rare mutations are in consideration followed by deep molecular sequencing. So, the screening program is more important for rare mutations of thalassemia.

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