Trakia Journal of Sciences, Vol, 5, .Suppl. 1, pp7-8, 2007

Available online at:
http://www.uni-sz.bg

ISSN 1312-1723

IL-12BPRO POLYMORPHISM BY TWO ETHNIC GROUPS IN BULGARIA

N. Chilingirova, L. Miteva, S. Stanilova

Department of Molecular Biology, Immunology and Genetics,
Faculty of Medicine, Trakia University, Stara Zagora, Bulgaria
nat_chilingirova@yahoo.com

ABSTRACT

Interleukin 12 (IL-12) is a disulphide-bonded heterodimer consisting of a 35kDa alpha and a 40kDa beta subunit. It is involved in the stimulation and maintenance of Thl cellular immune responses, including the normal host defense against various intracellular pathogens.

The aim of this study was to determine the distribution of IL-12Bpro polymorphism in two ethnical groups - Bulgarians (n=76) and Turkish minority (n=31)—in Bulgaria. Genomic DNA was isolated from the blood of healthy Bulgarian citizens and genotyping was performed by ARMS-PCR using allele-specific primers: for 4bp-inserted allele *(IL 12Bpro-1)* and for shorter allele *(IL 12Bpro-2)*.

Our results showed no differences in genotype and allelic frequencies of lL-12bpro polymorphism by Bulgarians and Turkish minority in Bulgaria.

Key words: IL-12B; Promoter polymorphism; genotype and allelic frequencies; ethnic groups in Bulgaria

INTRODUCTION

The immune system is a complex system that is responsible for distinguishing the human body from everything foreign to it, and for protecting people and vertebrate animals against infections and foreign substances. The development of the immune system and the host response to microbial infection rely on the activation and silene ing of numerous, differentially expressed genes [1]. Interleukin (I L)-12 for instance plays a key role in the induction of Th 1 cells, a T cell subset involved in many autoimmune diseases. It is a heterodimeric 70 kDa glycoprotein consisting of a 40kDa subunit and a 35 kDa subunit and is naturally produced by monocytes/macrophages, and dendtiric cells in response to antigenic stimulation. IL-12 is involved in the differentiation of naive T cells into Thl cells, which is important in resistance against pathogens. It is known as a T cell stimulating factor, which can stimulate the growth and function of T cells. It stimu lates the production of interferon gamma and tumor necrosis factor alpha from T and natural killer (NK) cells]. Some cells such as macrophages and microglial cells have been shown to produce specifically the 1L 12p-40 subunit but not IL12 under certain conditions |2, 3]

Recently, a complete genomic sequence analysis of the IL-12 gene encoding its p40 subunit (I L - 1 2 B) identified several intronic polymorphisms, a single-nucle otide polymorphism (SNP) at position +16974 (+16974 A/C) in the 3'-untranslated region (UTR) of 1L-12B [4,5] and a promoter polymorphism (//.-*12Bpro)* [6]. IL-12B is located at chromosome 5q31 —33, with Genebank accession number: AY008847. In respect to an important role of promoter region in the expression of many eukaryotic genes, many studies

demonstrated association of *IL 12Bpro* polymorphism with gene expression, and Thl mediated diseases [6-10], On the other hand, a wide array of studies has demonstrated differences in genotype and allele frequencies of cytokine gene polymorphisms depending on ethnicity and race [11, I 2]

Against the background, we examined for the first time the genotype and allele frequencies of *IL- 12Bpro* polymorphism of IL-12B in two ethnic subgroups from Bulgaria.

MATERIALS AND METHODS

Subjects

A total group of 107 healthy volunteers were included in the study of the frequency distribution of *IL12Bpro* polymorphism. The mean age of the group was 36.3 ±12.1 years and contained 42 (39%) males and 65 (61%) females. This group was divided in two subgroups, according to the ethnic affiliations - Bulgarians (n=76) and Turkish minority in Bulgaria (n= 31). The mean age of the subgroup of Bulgarians was 39.3± 11,9 years (43 % males and 57 % females), and the mean age of the subgroup of Turkish minority in Bulgaria was 30±9.8 years (29% males and 71% females).

Informed consent was obtained from all subjects and authorization was given by the Ethics Review Board of the Faculty of Medicine, Trakia University.

DNA extraction and genotyping

First of all we isolated genomic DNA from the blood of 107 volunteers of both ethnic groups. Blood specimens were collected in tripotassium EDTA sterile tubes. Genomic DNA in our study was extracted using two main methods: standard salting out method according to *Miller et al* [12], normally used for DNA isolation, and column chromatography using GFX genomic blood DNA purification kit (Amersham, UK) and stored at -20°C until use.

In our case genotyping for the *!L-12Bpro* polymorphism in promoter region of the IL-12 B gene was performed with amplification refractory mutation system (ARMS)-PCR methodology as previously described with modifications by *Muller-Berghaus et al* [7].

Briefly, 5' primers specific for the I*L-UBpro* allele were used in combination with a generic 3' primer: 1L12B5-8 (TGT CTC CGA GAG AGG CTC TAA), IL12B5-10 (TGT CTC CGA GAG AGG GCT GT) and ILI2B3-5 (TGG AGG AAG TGG TTC TCG TAC). ILI2B5-8 and ILI 2B3-5 amplify the longer allele with 4bp insertion (referred to as *IL*l*2Bpro-1)* and 1L12B5 10 and IL12B3-5 amplify the short allele with GC transition (referred to as *IL 12Bpro-2).* PCR amplification was carried out in 10 pL volumes containing GeneAmp lOx PCR buffer, 0.25 U of AmpliTaq Gold polymerase, 2.5 mmol/1 MgC12, 100 mmol/l of each dNTP, 0.4 mmol/1 of each primer (primers and reagents were supplied by Applied Biosystems, USA), and 0.1 - 0.5 mg of genomic DNA. Amplifications were performed in a GeneAmp PCR System 9700 (Applied Biosystems). After the initial incubation step (95°C for 10 min), PCR was performed for 30 cycles: 30 sec at 95°C, 30 sec at 65°C, and 30 sec at 72°C. A final extension step of 7 min at 72°C completed the reaction. The PCR products, 196 bp for allele I and/or 192 bp for al lele 2, were visualized on a 2.5% agarose gel stained with ethidium bromide (0.5 mg/ml). Ethidium bromide and agarose were supplied by Sigma Chemical Co. The 100 bp DNA marker ladder was supplied by Amersham- Pharmacia, Biotech.

Statistical analysis

Statistical analyses were performed using StatSoft version 6. Comparison of the distribution of *IL-12Bpro* polymorphism was performed using the chi-square test. A *P*-value < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The PCR products from the successful amplification using *IL-12Bpro*-specific primers were electrophoresed on 2.5% agarose gels and visualized with ethidium bromide staining (0.5 mg/ml) and the electrophoresis pattern are presented in Figure 1, The alleles were designated as "allele-1" and "allele-2", respectively - three different genotyping variations for IL-12B promoter polymorphism, homozygous (1/1 and 2/2) and heterozygous (1/2) as a result from ARMS-PCR are indicated,

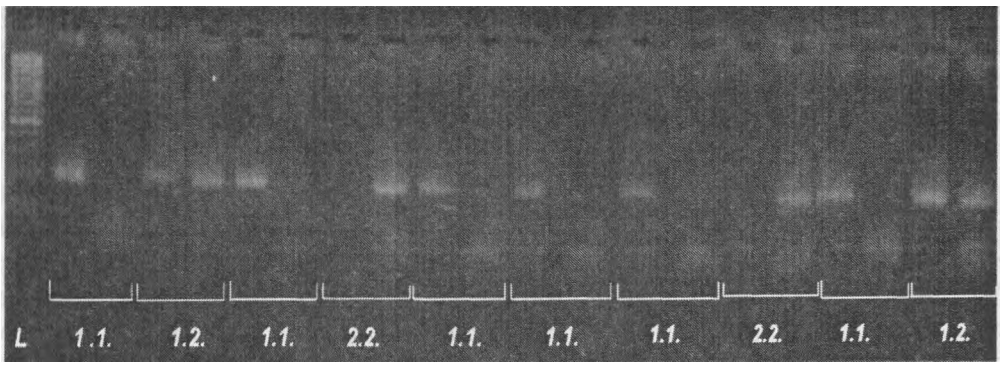

Figure 1. The ARMS-PCR products electrophoresed on 2.5% agarose gels performed for genotyping of IL12Bpro polymorphism.

When the amplification runs only in one reaction mixture containing specific primer set for lL12Bpro-l allele or specific for IL12Bpro-2 allele, the examined genotypes are homozygous (1/1 and 2/2 respectively). When the amplification runs in both reaction mixtures, the examined genotype is heterozygous (genotype 1/2). L- indicates the100 bp DNA marker ladder

The genotype and allele frequencies of the *IL-12Bpro* polymorphism in our study group comprised 107 healthy volunteers from two ethnic subgroups - Turkish minority in Bulgaria and Bulgarians are presented in Table 1.

The distribution of *IL-12B* promoter genotypes in all investigated groups did not deviate from the expected Hardy-Weinberg equilibrium (P >0.05).

	Bulgarians		Turkish minority		Total group of Bulgarian citizens	
	n	%	n	%	n	%
Genotype frequencies						
Genotype -1.1.	25	33%	11	35%	36	34
Genotype -1.2.	38	50%	16	52%	54	50
GenotyDe - 2.2.	13	17%	4	13%	17	16
Allelic frequencies						
Allele-1	88	58%	38	61%	126	59
Allele-2	64	42%	24	39%	88	41

Table 1: Genotype and allelic frequencies of the IL-12Bpro polymorphism in Bulgarian citizens

The distribution of *IL-12Bpro* polymorphism among two ethnic groups from Bulgaria was very similar. No significant differences of genotype ($\chi 2$=0.262; p=0.731), and allele frequencies ($\chi 2$=0.21; p=0.647), were observed in comparison between Bulgarians and the Turkish minority in Bulgaria, using the $\chi 2$ -test.

Bearing in mind that Turkish minoritty is a major ethnic group in Bulgaria country, since 16th century, we were interested in genotype distribution of one of high polymorphic cytokine gene- 1L-12B, of the people living on territory of Bulgaria, who are members of these two different ethnic groups - Bulgarians and Turks.

Our results, concerning genotype and allelic frequencies of *IL-l2Bpro* polymorphism among Bulgarians and the Turkish minority, showed no significant differences between them. These results are in principal agreement with other studies, demonstrated lack of differences between Bulgarians and the Turkish minority in Bulgaria

for other polymorphism in the same gene, precisely +16974A/C SNP in 3'-untraslated region [14 -16], as well as for other genes like the multi-locus haplotypes of HLA class I and class 11 loci [17],

Based on our results, both groups of Turkish minority in Bulgaria and Bulgarians can be included in a total group from the overall Bulgarian population. The frequencies of the 1,1, 1,2, and 2,2 genotypes in total cohort from Bulgarian citizens were 34%, 50%, and 16%, respectively, which were similar to the frequencies reported for other populations from Caucasian race, for example for two hundred forty-four individuals from the Melbourne cohort [9], for healthy donors from Netherlands [10] and Germany [7].

We investigated the distribution of *IL-12Bpro* polymorphism in total cohort of Bulgarians in respect to sex and the results are present in table 2,

	Genotype frequencies			Allelic frequencies	
	Genotype 11, n(%)	Genotype 1.2. n(%)	Genotype 2.2. n (%)	Allele 1	Allele 2
Female (n = 65)	23 (35)	33 (51)	9(14)	79 (61)	51 (39)
Male (n = 42)	13(31)	21 (50)	8(19)	47(56)	37 (44) ,

Table 2. Genotype and allelic frequencies of the 1L-12Bpro polymorphism in attit ude of the sex

The genotype frequencies among female and male in total group of Bulgarian population were very similar (genotype 1.1- 35% vs. 31%; genotype 1,2- 51% vs. 50%; genotype 2,2- 14% vs. 14%; $\chi 2 = 0.586$; p=0,746). Also, the established allele frequency of the allele-1 in female (61%) was did not different to those in male (56%); $\chi 2=0.489$; p=0.484.

These results demonstrated that *IL-12Bpro* polymorphism does not depend on the sex, in contrast to some other cytokine genes' polymorphisms, precisely IL-10, IFN-gamma genes, that had been associated with gender [18, 19].

In conclusion, in the present study, we demonstrated that there is no difference in attitude of the *!L- 12Bpro* polymorphism between Bulgarians and Turkish minority in Bulgaria. The genotype and allelic frequencies for *IL-12Bpro* by Bulgarians do not deviate from frequencies reported for other populations of the white race and the distribution of *IL-12Bpro* polymorphism does not associated with gender. The present study may facilitate the investigations of cytokine polymorphisms and differences in immune response between ethnic groups and populations.

REFERENCES

t. Janeway C.A. Travers P., Walport M., Shlomchik M. Immunobiology New York and London: Garland Science ; c2001

2. HoElscher C: The power of combinatorial immunology: dimeric cytokines in infectious diseases, Med Microbiol Immunol 2004, 193: 1-17

3. Trinchieri G: Interleukin-12 and the regulation of innate resistance and adaptive immunity Nature Reviews, Immunology 2003, 3: 133 - 146

4. Huang D, Cancilla MR, Morahan G. Complete primary structure, chromosomal localization and definition of polymorphisms of the gene encoding the human interleukin- 12p40 subunit. Genes Immun 2003; 1: 515-520.

5. Hall MA, McGlinn E, Coakley G et al. Genetic polymorphism of 1L-12 p40 gene in immune-mediated disease. Genes Immun 2000; 1: 219-224.

6. Morahan G, Boutlis CS, Huang D et al. A promoter polymorphism in the gene encoding interleukin-12 p40 (IL-12B) is associated with mortality from cerebral malaria and with reduced nitric oxide production. Genes Immun 2002; 3:414—418.

7. Muller-Berghaus J, Kern K, Paschen A et al. Deficient IL-12p70 secretion by dendritic cells based on IL12B promoter genotype. Genes Immun. 2004; 5: 431 — 4.

8. Morahan G, Huang D, Wu M et al. Association of IL12B promoter polymorphism with severity of atopic and non-atopic asthma in children. Lancet 2002; 360: 455-9.

9. Khoo SK, Hayden CM, Roberts M, Horák E, de Klerk N, Zhang G, Robertson CF, Goldblatt J, Le Souef P. Associations of the IL12B promoter polymorphism in longitudinal data from asthmatic patients 7 to 42 years of age. J Allergy Clin Immunol, 2004, 113:475-481

10. Litjens NH, van der Plaš MJ, Ravensbergen B, Numan-Ruberg SC, van Assen Y, Thio HB, van Dissel JT, van de Vosse E, Nibbering PH. Psoriasis is not associated with IL-12p70/IL-12p40 production and ILI2B promoter polymorphism. J Invest Dermatol. 2004 Apr; 122(4):923-6,

11. Golovleva 1, Saha N, Beckman L. Ethnic differences in interferon-alpha allele frequencies. Hum Hered 1997; 47:185-188.

12. De Capei MU, Dametto E, Fasano ME, Rendine S, Curtoni ES.Genotyping for cytokine polymorphisms: allele frequencies in the Italian population, Eur J Immunogenet 2003; 30: 5—10.

13. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucl Acid Res 1988; 16: 1215-1220.

14. L. Miteva, S. Stanilova Genotype and allelic frequency of a Taql polymorphism in 3'UTR of the IL-12p40 gene in Bulgarians. Trakia Journal of Sciences 2004, Vol. 2, No. 3, pp 31-35.

15. Stanilova S, Miteva L. Taq-I polymorphism in 3'UTR of the IL-12B and association with IL-12p40 production from human PBMC. Genes Immun. 2005;6:364-6.

16. Yilmaz V, Parmaan YG, Serdaroglu P, Deymeer F, Saruhan-Direskeneli G. IL-12 and IL-10 polymorphisms in Myastenia gravis. Neuroanatomy 2003; 2 (Suppl 1): 1 (abstr. 4).

17. Uyar FA, Dorak MT, Saruhan-Direskeneli G. Human leukocyte antigen-A, -B and -C alleles and human leukocyte antigen haplotypes in Turkey: relationship to other populations. Tissue Antigens 2004; 64: 180-187.

18. Lio D, Scola L, Crivello A, Colonna-Romano G, Candore G, Bonafe M, Cavallone L, Franceschi C, Caruso C. Gender-specific association between -1082 IL-10 promoter polymorphism and longevity. Genes Immun. 2002;3:30-3.

19. Lio D, Scola L, Crivello A, Bonafe M, Franceschi C, Olivieri F, Colonna-Romano G, Candore G, Caruso C. Allele frequencies of +874T— >A single nucleotide polymorphism at the first intron of interferon-gamma gene in a group of Italian centenarians. Exp Gerontol. 2002; 37:315-9.