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Full Length Research Paper

Frequency of Gγ-globin promoter -158 (C>T) Xmn I polymorphism in Denizli, Turkey

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The effect of -158 (C>T) Xmn I polymorphism on expression of Gγ-globin gene has been the subject of considerable interest. This study aims to determine the frequency of the Gγ Xmn I polymorphism in β-thalassemia patients in Denizli province of Turkey. We studied Xmn I polymorphism in the DNA samples of 27 with β-thalassemia major, 210 β-thalassemia minor patients and 100 healthy subjects as the control group. According to our results, 4/54 chromosomes (7.4%) from homozygous β-thalassemia patients, 37/210 chromosomes (17.6%) from heterozygous β-thalassemia carriers and 43/200 chromosomes (21.5%) from the control group were found to be positive for the Xmn I polymorphism. Besides, Xmn I polymorphism frequency of β° IVS II-1 (89.0%), β° codon 44 (75.0%) and β^+ -87 (66.0%) mutations showed relatively higher Xmn I polymorphism frequency regarding to the other β-thalassemia mutations. Xmn I polymorphism was fairly low in β-thalassemia patients and in the normal population in the region. We think that will be for the better explained with the other single nucleotide polymorphisms (SNPs) or the combinations associated with Xmn I polymorphism of β-globin gene interactions for the region.

Key words: Denizli, β-thalassemia mutations, Gγ- Xmn I polymorphism.

INTRODUCTION

The β-thalassemia syndromes originate from the absence of or from a reduction in the synthesis of structurally normal β-globin chains. The clinical presentation of β-thalassemia is highly variable, ranging from severe transfusion-dependent anemia to milder conditions (Weatherall, 2001). The patient's phenotype was determined by the β-chain deficiency and α/β-globin imbalance, and is influenced by a variety of genetic factors linked or unlinked to the β-globin gene cluster. Increased synthesis of γ-globin in adult life was used as an explanation of the mild phenotype in the β-thalassemia individuals in a growing list of studies. The increased γ-chain production is genetically determined and partially associated with β-haplotypes characterized by the presence of particular microsatellite sequences and/or the Xmn I polymorphism (Agouti et al., 2007; Grosso et al., 2008; Papachatzopoulou et al., 2006).

For many years, the search for treatment aimed at the reduction of globin chain imabalance in patients with β-thalassemia and hemoglobin S (Hb S) (sickle cell anemia) cases has focused on the pharmological manipulation of fetal hemoglobin ($\alpha_2\gamma_2$; Hb F). Clinical trials that is aimed at augmentation of fetal hemoglobin synthesis in β-thalassemia and Hb S (sickle cell anemia) patients, included those of 5-azacytidine, hydroxyurea (HU), recombinant human erythropoietin, butyric acid compounds and combinations of these agents (Italia et al., 2009; Olivieri and Weatherall, 1998a, b). Some studies suggested the possible association between genotype and response to HU, for patients who were homozygous for Xmn I polymorphism in different countries (Alebouyeh et al., 2004; Bradai et al., 2007; Yavarian et al, 2004). But in the work of other researchers, there was no correlation between the presence of Xmn I polymorphism and response to

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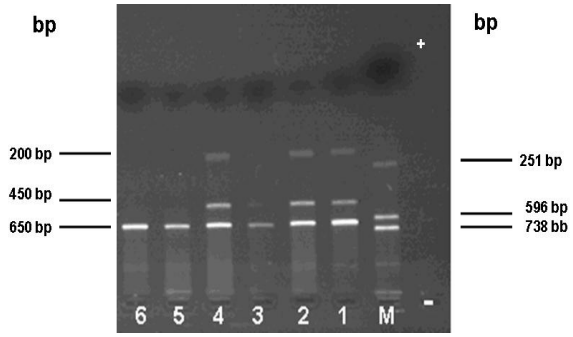

M: Marker (738 bp, 596 bp, 251 bp)

Lane 1,2,4: 650 bp, 450 bp and 200 bp fragments from the patients for the Xmn I (+/-)

Lane 3,5: 650 bp fragment from a patient with Xmn I (-/-)

Lane 6: Undigested 650 bp amplified sequence

Figure 1. Agarose gel electrophoresis of amplified DNA after digestion with Xmn I for the detection of the Gγ-globin promoter -158 (C>T) polymorphism alleles.

theraphy in β-thalassemia patients in different countries (Dixit et al., 2005; Koren et al., 2008).

The presence of the Xmn I polymorphism in the Gγ-globin gene promoter (the C>T substitution at position -158) has been shown to ameliorate the severity of the disease, because of its strong association with an increased production of fetal Hb. This particular polymorphism can be detected by the presence (+) or absence (-) of an Xmn I restriction enzyme site (Labie et al., 1985a, b). The effect of -158 (C>T) polymorphism on expression of Gγ-globin gene has been the subject of considerable interest. The association of β-thalassemia mutations with Xmn I Gγ polymorphism with elevated Hb F expression has been previously published (Dedoussis et al., 2000).

β-thalassemia is the most common single gene disorder in Denizli province of Turkey. The average frequency of the β-thalassemia was reported to be 2.6 to 3.7% in Denizli province by different researchers (Keskin et al., 2000; Yildiz et al., 2005). The β-globin gene cluster haplotypes linked with the β-thalassemia gene in Turkish population has been reported in previous studies (Atalay et al., 2007; Bahadir et al., 2009; Ozturk et al., 2007). But Xmn I polymorphism frequency in Turkish population has not been studied before. In the present study, our aim was to investigate the relationship between Xmn I polymorphism and β-thalassemia mutations in comparison with the normal population in Denizli province of Turkey.

MATERIALS AND METHODS

We studied 27 homozygous β-thalassemia patients, 210 heterozygous β-thalassemia carriers and DNA samples from 100 healthy control subjects. The DNA samples were collected from unrelated individuals. Written informed consent had been obtained from every individual and/or their parents. Samples from cases who had been diagnosed at the molecular level were deposited in the Pamukkale University Biophysics Department DNA bank as anonymous samples for further investigations.

Blood samples were collected in ethylenediaminetetraacetic acid (EDTA) vacutainers and DNA was extracted from peripheral blood using the standard phenol-chloroform procedure. Then, mutation analysis for the β-globin gene was done with β-globin Strip assay (ViennaLab, Austria). This beta thalassemia mutations were determined as β^+ IVS I-110 (G>A), β° IVS I-1 (G>A), β^+ IVS I-6 (T>C), β° IVS II-1(G>A), β^+ IVS I-5 (G>C), β° IVS I-116 (T>G), β° IVS I-130 (G>C), β^+ IVS II-745 (C>G), β^+-87 (C>G), β° frameshift codons (FSC) 8 (-AA), β° FSC 8/9 (+G), β° FSC 6 (-A), β° Codon 39 (C>T), β° Codon 44(-C) in Denizli province.

The promoter region at -158 position of the Gγ-globin gene was confirmed by Xmn I restriction enzyme digestion of a 650 bp amplified DNA sequence from the promoter region of the Gγ-globin gene. The 650 bp target DNA sequence was first amplified using the oligonucleotide primers BFH 003 (5'- AAC TGT TGC TTT ATA GGA TTT T-3') and BFH 004 (5'- AGG AGC TTA TTG ATA ACC TCA GAC-3') at a final concentration of 10 pmol each (Sutton et al., 1989). Polymerase chain reaction (PCR) components were purchased from Bioron (Ludwigshafen, Germany) and New England Biolabs, Inc. (Beverly MA, U SA). The PCR conditions were 30 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 15 s and elongation at 72°C for 30 s. A final extension at 72°C for 5 min was included.

PCR products (10 µl) were digested overnight at 37°C with Xmn I restriction enzyme (Fermentas Life Sciences, Vilnius, Lithuania, 10 U/µl). Digested fragments were analyzed with 1.2% agarose electrophoresis and visualized using ultraviolet light illumination.

RESULTS

β-thalassemia mutations included in this study were β^+ IVS I-110 (G>A), β° IVS I-1 (G>A), β^+ IVS I-6 (T>C), β° IVS II-1(G>A), β^+ IVS I-5 (G>C), β° IVS I-116 (T>G), β° IVS I-130 (G>C), β^+ IVS II-745 (C>G), β^+-87 (C>G), β° FSC 8 (-AA), β° FSC 8/9 (+G), β° FSC 6 (-A), β° Codon 39 (C>T), β° Codon 44 (-C) from Denizli. DNA amplification using the primers and the PCR protocol developed in our laboratories produced very distinct PCR products (Figure 1, lane 6). Digestion with PCR products with Xmn I produced 650, 450 and 200 bp bands and were observed when only one chromosome possessed site (+/-) (Figure 1, Lanes 1, 2 and 4). Absence of Xmn I site in both chromosomes (-/-) showed only the original undigested 650 bp fragment (Figure 1, Lanes 3 and 5).

The Xmn I polymorphism was divided into two categories (Xmn I [+] and Xmn I [-]). Xmn I allele frequency analysis showed that 4/54 chromosomes (7.4%) from homozygous β-thalassemia patients, 37/210 chromosomes (17.6%) from heterozygous β-thalassemia carriers and 43/200 chromosomes (21.5%) from the control group were found to be positive for the Xmn I polymorphism (Table 1). Besides, Xmn I polymorphism frequency of β° IVS II-1 (89.0%), β° codon 44 (75.0%)

Table 1. Xmn I polymorphism frequency in β-thalassemia and normal chromosomes.

Total N	Genotype type	Xmn I allele frequency {N (%)}	
		Xmn I [-]	Xmn I [+]
200	Normal	157 (78.5)	43 (21.5)
54	Homozygotes	50 (92.6)	4 (7.4)
210	Heterozygotes	173 (82.4)	37 (17.6)

N: No of chromosomes

Table 2. Xmn I polymorphism frequency of β-thalassemia mutation types in Denizli province of Turkey.

S/N	β-thalassemia type mutation	Codon	Total N	Xmn I [+]
				N (%)
1	IVS II-1(G>A)	β°	19	17 (89.0)
2	Codon 44 (-C)	β°	4	3 (75.0)
3	-87 (C>G)	β^+	3	2 (66.0)
4	IVS I-5 (G>C)	β°	6	3 (50.0)
5	IVS I-130 (G>C)	β°	2	1 (50.0)
6	IVS II-745 (C>G)	β^+	7	3 (42.0)
7	FSC 8 (-AA)	β°	15	6 (40.0)
8	Codon 39 (C>T)	β°	13	4 (30.0)
9	IVS I-6 (T>C)	β^+	23	4 (17.0)
10	IVS I-1 (G>A)	β°	42	6 (14.0)
11	IVS I-110 (G>A)	β^+	121	16 (13.0)
12	IVS I-116 (T>G)	β°	2	0 (0)
13	FSC 8/9 (+G)	β°	5	0 (0)
14	FSC 6 (-A)	β°	2	0 (0)

N: No. of chromosomes.

and β^+ -87 (66.0%) mutations showed relatively higher Xmn I polymorphism frequency regarding to the other β-thalassemia mutations observed in Denizli region (Table 2).

DISCUSSION

The Xmn I cleavage site is located at 158 bases upstream from the transcription start site of the Gγ gene in the β-globin gene cluster (Neishabury et al., 2010). In several reports, positive Xmn I Gγ polymorphism is associated with the main phenotype modifying factor of beta thalassemia major (Agouti et al., 2007; Boudrahem-Addour et al., 2009; Grosso et al., 2008; Neishabury et al., 2010; Panigrahi and Agarwal, 2008; Thein, 2004). Among these studies, β° codon 44 (-C), β^+ IVS I-5 (G>C), β° IVS II-1 (G>A) β^+ - 87 (C>G) and β° FSC 8 (-AA) mutations were observed to be positive for the Xmn I [+] polymorphism in different country (Agouti et al., 2007; Bradai et al., 2007; Boudrahem-Addour et al., 2009; Dixit et al., 2005; Labie et al., 1985; Panigrahi and Agarwal, 2008; Neishabury et al., 2010; Thein, 2004, 2008). In our study, we observed that Xmn I polymorphism was fairly low in beta thalassemia patients and in the normal population in Denizli region. Also, we observed that Xmn I polymorphism frequency of β° IVS II-1 (89.0%), β° codon 44 (75.0%) and β^+ -87 (66.0%) mutations showed relatively higher Xmn I polymorphism frequency regarding to the other β-thalassemia mutations (Agouti et al., 2007; Bradai et al., 2007; Boudrahem-Addour et al., 2009; Dixit et al., 2005; Labie et al., 1985; Panigrahi and Agarwal, 2008; Neishabury et al., 2010; Thein, 2004, 2008). This study were demonstrated in low presence of Xmn I polymorphism as accepted important genetic ameliorating factors in patients with thalassemia intermedia and thalassemia major in Denizli province.

To study the frequency of Xmn I polymorphism in β-thalassemia, we took the advantage of having a large number of individuals with thalassemia alleles. Xmn I polymorphism frequency was determinated between 27 β-thalassemia homozygotes, 210 β-thalassemia heterozygotes and 100 control subjects. Positive Gγ Xmn I polymorphism was not apperad in both thalassemia

cases and normal healthy cases with no statistically significant difference in frequency. To our knowledge, this study is the first report on Gγ-globin gene promoter Xmn I polymorphism in Turkey in relation with β thalassemia chromosomes. We observed that effective Xmn I polymorphism were relatively low, both in beta thalassemia mutations and normal population in Denizli region.

In some studies, researchers put forward to Xmn I polymorphism which is associated with increased expression of Gγ gene and response to HU was significantly correlated with the presence of Xmn I polymorphism (Alebouyeh et al., 2004; Bradai et al., 2007; Yavarian et al, 2004). However, according to other researchers, because of the fact that there was no correlation between the presence of Xmn I polymorphism and response to theraphy in β-thalassemia patients, they suggested that the presence of $\alpha^{-3.7}$ deletion is a predictor of good response (Dixit et al., 2005; Koren et al., 2008). This literature was also enabled us to evaluate the role of Xmn I polymorphism frequency not important genetic factors that could ameliorate the clinical course of β-thalassemia cases observed for Denizli province of Turkey. The results of our study implicated that Xmn I Gγ polymorphim alone could not be relied on for severe case of β-thalassemia disease. Consequently, we observed that if Xmn I polymorphism is the major cis-acting element in the HU theraphy, the region is not suitable locus for such an approach due to the low existence of the effective Xmn I polymorphism, both in β-thalassemia mutations and normal population. For this reason, other SNPs or the combinations associated with Xmn I polymorphism of β-globin gene interactions should be further analyzed in Denizli province.

Our results demonstrated the necessity to consider other possible mechanism for treatment of the diseases in this region. These mechanisms include repetitive sequences or single nucleotide polymorphism within the β-globin cluster locus control region (LCR), mutations in binding for transcription regulatory elements or mutations encoding β-globin transcription regulatory elements (Thein, 2008). Larger studies are needed to validate mechanisms for the genetic interactions underlying the genotype-phenotype relationship of β-thalassemia types, because these studies will allow the evaluation of different genetic factors or co-inheritance of Xmn I polymorphism involved in the amelioration of the clinical severity of β-thalassemia patients (Boudrahem-Addour et al, 2009). In conclusion, we hope that future studies will provide a basis for better understanding of the molecular mechanisms of β-thalassemia disease.

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