

Diverse phenotypes and transfusion requirements due to interaction of β -thalassemias with triplicated α -globin genes

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Abstract Co-inheritance of triplicated α -genes can alter the clinical and hematological phenotypes of β -thalassemias. We evaluated the phenotypic diversity and transfusion requirements in β -thalassemia heterozygotes, homozygotes, and normal individuals with associated α -gene triplication. Clinical and hematological evaluation was done and the β -thalassemia mutations characterized by a covalent reverse dot blot hybridization/amplification refractory mutation system. Alpha-globin gene triplication was assessed by multiplex PCR. During the last 2.5 years, 181 β -thalassemia patients and β -thalassemia carriers with an unusual clinical presentation were referred to us for screening for the presence of associated α -globin gene triplication. Twenty-nine of them had associated α -gene triplication (3 β -thalassemia homozygotes or compound heterozygotes and 26 β -thalassemia heterozygotes). One β -thalassemia compound heterozygote [IVS 1–5 (G→C)+CD 41/42 (–CTTT)] was anemic at birth and required blood transfusions unusually early by 6 weeks of age. The second patient (4.5 years) was also clinically severe and became transfusion dependent in spite of having one mild β -thalassemia mutation [Capsite +1 (A→C)]. The third case (3.5 years) who was homozygous for a mild β -gene mutation [–88 (C→T)] with α gene triplication was untransfused. The 26 β -thalassemia heterozygotes with associated triplicated α -genes presented variably, with a β -thalassemia intermedia-like presentation. While screening the family members of all

these cases, we found another 10 β -thalassemia heterozygotes and 9 normal individuals with α -globin gene triplication; however, all of them were asymptomatic. Beta-thalassemia carriers, homozygotes, and compound heterozygotes with an unusual presentation should be screened for the possible presence of associated α -globin gene triplication which could influence the clinical and hematological presentation.

Keywords Beta-thalassemia · α -Gene triplication · Clinical presentation · Blood transfusion

Abbreviations

HPLC	High-performance liquid chromatography
CRDB	Covalent reverse dot blot hybridization
ARMS	Amplification refractory mutation system
TI	Thalassemia intermedia

Introduction

Beta-thalassemias are a heterogeneous group of autosomal recessive disorders characterized by reduced or absent β -globin chain synthesis. The resulting relative excess of unbound α -globin chains precipitates in erythroid precursors in the bone marrow, leading to their premature death and hence to ineffective erythropoiesis. β -Thalassemia patients have variable clinical phenotypes, ranging from the severe transfusion-dependent thalassemia major to the milder form of thalassemia intermedia. Patients with the major form of the disease have severe microcytic and hypochromic anemia and hepatosplenomegaly and usually come to medical attention within the first 2 years of life [1].

Individuals with β -thalassemia intermedia present later in life with milder anemia that may require occasional

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transfusions, liver and spleen enlargement, typical bone changes, and mild to moderate jaundice. Few β -thalassemia intermedia patients may become transfusion dependent at a later age. Beta-thalassemia heterozygotes are usually asymptomatic [1]. Triplication of the α -globin genes leads to increased production of α -globin chains. This is usually not a problem in otherwise normal individuals [2, 3]; however, when α -gene triplication is co-inherited with β -thalassemia, there may be sufficient α - and β -globin imbalance to produce a more severe phenotype [1, 2].

We report our experience on the co-inheritance of triplicated α -globin genes and β -thalassemia.

Materials and methods

One hundred and eighty-one individuals (β -thalassemia patients and carriers) with an unusual clinical presentation were referred to us over the last 2.5 years for investigating the possible co-inheritance of additional α -genes with β -thalassemia. They were either β -thalassemia homozygotes or compound heterozygotes who presented with severe anemia very early in life or they were β -thalassemia heterozygotes with a thalassemia intermedia-like presentation.

Clinical evaluation was done in all the cases and their past history noted from their medical records. Five to 6 ml of blood was collected in EDTA from all the individuals and their family members who were clinically normal after an informed consent was obtained. The study was approved by our Institutional Ethics Committee. The complete blood count was measured by an automated hematology counter (Sysmex, K-1000, Sysmex Corporation, Kobe, Japan), and the percentage of HbA₂ and HbF were measured by HPLC on the Variant Hb Testing System (Bio-Rad Laboratories, Hercules, CA, USA). DNA was extracted from the leucocytes using a standard method. Beta-genotyping was done by covalent reverse dot blot (CRDB) hybridization [4] or by amplification refractory mutation system (ARMS) [5]. Screening for α -gene triplication was done by multiplex PCR [6].

Results

Among the 181 individuals with different β -thalassemia phenotypes evaluated, 29 had triplicated α -globin genes. They included 3 β -thalassemia homozygotes or compound heterozygotes and 26 β -thalassemia heterozygotes. Ten β -thalassemia heterozygotes and 9 normal individuals from the family members who were tested also had α -gene triplication.

The 3 β -thalassemia homozygotes/compound heterozygotes with associated α -globin gene triplication had variable clinical phenotypes. Their hematological and molecular findings are shown in Table 1.

Table 1 Hematological and molecular findings and transfusion requirements of β -thalassemia homozygotes/compound heterozygotes with associated α -globin gene triplication

Sr. no.	Age	Hb (g/dl)	RBC ($\times 10^{12}/l$)	MCV (fl)	MCH (pg)	MCHC (g/dl)	RDW (%)	HbA ₂ (%)	HbF (%)	β -Gene mutation	Frequency of blood transfusions
1	6 weeks/F	5.5	1.78	84.3	30.9	36.7	27.9	0.6	99.1	IVS 1-5 (G→C)+ CD 41/42 (-CTTT)	Required regular transfusions subsequently
2	4.5 years/M	5.9	2.19	74.9	26.9	36.0	20.1	3.8	15.0	Capsite +1 (A→C)+ CD 41/42 (-CTTT)	10 times
3	3.5 years/M	9.0	4.16	67.5	21.6	32.0	27.6	4.4	80.7	-88 (C→T)+-88 (C→T)	Nil

Hb hemoglobin, RBC red blood cell, MCV mean corpuscular volume, MCH mean corpuscular hemoglobin, MCHC mean corpuscular hemoglobin concentration, RDW red cell distribution width, HbA₂ hemoglobin A₂, HbF fetal hemoglobin

The first case was a 6-week-old female baby who was anemic at birth (Hb 12.1 g/dl) and required transfusions at the age of 6 weeks and subsequently became transfusion dependent. She had inherited a severe β^+ -thalassemia mutation [IVS 1–5 (G→C)] and a β^0 -thalassemia mutation [CD 41/42 (–CTTT)] along with the triplicated α -globin gene.

The second case was a 4.5-year-old male child who clinically presented with anemia at the age of 8 months and gradually became transfusion dependent. He had hepatosplenomegaly, and his liver and spleen were palpable 10 cm below the costal margin. He had inherited one mild β^{++} mutation [Capsite +1 (A→C)] and a β^0 mutation [CD 41/42 (–CTTT)] with the triplicated α -globin gene.

The third case was a 3.5-year-old male child who was also anemic at birth (Hb 12.5 g/dl). However, he is untransfused till now and is maintaining a hemoglobin level of around 9 g/dl. His β -globin genotype is β^{++}/β^{++} , being homozygous for a mild β -thalassemia mutation [–88 (C→T)] with co-inherited α -globin gene triplication.

Of the 36 β -thalassemia heterozygotes with triplicated α -globin genes, 26 were clinically symptomatic whereas 10 were asymptomatic. Eighteen of the 26 symptomatic individuals required intermittent transfusions, while 2 of them needed repeated transfusions only during their pregnancy. Fourteen of them had splenomegaly (spleen 4 to 5 cm below the costal margin), hepatomegaly (liver 2–3 cm below the costal margin), or both. One of the 14 cases was splenectomized (spleen 18 cm below the costal margin). One β -thalassemia heterozygote with triplicated α -globin genes was a 3-month-old female baby, heterozygous for the IVS 1–5 (G→C) β^+ -thalassemia mutation with splenomegaly (spleen 5 cm below the costal margin) and a Hb level of 5.2 g/dl. This child was subsequently transfused once and is now 1 year old. On screening her parents, the mother had β -thalassemia trait and α -globin gene triplication while the father had a normal β genotype but had α -globin gene triplication.

Twenty-one of the 26 symptomatic β -thalassemia heterozygotes had the severe β^+ mutation IVS 1–5 (G→C), while 5 of them had β^0 mutations [IVS 1–1 (G→T)—1, CD 15 (G→A)—2, and CD 41/42 (–CTTT)—2].

Of the 10 asymptomatic β -thalassemia heterozygotes, 8 had the IVS 1–5 (G→C) mutation, and one case each had the milder Capsite +1 (A→C) and the milder –88 (C→T) mutation, respectively.

We compared the hematological parameters of 26 symptomatic β -thalassemia heterozygotes having α -gene triplication with those of the 10 asymptomatic heterozygotes having α -globin gene triplication as well as with 30 β -thalassemia heterozygotes with a normal α genotype.

Their hematological and molecular findings and requirements of blood transfusion are shown in Table 2.

The group of symptomatic β -thalassemia heterozygotes with α -globin gene triplication had significantly lower ($p \leq$

0.05) Hb levels and higher HbF levels as compared to the asymptomatic group of heterozygotes with α -globin gene triplication. However, no significant difference was observed in the mean Hb level and HbF level with the group of β -thalassemia heterozygotes without α -globin gene triplication. The RBC count was lower in the symptomatic β -thalassemia heterozygotes with α -gene triplication compared to the other two groups. The family members with β -thalassemia trait and α -globin gene triplication were clinically asymptomatic, but they had high RBC counts ($3.82\text{--}6.08 \times 10^{12}/l$) and a similar degree of hypochromia and microcytosis as compared to the symptomatic carriers.

Nine individuals with a normal β genotype but with triplicated α -globin genes were also encountered among the family members of these cases. All of them were clinically asymptomatic and their hematological parameters did not show any difference when compared with a group of 30 clinically normal individuals with a normal β and α genotype (Table 3).

Discussion

The different phenotypes of β -thalassemia are mainly distinguished by the clinical severity of the disease. The non-transfusion-dependent thalassemias primarily include cases of β -thalassemia intermedia, HbE- β -thalassemia, and HbH disease. Unlike the β -thalassemia major patients, they do not require regular blood transfusions for survival. Majority of the thalassemia intermedia patients are homozygotes or compound heterozygotes for β -thalassemia, but they could have a single dominant β -thalassemia mutation or concurrent inheritance of an α -gene deletion or triplication or a mutation in the γ -gene [7–9]. Thus, associated primary, secondary, or tertiary modifiers can alter the phenotypic presentation.

Unequal crossing over between the linked α -globin genes on chromosome 16 may generate single or triplicated α -globin genes [1]. The presence of α -globin gene triplications is known to have aggravating effects on the phenotype of β -thalassemia heterozygotes [3, 10–12]. Beta-thalassemia heterozygotes with co-inherited α -globin gene triplication have moderate anemia, and these individuals may also present clinically like β -thalassemia intermedia cases (β -TI) [13, 14]. The effect of a single or a couple of additional α -globin genes in homozygous β -thalassemia cases would aggravate the severity even further; however, this has not been investigated in detail [1].

We identified 29 individuals with triplicated α -genes among 181 β -thalassemia patients and carriers with an unusual clinical presentation who were investigated. Among the 3 β -thalassemia homozygotes/compound heterozygotes with associated α -globin gene triplication, the first case presented unusually early at 6 weeks. β -Thalassemia major cases generally do not present till 3 to 6 months of age. There is,

Table 2 Hematological and molecular findings and transfusion requirements of β -thalassemia heterozygous with and without triplicated α -globin genes

No. of individuals	Hb (g/dl)	RBC ($\times 10^{12}/l$)	MCV (fl)	MCH (pg)	MCHC (g/dl)	RDW (%)	HbA ₂ (%)	HbF (%)	β -Gene mutations	Frequency of blood transfusions
β -Thalassemia heterozygotes with α -globin gene triplication ($n=36$)	8.2 \pm 1.5 ^a	4.0 \pm 0.9	67.6 \pm 7.2	20.6 \pm 2.7	30.5 \pm 2.6	24.2 \pm 6.4	4.7 \pm 0.6	2.6 \pm 1.7 ^a	IVS 1-5 (G \rightarrow C)—20, IVS 1-1 (G \rightarrow T)—1, CD 15 (G \rightarrow A)—2, CD 41/42 (-CTTT)—2	Transfusions only during pregnancy—2, Transfused once—10, Transfused 3–4 times—5, Transfused 8–10 times—2, No transfusions- 6.
Early presentation ($n=1$) Age, 3 months	5.2	2.77	61.4	18.8	30.6	33.0	3.2	21.4	IVS 1-5 (G \rightarrow C)	Transfused once
Asymptomatic ($n=10$) Age (years), 10–40	10.4 \pm 1.3 ^a	5.1 \pm 0.8	66.7 \pm 9.2	20.8 \pm 3.9	31.2 \pm 2.6	21.6 \pm 7.1	4.7 \pm 0.9	1.05 \pm 0.7 ^a	IVS 1-5 (G \rightarrow C)—8 Capsite +1 (A \rightarrow C)—1 -88 (C \rightarrow T)—1	Nil
β -Thalassemia heterozygotes without α -globin gene triplication ($n=30$)	9.1 \pm 2.6	4.6 \pm 1.3	65.3 \pm 7.5	20.6 \pm 2.7	30.9 \pm 1.6	21.0 \pm 5.8	5.0 \pm 0.8	1.8 \pm 1.7	IVS 1-5 (G \rightarrow C)—19 CD 15 (G \rightarrow A)—1 619 bp deletion—5 CD 41/42 (-CTTT)—1 CD 8/9 (+G)—1 CD 30 (G \rightarrow C)—1 IVS 1-1 (G \rightarrow T)—1 IVS 2-837 (T \rightarrow C)—1	Nil

Hb hemoglobin, RBC red blood cell, MCV mean corpuscular volume, MCH mean corpuscular hemoglobin, MCHC mean corpuscular hemoglobin concentration, RDW red cell distribution width, HbA₂ hemoglobin A₂, HbF fetal hemoglobin

^a Statistically significant at $p \leq 0.05$

Table 3 Hematological and molecular findings and transfusion requirements of normal individuals with and without triplicated α -globin genes

	No. of individuals	Hb (g/dl)	RBC ($\times 10^{12}/l$)	MCV (fl)	MCH (pg)	MCHC (g/dl)	RDW (%)	HbA ₂ (%)	HbF (%)	β -Gene mutation	Frequency of blood transfusions
Normal individuals with α -globin gene triplication	Asymptomatic (n=9) Age (years), 9–60	13.0 \pm 1.2	4.4 \pm 0.3	85.3 \pm 5.3	29.7 \pm 1.7	34.8 \pm 1.1	13.6 \pm 3.8	2.7 \pm 0.1	0.6 \pm 0.6	Nil	Nil
Normal individuals without α -globin gene triplication	Asymptomatic (n=30) Age (years), 16–39	13.9 \pm 1.3	4.7 \pm 0.3	83.3 \pm 10.3	29.5 \pm 1.9	34.7 \pm 1.3	13.6 \pm 1.0	2.6 \pm 0.1	0.2 \pm 0.1	Nil	Nil

Hb hemoglobin, RBC red blood cell, MCV mean corpuscular volume, MCH mean corpuscular hemoglobin, MCHC mean corpuscular hemoglobin concentration, RDW red cell distribution width, HbA₂ hemoglobin A2, HbF fetal hemoglobin

however, some data to indicate that β -thalassemia homozygotes with triplicated α -genes show evidence of hemolytic anemia earlier in life and anticipated transfusion dependency [15]. The β genotype would also influence the clinical presentation. Our second case (Table 1) was transfused 10 times in 4.5 years. The Capsite mutation when associated with a second severe β -gene mutation leads to a varied phenotype, and these patients are often transfusion independent. However, the triplicated α -gene may have further aggravated the condition. The third case was homozygous for a mild β -thalassemia mutation –88 (C \rightarrow T) with α -gene triplication and has remained untransfused till now (Hb 9.0 g/dl). However, he is only 3.5 years old yet.

β -Thalassemia heterozygotes with associated α -globin gene triplication show a variable phenotype. At one end of the spectrum, they are asymptomatic like most β -thalassemia heterozygotes although they may have slightly higher HbF values. At the other end, some individuals have a picture of mild thalassemia intermedia with hemoglobin values ranging from 8 to 10 g/dl and splenomegaly [1]. The clinical picture of heterozygous β -thalassemia with homozygous α -globin gene triplication has been of a moderate to severe form of thalassemia intermedia, with hemoglobin values in the range of 6–9 g/dl and moderate splenomegaly. In our study, the 3-month-old child, a β -thalassemia heterozygote with severe anemia (Table 2), could have inherited the triplicated α -globin genes from both her parents. As gene dosage studies were not done, we were not able to confirm this. In few earlier studies, the combination of homozygous α -gene triplication with β -thalassemia alleles has resulted in a moderate-to-severe β -thalassemia intermedia presentation with occasional blood transfusion requirements [13, 16, 17]. Fallah et al. [17] reported a 4-year-old child with an $\alpha\alpha\alpha/\alpha\alpha\alpha$ genotype and heterozygous β -thalassemia with the IVS 1–5 (G \rightarrow C) mutation, who had been transfused once for her anemia. All our symptomatic β -thalassemia heterozygotes had the common Indian β -globin gene mutations of the severe β^+ or β^0 type associated with a lower output or absence

of the synthesis of β -globin chains, whereas the asymptomatic carriers had the β^+ and β^{++} type of mutations with a higher amount of β -globin chain synthesis.

An earlier study showed that HbA₂ levels were moderately increased in the presence of α -globin gene triplications, either in association with β -thalassemia [13] or solely [18]. We did not observe this in our study. The HbA₂ level in β -thalassemia increases when excess of α -globin chains binds to excess δ -globin chains. As explained earlier in the case of α -globin gene triplication alone, the δ -globin chains are not overexpressed, so the small excess of α -globin chains after binding completely to the δ -globin chains would be eliminated by proteolysis whereas the HbA₂ level could be reduced because of the absence of α -globin chains which may in turn lead to proteolysis of the excess δ -globin chains [15].

The prevalence of the presence of α -globin gene triplication in β -thalassemia heterozygotes has been shown earlier to be around 2 % and in normal individuals around 3 % in India [19]. This study shows that α -gene triplication is not that infrequent in Indians and its interaction with a diversity of β -thalassemia mutations leads to variable phenotypes. Hence, the presence of triplicated α -globin genes should always be considered in β -thalassemia homozygotes and heterozygotes with an unusual presentation, which could influence the clinical and hematological presentation.

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Authorship contributions PM, DU, and MG carried out the molecular analysis for alpha-gene triplication, and PM wrote the first draft of the manuscript. PS helped with the molecular analysis for β -thalassemia, AN supervised the laboratory work, and CS clinically evaluated the patients. RC designed the study, helped with the analysis of the data, and finalized the manuscript. KG provided inputs for the manuscript. All the authors have read and approved the manuscript.

Conflict of interest The authors declare that they have no conflict of interest.

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