Association of polymorphic pattern of the (AT) × (T)_y motif of β-globin gene in North Indian thalassemia patients with variable clinical expression

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Received: 15 October 2008 / Accepted: 24 January 2009
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Abstract Among the polymorphic genetic markers of the β-globin gene cluster, the (AT) × (T)_y motif and a common C→T variation at -158 of the Gγ globin gene were investigated in 20 β-thalassemia IVS I-5 (G→C) homozygous patients with variable clinical manifestation and 10 normal subjects from north India to deduce any association with the co-inherited genetic polymorphism. We found (AT)₇(T)₇ repeat motif in all the 20 chromosomes of normal subjects and two different sequence configuration (AT)₈(T)₅, (AT)₉(T)₅ in the patient group whereas the presence of -158(C→T) Gγ was quite polymorphic. We conclude that polymorphism -158(C→T) Gγ and (AT) × (T)_y sequence variation other than (AT)₇(T)₇ could influence the phenotype by increasing Hb F expression.

Keywords (AT) × (T)_y motif · β-thalassemia · Polymorphism · Fetal hemoglobin

Letter to the editor

There are several reports which, indicates that the negative regulatory sequences, especially the sequences located between -610 nts and -490 nts of 5' β-globin gene modulate Hb F expression and clinical severity among β-thalassemia and sickle cell anemia patients [1]. This region spans the binding site for the trans acting BP1 nuclear protein that acts as negative regulatory protein [2].

In our north Indian population the commonest found mutation [65–70%] among β-thalassemia patients is IVS I-5 [G→C] [3] followed by 619bp deletion. However, variable clinical presentation was observed among patients having this mutation. To reveal variation in clinical severity in patients having IVS I-5 [G→C] mutation on both the chromosomes the present study is undertaken to evaluate the association of (AT) × (T)_y motif and -158 Gγ gene [C→T] polymorphism in transfused (12) and non-transfused (8) thalassemia patients.

Peripheral blood samples were taken from 12 transfusion dependent and 8 transfusion independent β-thalassemia patients homozygous for IVS I-5 (G→C) mutation after informed consent. All the subjects were hailed from Uttar Pradesh. Genomic DNA was isolated from the peripheral blood and all samples were characterized for red cell indices, HbA₂ and pre transfusion Hb F levels. Sequence variation in β-globin gene (1.8 kb) were examined by sequencing analysis of (AT) × (T)_y repeat motif at -540 region of β-globin gene and polymorphic (C→T) substitution at -158 of Gγ gene promoter region. Qiaquick PCR purification kit [Qiagen] was used for purification and sequencing was performed on ABI Prism 377 automated DNA sequencer (Applied Biosystems). Sequences were aligned with the corresponding wild type sequences using the Factura and Sequence navigator software programs.

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the normals where no mutation of β -globin gene is present. Eight of thalassemia patients had high levels of Hb F (>60–86.5%) and did not require transfusion (Table 1). The other twelve were transfusion dependent. The results indicated highly polymorphic pattern at Xmn I site. However, (AT)_x(T)_y motif show interestingly different pattern in thalassemia vs normal group.

Though, we observed various configurations of the motif like (AT)₈(T)₅, (AT)₇(T)₇, (AT)₉(T)₅ prevalent among thalassemia chromosomes. The (AT)₇(T)₇ motif seemed to be the prevalent configuration in normal chromosome only [6]. Bandyopadhyay, et al. (2005) [7] reported 65% prevalence of this motif in East India normal population. In our group of patient (AT)₇(T)₇ configuration seemed rare in thalassemia group like Kukreti, et al. and most of the chromosome being of (AT)₈(T)₅ variety.

Our results are in concordance of Kukreti, et al. (2002) [6] where they analyzed only two IVS I-5 chromosome & found association with (AT)₈(T)₅ motif. We also found that this mutation is associated chiefly with (AT)₈(T)₅ motif at a frequency of 28/40 (70%), (AT)₉(T)₅ 4/40 (10%) and (AT)₇(T)₇ 8/40 (20%). The diverse motif associated with IVS I-5 (G→C) mutation may be explained on the basis of inter-allelic gene conversion as well as separate origins of the mutation.

The frequency of the (AT)₇(T)₇ configuration in normal chromosomes of our studied Indian population is 100% and in thalassemia chromosome are 20%.

The two chromosome carrying the IVS I-5(G→C) mutation had (AT)₇(T)₇ rearrangement with a C→T substitution at position –543 in contrast to Dimovski, et al. (1994) [1] where this substitution was reported along with (AT)₈(T)₅ in IVS I-5(G→C) mutation (Table 2).

In our population, though the (AT)₇(T)₇ motif occurs at a frequency of 1.0 in normal chromosome it is rare among the thalassemia chromosome. It does not occur at all in the 32 chromosome bearing IVS I→5(G→C) indicating that the mutations originally occurred in chromosome bearing motif other than (AT)₇(T)₇. The (AT)₉(T)₅ motif occurred in our population in thalassemia chromosomes at a frequency of 0.10. The (AT)₈(T)₅ configuration of the motif, which did not occur in the European study at all, is present in Indian population in thalassemia chromosome at frequency of seven.

The (AT)₉(T)₅ motif and T allele of the –158 Gγ polymorphism were found to be associated with increased level of Hb F in β -thalassemia carriers (Guida, et al. 2006) [8]; this is in concordance of our results where two cases with (AT)₉(T)₅ motif and T allele of the –158 Gγ polymorphism have high level of Hb F.

Dimovski, et al. [1] reported (AT)₉(T)₅ motif found in

ten chromosomes and all had T→C substitution at position –551. Interestingly in our studied subjects this T→C substitution was found in only those two subjects which having (AT)₉(T)₅ motif on both the chromosome.

We conclude that in homozygous β -thalassemia patients, the severity of the mutations is the most important factor influencing the phenotype, but some polymorphisms such as –158(C→T) Gγ & (AT) × (T)_y repeat motif other than (AT)₇(T)₇, increasing the Hb F expression and ameliorate the clinical course of the disease.

Acknowledgement The authors are thankful to Council of Science & Technology, UP, India for providing financial assistance and Dr Ritushree Kukreti for sequence alignment.

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