Co-inheritance of α and β-Thalassemia in a Jordanian Family

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Additional α-genes may increase the severity of heterozygous β-thalassemia. Conversely, the co-inheritance of α-thalassemia with homozygous β-thalassemia and the consequent reduction in α-globin chain excess often results in a milder clinical and haematological phenotype. This study describes the hematological and the molecular data resulting from the interaction between α and β^+-thalassemia determinants in a Jordanian family. The parents are double heterozygotes for α and β-thalassemia. DNA analysis of four children characterized homozygosity for β^+ IVS 1.6 thalassemia mutation in three of them. Co-existing heterozygosity for $-\alpha^{3.7}$ was detected in two of them (Children 1 and 2). Those two children have a less severe clinical course than that of the third child (Child 3) with homozygosity for β-thalassemia only. The co-existence of $-\alpha^{3.7}$ mutations with homozygous β-thalassemia may have converted a transfusion-dependent thalassemia major to non transfusion-dependent thalassemia intermedia. The fourth child (Child 4) was heterozygous for $-\alpha^{3.7}$ but lacked β^+ IVS 1.6 mutation and appeared normal.

ABBREVIATIONS: MCH = mean corpuscular hemoglobin; MCV = mean corpuscular volume; PCR = polymerase chain reaction.

INDEX TERMS: α-thalassemia; β-thalassemia; co-inheritance; Jordan; PCR.

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Human globin-gene mutations are amongst the most common genetic disorders observed in human populations.[1] At the molecular level β-thalassemia comprises a heterogeneous group of hemoglobin disorders resulting from mutations eliminating (β^0) or reducing (β^+) the β-globin gene expression. Over 200 different mutations can cause β-thalassemia phenotype.[2]

Of the numerous mutations that have been described to cause α-thalassemia phenotype, deletions at the α-gene locus account for the vast majority of α-thalassemia alleles.[3] The most widely occurring single-gene deletions are the $-\alpha^{3.7}$ and the $-\alpha^{4.2}$, while the double α-gene deletions *in cis*, such as the $--^{SEA}$, $--^{FIL}$, and $--^{THAI}$ alleles are most common in Southeast Asia and the $--^{MED}$ and the $--\alpha^{20.5}$ double gene deletions occur most frequently in the Mediterranean area.[4]

Imbalance in the relative amounts of β and α-globin chains plays a major role in the pathophysiology of thalassemia. In homozygous β-thalassemia, the excess α-chains precipitate in the red blood cell precursors causing membrane damage, red cell destruction, ineffective erythropoiesis, and consequently anemia.

In heterozygous β-thalassemia, the deleterious effects of excess α-chains have been implicated through the correlation of β-thalassemia mutations with the mean corpuscular volume (MCV) of the red blood cells.[5]

Unlike β-thalassemia, the detection of α-thalassemia carrier status has always been problematic because of the limitations in traditionally existing techniques in molecular characterization of α-thalassemia carriers. Currently, polymerase chain reaction (PCR) based assays provide a simple, sensitive, and rapid detection of α-thalassemia trait carriers. [6, 7]

It has been shown that the excess α-genes increase the severity of heterzygous β- thalassemia.[8,9] Conversely, heterozygousity for α-thalassemia in conjunction with homozygous β-thalassemia ameliorates the clinical condition.[10]

In the present study we reported the clinical, hematological, and molecular data resulting from the interaction between α and β-thalassemia in a Jordanian family of six affected members.

MATERIALS AND METHODS

Blood samples were vacuum collected in Na–EDTA. Hematological parameters were obtained from an automated counter Sysmex SE 9000™ (Sysmex-Toa Medical Electronics Co.: Kobe, Japan). Red cell lysates were examined on cellulose acetate electrophoresis at pH 8.6. Hb A2 and Hb F fractions were measured by High Performance Liquid Chromatography using BioRad. Variant II™. Genomic DNA was isolated by commercial DNA isolation kit (instagene genomic isolation kit, BioRad.: USA). Samples were tested for 22 most common β-thalassemia mutations by reverse hybridization (β-Globin StripAssay, ViennaLab: Vienna, Austria). Positivity for β-thalassemia mutations was confirmed by a micro well hybridization technique MDX- Betha Gene 1 Kit™ (BioRad.: USA) according to manufacturer instructions. Detection of α-thalassemia mutation $-\alpha^{3.7}$ was achieved by PCR-based technique using published primer sequences[6] with some modifications to the original technique. Each 50 µl reaction contained 200 µM of each dNTP, 1.5 µM MgCl2, 2.5 µg BSA, 10% DMSO, 250 ng – 500 ng of genomic DNA, and two units of Taq DNA polymerase (Ampli Taq Gold polymerase, Perkin Elmer) in the supplied reaction buffer. Reactions were performed in a Perkin Elmer 9600™ thermal cycler. The program was initiated with denaturation at 96°C for 12 minutes followed by 32 cycles of 96°C denaturation for two minutes, 62°C for 75 seconds, and 72°C extension for 135 seconds. The reaction was completed with final extension at 72°C for ten minutes. After amplification, 10 µl of product was electrophoresed through one percent agarose gel in 1x Tris-EDTA-Borate-buffer at 10 volts/cm for one hour. The ethedium bromide stained gel was visualized and photographed under a UV transilluminator.

RESULTS

Clinical picture and hematological data

Parents

Both parents were apparently normal. Their Hb and Hct levels were normal for their ages. Only mild reduction in their MCV and mean corpuscular hemoglobin (MCH) values was noticed. The blood picture of both parents including red cell morphology was consistent with thalassemia trait. Cellulose acetate electrophoresis (pH 8.6) showed mild increase in the Hb A2 values for both parents with no other abnormal hemoglobin fraction. DNA analysis (Figure 1) revealed that both parents were double heterozygotes for β-thalassemia mutation β^+ IVS1-6 and α- thalassemia mutation $-\alpha^{3.7}$. The hematological data and genotypes for the family are shown in Table 1. Figure 1 shows the PCR amplification product for the $-\alpha^{3.7}$ mutation.

Children

Children 1 and 2: These two children were referred to the pediatric hematology clinic at the ages of three and four years respectively. They were presented with similar clinical and hematological phenotype consistent with a diagnosis of thalassemia intermedia. Both children showed mild spleenomegaly with occasional requirement for blood transfusion. The hematological data for Children 1and 2 were as follows; (MCV = 62 fl and 50.5 fl, MCH = 15.8 pg and 20.2 pg, Hb A2 = 5.2% and 7.2%, Hb F = 1.8% and 3.1% respectively). Genotype analysis for the two children demonstrated homozygosity for β^+ IVS 1-6 mutation and heterozygosity for $-\alpha^{3.7}$ mutation.

Table 1. Hematological findings and thalassemia genotypes of the family

Patient	Age years	Hb (g/dl)	Hct%	MCV (fl)	MCH (pg)	HbA2%	HbF%	Serum ferritin ng/ml	β-globin genotype	α-globin genotype
Mother	30	12.1	30.7	70.1	22	3.7	0.4	64	Het IVS1-6	- α/αα
Father	41	15.3	46.5	72.1	23.7	4.1	0.3	124	Het IVS1-6	- α/αα
Children										
1	6	9.6	29.5	62.0	20.2	5.2	1.8	262	Homo IVS1-6	- α/αα
2	8	88.8	28.1	50.5	15.8	7.2	3.1	80	Homo IVS1-6	- α/αα
3	5	7.8	24.4	65.9	24.4	4.7	14.0	1490	Homo IVS1-6	αα/αα
4	7	12.4	35.4	65.0	22.8	2.8	0.3	65	Normal	- α/αα

Child 3: At the age of one year, this child presented with clinical manifestations consistent with thalassemia major: severe anemia, spleenomegaly. This child was diagnosed as β-thalassemia major based on the increase in Hb F fraction, the typical red cell morphology, and the suggestive family history. He showed consistent requirement for blood transfusion almost every month. This child was found to be homozygote for β^+-thalassemia mutation IVS 1-6 while the $-\alpha^{3.7}$ mutation was not detected.

Child 4: DNA analysis for this apparently normal child revealed the presence of-$\alpha^{3.7}$ mutation with the absence of β^+ IVS1-6 mutation. He had a hemoglobin value of 12.4 gm/dl, MCV value of 65 fl, MCH value of 22.8 pg, Hb A2 concentration of 2.8% and Hb F concentration of 0.3%. Child 4 was diagnosed as $-\alpha^{3.7}$ trait carrier.

DISCUSSION

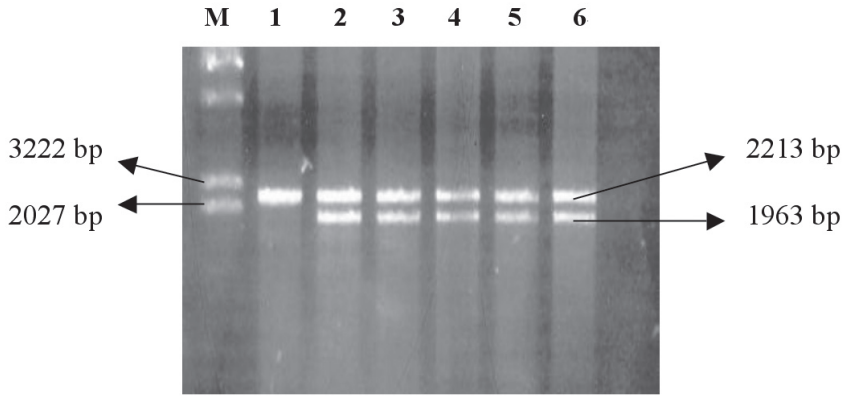
Homozygosity for β-thalassemia mostly results in thalassemia major, characterized by severe anemia, splenomegaly, and requirement for regular blood transfusion from early infancy. However, some homozygotes present with mild hematological and clinical phenotype without requirement for regular blood transfusion. This condition is called thalassemia intermedia.[1] Thalassemia intermedia may result from the inheritance of mild β-thalassemia mutations, or as a result of interaction between triplicated α-globin gene with heterozygous β-thalassemia, compound heterozygosity for β-thalassemia, or as a result of co-inheritance of α-thalassemia with homozygous β-thalassemia.[11]

Except for mild reduction in the MCV and MCH, both parents are phenotypically normal, with α-thalassemia genotype of –α/ αα indicating the loss of a single α-gene. Both parents also are heterozygotes for the β^+ IVS1-6 mutation, which is of mild severity, thus, the ameliorating effect resulting from the interaction between these two mutations in heterozygotes might explain the borderline Hb A2 levels (Mother Hb A2 = 3.7%, Father Hb A2 = 4.1%). It has been suggested that the levels of Hb A2 in α-thalassemia are lower than in normal individuals,[12] raising the possibility that the level may be lower in double heterozygotes for α and β-thalassemia than in those with β-thalassemia alone.

Both Children 1 and 2 presented with β-thalassemia intermedia phenotype. Their symptoms were considerably less severe than those seen in Child 3. Both children were found to be homozygous for β^+-thalassemia mutation IVS 1-6 and heterozygous for $-\alpha^{3.7}$ mutation. Both children have received only occasional blood transfusions. The hematological data and the clinical phenotypes may seem quite mild compared to Child 3 with homozygosity for β-thalassemia only. The clinical and hematological findings for Child 3 were consistent with the diagnosis of β-thalassemia major. These findings include severe anemia, splenomegaly (4 cm below the coastal margin) and iron overload. DNA analysis for this child showed homozygosity for β^+-thalassemia mutation IVS 1-6 mutation while the $-\alpha^{3.7}$ mutation was not detected. His Hb F is moderately increased (14%), which is relatively lower than that usually observed in β-thalassemia homozygotes. This is not uncommon in case of the inheritance of the mild β-thalassemia mutation IVS1-6.[1] This child receives regular blood transfusions at monthly intervals.

Child 4 represents a typical case of α-thalassemia trait. His MCV and MCH are slightly reduced (MCV = 65 fl, MCH = 22.8 pg). DNA analysis confirmed heterozygosity for the $-\alpha^{3.7}$ and the absence of β^+-thalassemia mutation IVS 1.6.

Figure 1. One percent agarose gel electrophoresis of PCR product for α-thalassemia ($-\alpha^{3.7}$) mutation



M: DNA marker, λ DNA/ Hind III
Lane 1: Child 3. Normal genotype, (αα/αα)
Lanes 2, 3, 4: Children 1, 2, and 4. Heterozygous genotypes, (-α/αα)
Lanes 5, 6: Mother and father. Heterozygous genotypes, (-α/αα)

Hb F levels for Children 1 and 2 (1.8 % and 3.1 % respectively), are significantly less than that seen in Child 3 which agrees with the concept that Hb F level is decreased when a deletional α-thalassemia mutation coexists with other β-globin gene mutations and the value of HbF is conversely related to the degree of anemia.[13]

These findings demonstrate clearly that the co-inheritance of α-thalassemia plays an important role in modifying the clinical course of homozygous β-thalassemia.

It seems highly probable that many of thalassemia intermedia phenotypes can be explained by the co-inheritance of α-thalassemia,[14,15] and the co-inheritance of triplicated α-globin gene with heterozygous β-thalassemia. Local studies in Jordan estimated the prevalence of β-thalassemia at three percent to four percent, with a wide range of β-thalassemia mutations existing in the country.[16,17,18] The homozygous β-thalassemia patients in Jordan resemble a group with significant clinical heterogeneity. It is important to determine whether the presence of single α-thalassemia determinant explains the clinical heterogeneity among those patients. Our study suggests that this may be the case, but it will be necessary to analyze a series of transfusion dependent homozygotes for β^+ and β^o-thalassemia and compare the incidence of α-thalassemia in that group with a large series of patients known as mild homozygotes for β^+ and β^o-thalassemia. This will enable us to define fully the clinical spectrum resulting from the interaction between α and homozygous β-thalassemia.

Furthermore, in fetuses homozygous for β-thalassemia, the detection of α-thalassemia determinant should become an important part of the program for the prevention of these disorders, especially with the development of PCR technology which allows an easy identification of α-thalassemia trait carriers making it now possible to predict more accurately the clinical outcome of the interaction between different α and β-thalassemia determinants.

This case represents an extremely rare condition, as both parents are double heterozygous for α and β-thalassemia. Furthermore, the two determinants were clearly defined. Such co-inheritance of α and β-thalassemia might be due to the high prevalence of thalassemia determinants, and the high rate of consanguineous marriages among Jordanians.[19] The parents in this case are first-degree relatives from both sides.

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