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Full Length Research Paper

Analysis of Interleukin-10 polymorphic variants in Pakistani population

Muhammad Sohail Afzal*, Sadia Tahir, Amna Salman, Talha Shafi, Tahir Ahmed, Faiza Rasheed and Ishtiaq Qadri

NUST Center of Virology and Immunology (NCVI), National University of Sciences and Technology (NUST), Islamabad, Pakistan.

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Interleukin-10 (IL-10) is a Th2 cytokine that down regulates the Th1 cytokine, functions as immunosupressor for innate arm of the immune system and plays a significant role in a wide range of human diseases. IL-10 plasma levels vary inter-individually and three-quarters of this variability is contributed by genetic factors. For analysis of pattern of inheritance of IL-10 polymorphism in Pakista, 419 individuals were enrolled. In this study, investigation of promoter polymorphism (AF295024.1:c.-1082 G>A, AF295024.1:c.-819 C>T, AF295024.1:c.-592 C>A) was performed by amplification refractory mutation system-polymerase chain reaction (ARMS-PCR). Results show that heterozygous genotypes AF295024.1:c.-1082 G>A (84%), AF295024.1:c.-819 C>T (86%), and AF295024.1:c.–592 C>A (86%) at all studied loci were the major genotypes inherited in Pakistan. Four haplotypes AF295024.1:c. [-1082 G>A; -819 C>T; -592 C>A] [G; C; C], [G; T; A], [A; C; C], [A; T; A] were found in proportion of 43.7, 9.9, 3.7 5 and 43%, respectively. The haplotype [G; T; A] had been reported previously only from China. Nine different diplotypes were identified and [G; C; C/A; T; A] (73.5 %) was the major player. Results were compared with other published reports from different ethnic groups which suggest that ethnic differences influence the pattern of inheritance of IL-10 polymorphism in a significant way. This difference in inheritance pattern based on ethnicity may contribute to human diseases outcomes.

Key words: IL-10, polymorphism, ethnicity.

INTRODUCTION

Cytokines have been the focus of scientific interest for the last two decades. Cytokines play an important role in pathogenesis of various diseases so analyzing their expression enables one to understand disease pathogenesis. There are two subsets of T- helper cells, Th1 and Th2, which secrete cytokines (Mosmann et al., 1986). Th1 and Th2 CD4+ T- cells differ in cytokine expression. Th1 cells produces INF-y, INF-a, IL-12, and IL-2 whereas Th2 cells express IL-10, -4, -6, -5 , and -13 (Romagnani .,1995). IL-10 was first described as cytokine synthesis inhibitory factor (CSIF) produced by Th2 cell clones and it inhibits the synthesis of several cytokines (INF-y, IL-2) produced by Th1 cell clones (Fiorentino et al., 1989) and disturbs the Th1/Th2 balance. IL-10 production capacity has been demonstrated for various cell population, that is certain T-cell subsets (Th2, Tc2, Tr1) (Fiorentino et al., 1989), monocytes/macrophages (De Waal Malefyt, 1991; Spite and Dewell, 1992), B cells (Pistoia, 1997), eosinophils (Nakajima et al., 1996) and mast cells (Lin and Befus, 1997). Macrophages seem to be the major source of IL-10 (Asadullah et al., 2003).

Human IL-10 is a homo dimer with molecular mass of 37 KD (Asadullah et al., 2003). The human IL-10 gene is located on chromosome 1, 5.1 Kb in size and encodes for 5 exons (Spite and Dewell, 1992). Biological effects of IL-10 are quite complex. Debates are going on to establish its role as either an immunosuppressive or

*Corresponding author. E-mail: Sohail.ncvi@gmail.com. Tel: +92-321-5244808, +92-51-90856144. Fax: +92-51- 90856122.

immunomodulatory. Both overexpression and deficiencies of IL-10 are likely to have pathophysiological significance in infectious diseases, autoimmunity, allergy, cancer and transplantation. IL-10 mediates cell signaling through Jak/Stat and NFkB pathways and controls many biological process of the body (Finbloom and Winestock, 1995).

IL-10 promoter is highly polymorphic. It contains two informative micro satellites, IL-10 G and IL-10 R; 1.2 Kb and 4 Kb upstream of transcription start site (Eskdale et al., 1996). It also possess several other polymorphic sites including three frequent point mutations at AF295024.1:c.-1082 G>A, AF295024.1:c.-819 C>T, and AF295024.1:c.-592 C>A. These SNPs showed linkage disequilibrium and form haplotypes AF295024.1:c. (-1082 G>A; -819 C>T; -592 C>A) (Turner et al., 1997). Based on these haplotypes, individuals are divided into high producers [G; C; C], intermediate producers [A; C; C] and low producers [A; T; A] of IL-10 (Hulkkonen et al., 2001). IL-10 polymorphism distribution varies among different population and results in differential genotype, haplotypes and diplotypes (haplotype inherited) prevalence among different populations. This study investigates the presence and ratios of different IL-10 alleles, genotypes, haplotypes and diplotypes in Pakistani population.

MATERIALS AND METHODS

Study group

For determining the IL-10 SNPs and haplotypes in Pakistan blood samples from 419 individuals enrolled in the study from different areas of the country, blood samples were collected in EDTA tubes and were stored at 4°C till extraction. All the participants were of same ethnicity and from the same geographical area. The study was approved by the Ethical Committee of NUST Center of Virology and Immunology (NCVI), Islamabad, Pakistan and written consent was obtained, for each participant.

DNA extraction

Genomic DNA from venous blood of subjects included in the study were extracted using genomic DNA extraction kit according to the manufacturer's protocol (Gentre, USA). DNA quantification was done by Bio Photometer (Eppendorf, USA). DNA was stored at -20°C till further usage.

Genetic analysis

For IL-10 haplotypes determination, ARMS-PCR method was used as described by Perry et al. (1998). For each polymorphism, two separate reactions were performed. Each reaction contained one of the two allele specific forward primers and a generic anti sense primer. PCR amplification was performed in 15 µl reaction volume containing 40 ng genomic DNA, 1.5 µl 2 mM dNTP, 25 mM $MgCl_2$, 1 µl 10 pmol each primer and 0.7 units of Taq polymerase in 1X reaction buffer with cycling conditions 94°C for 5 min, followed by 35 cycles at 94°C for 45 s, 58°C for 40 s, 72°C for 50 s and finally 7 min extension at 72°C. To ensure PCR success, an internal control region was amplified from the human growth hormone. The amplified products were analyzed on 2% agarose gel.

Statistical analysis

Statistical analysis was performed by Study Result Software Version 1.0.4 (CreoStat HB Frolunda, Sweden). The distribution of cytokine genes polymorphisms among Pakistani population and other populations were compared by the χ^2 or Fischer's exact test. P values smaller than 0.05 were considered significant.

RESULTS

The study was able to establish that the allele frequencies of AF295024.1:c.-1082 G and AF295024.1:c.-1082 A; 0.53 and 0.47. The C, T (C, A) alleles at AF295024.1:c.-819 C>T, and AF295024.1:c.-592 C>A was almost similarly inherited in Pakistani population, that is, 49.4 and 50.6%, respectively (Table 2). The genotype frequencies (Table 4) of AF295024.1:c.-1082 GG, AF295024.1:c.-1082 GA and AF295024.1:c.-1082 AA was 11, 84 and 5%, respectively. The frequencies for AF295024.1:c.-819 CC, AF295024.1:c.-819 CT and AF295024.1:c.-819 TT (AF295024.1:c.-592 CC, AF295024.1:c.-592 CA and AF295024.1:c.-592 AA) at AF295024.1:c.-819 (AF295024.1:c.-592) were 0.04, 0.86 and 0.1%, respectively. It was also found that four haplotypes in these SNPs AF295024.1:c.[-1082 G>A; –819 C>T; -592 C>A] were present in Pakistani population. Table 6 shows the frequencies of haplotypes which were 43.7, 9.9, 3.7 and 43% of [G; C; C], [G; T; A], [A; C; C] and [A; T; A] respectively. There were nine different diplotypes found in our results (Table 8) among those [G; C; C/A; T; A] was the major diplotype (73.5%) followed by [G; C; C/G; T; A] (8.8%), [G; T; A/A; T; A] (7.2%), [A; C; C/A; T; A] (4.3%), [G; C; C/A; C; C] (3.3%) and [G; T; A/G; T; A] (1.9%). The diplotypes [A; T; A/A; T; A], [G; C; C/G; C; C] and [A; C; C/A; C; C] were the minor ones (0.5, 0.2 and 0.2%, respectively).

It was found that there is a significant difference ($p > 0.05$) in all studied IL-10 polymorphic alleles between Pakistan and other countries reports, that is China (Zhang et al., 2006), Republic of Macedonia (Trajkov et al., 2009), Turkey (Ates et al., 2008) and Italy (Scassellatia et al., 2004) except AF295024.1:c.-1082 A which showed insignificant difference between Pakistan and Italy (frequencies in Table 1, p values in Table 2).

Analysis of IL-10 genotypes (frequencies in Table 3, p values in Table 4) at AF295024.1:c.-1082 G>A, AF295024.1:c.-819 C>T, AF295024.1:c.-592 C>A (AF295024.1:c.-819 C>T and AF295024.1:c.-592 C>A are in linkage disequilibrium) among Pakistan and other published studies on Chinese (Zhang et al., 2006), Macedonians (Trajkov et al., 2009), Turkish (Ates et al., 2008) and Italian (Scassellatia et al., 2004) population illustrated that AF295024.1:c.-1082 GG was significantly different between Pakistan, China (Zhang et al., 2006), Pakistan, and Italy (Scassellatia et al., 2004). There was no difference found at the same locus for same combination of alleles among Pakistan, Republic of Macedonia (Trajkov et al.,2009) and Pakistan and Turkey

Table 1. Comparison of IL-10 polymorphic alleles among different populations.

IL-10 polymorphism	Pak 419	F (%)	Chi 322	F (%)	Mac 374	F (%)	Tur 208	F (%)	Ita 368	F (%)
AF295024.1:c.-1082 G	444	53	44	6.8	314	42	161	39.7	295	40.1
AF295024.1:c.-1082 A	394	47	600	93.2	434	58	255	61.3	441	59.9
AF295024.1:c.-819 C	414	49.4	269	41.8	540	72.2	292	70.2	547	74.3
AF295024.1:c.-819 T	424	50.6	375	58.2	208	27.8	124	29.8	189	25.7
AF295024.1:c.-592 C	414	49.4	269	41.8	534	71.4	292	70.2	547	74.3
AF295024.1:c.-592 A	424	50.6	375	58.2	214	28.6	124	29.8	189	25.7

Pak= Pakistan; Chi = China; Mac = Republic of Macedonia; Tur = Turkey; Ita = Italy; F = frequencies.

Table 2. P values of IL-10 polymorphic alleles from Pakistani population with different ethnic groups.

IL-10 polymorphism	Pakistan	China	Macedonia	Turkey	Italy
AF295024.1:c.-1082 G	Pakistan	0.00001	0.00001	0.00001	0.00001
AF295024.1:c.-1082 A	Pakistan	0.00001	0.00001	0.00001	0.237
AF295024.1:c.-819 C	Pakistan	0.00348	0.00001	0.00001	0.00001
AF295024.1:c.-819 T	Pakistan	0.00348	0.00001	0.00001	0.00001
AF295024.1:c.-592 C	Pakistan	0.00348	0.00001	0.00001	0.00001
AF295024.1:c.-592 A	Pakistan	0.00348	0.00001	0.00001	0.00001

Table 3. Comparison of IL-10 polymorphic genes among different populations.

IL-10 Polymorphism	Pak 419	F (%)	Chin 322	F (%)	Mac 374	F (%)	Turk 208	F (%)	Ita 368	F (%)
AF295024.1:c.-1082 GG	46	11	2	0.6	27	7.2	32	15.4	61	16.6
AF295024.1:c.-1082 GA	352	84	281	87.3	260	69.5	97	46.6	173	47
AF295024.1:c.-1082 AA	21	5	39	12.1	87	23.3	79	38	134	36.4
AF295024.1:c.-819 CC	17	4.1	159	49.4	190	50.8	99	47.6	127	34.5
AF295024.1:c.-819 CT	362	86.4	55	17.1	160	42.8	94	45.2	210	57.1
AF295024.1:c.-819 TT	40	9.5	108	33.5	24	6.4	15	7.2	31	8.4
AF295024.1:c.-592 CC	17	4.1	159	49.4	193	51.6	99	47.6	127	34.5
AF295024.1:c.-592 CA	362	86.4	55	17.1	148	39.6	94	45.2	210	57.1
AF295024.1:c.-592 AA	40	9.5	108	33.5	33	8.8	15	7.2	31	8.4

Pak= Pakistan; Chi = China; Mac = Republic of Macedonia; Tur = Turkey; Ita = Italy; F = frequencies.

(Ates et al., 2008). Inheritance of AF295024.1:c.-1082 AA showed significant difference among all the studied groups. AF295024.1:c.-1082 GA was inherited in significantly different manner among Pakistan and China (Zhang et al., 2006), but showed similar behavior in Pakistani, Macedonians (Trajkov et al., 2009), Turkish (Ates et al., 2008) and Italians (Scassellatia et al., 2004). There was a significant difference found in AF295024.1:c.-819 CC and AF295024.1:c.-819 CT (AF295024.1:c.-592 C>A and AF295024.1:c.-592 CA) inheritance among all ithe ncluded populations in comparison with Pakistan. But the pattern of inheritance of AF295024.1:c.-819 AA (AF295024.1:c.–592 TT) at the same loci did not show difference in Macedonians (Trajkov et al., 2009), Turkish (Ates et al., 2008) and Italians (Scassellatia et al., 2004) while it showed difference with Chinese (Zhang et al., 2006) in comparison with our results from Pakistan.

As illustrated in Tables 5 and 6, IL-10 haplotypes [A; C; C] and [A; T; A] was significantly different between Pakistan and China (Zhang et al., 2006), Republic of Macedonia (Trajkov et al., 2009), Turkey (Ates et al., 2008) and Italy (Scassellatia et al., 2004) while [G; C; C] haplotype was only differently inherited among Pakistan and China (Zhang et al., 2006). According to our results, presence of [G; T; A], a high IL-10 producing haplotypes was 9.9% in Pakistan which was not found in the included studies. Macedonian population (Trajkov et al., 2009) was the only who has [A; C; A] and [A; T; C] haplotypes.

Table 7 (frequencies) and Table 8 (p values) show the difference in inheritance of diplotypes among Pakistan and other reported results from China (Zhang et al., 2006), Republic of Macedonia (Trajkov et al., 2009), Turkey (Ates et al., 2008) and Italy (Scassellatia et al., 2004). All combination of diplotypes was significantly

Table 4. P values of IL-10 polymorphic genes from Pakistani population with different ethnic groups.

IL-10 olymorphism	Pakistan	China	Macedonia	Turkey	Italy
AF295024.1:c.-1082 GG	Pakistan	0.00001	0.0675	0.1155	0.022
AF295024.1:c.-1082 GA	Pakistan	0.21287	0.00001	0.00001	0.00001
AF295024.1:c.-1082 AA	Pakistan	0.00045	0.00001	0.00001	0.00001
AF295024.1:c.-819 CC	Pakistan	0.00001	0.00001	0.00001	0.00001
AF295024.1:c.-819 CT	Pakistan	0.00001	0.00001	0.00001	0.00001
AF295024.1:c.-819 TT	Pakistan	0.00001	0.1063	0.33048	0.58336
AF295024.1:c.-592 CC	Pakistan	0.00001	0.00001	0.00001	0.00001
AF295024.1:c.-592 CA	Pakistan	0.00001	0.00001	0.00001	0.00001
AF295024.1:c.-592 AA	Pakistan	0.00001	0.1063	0.33048	0.58336

Table 5. Comparison of IL-10 haplotypes among different populations.

IL-10 polymorphism	Pak 419	F (%)	Chi 322	F (%)	Mac 374	F (%)	Tur 208	F (%)	Ita 368	F (%)
[G; C; C] (high)	364	43.4	43	6.9	314	42	161	38.7	295	40.1
[G; T; A] (high)	83	9.9	-	-	-	-	-	-	-	-
[A; C; C] (intermediate)	31	3.7	226	35.1	214	28.6	131	31.5	253	34.4
[A; T; A] (low)	360	43	375	58	202	27	124	29.8	188	25.5
[A; C; A] (low)	-	-	-	-	12	1.6	-	-	-	-
[A; T; C](low)	-	-	-	-	4	0.5	-	-	-	-0.2

Pak= Pakistan; Chi = China; Mac = Republic of Macedonia; Tur = Turkey; Ita = Italy; F = frequencies.

Table 6. P values of IL-10 polymorphic haplotypes from Pakistani population with different ethnic groups.

IL-10 polymorphism	Pakistan	China	Macedonia	Turkey	Italy
[G; C; C]	Pakistan	0.00001	0.5579	0.10955	0.17822
[G; T; A]	Pakistan	-	-	-	-
[A; C; C]	Pakistan	0.00001	0.00001	0.00001	0.00001
[A; T; A]	Pakistan	0.00001	0.00001	0.00001	0.00001
[A; C; A]	Pakistan	-	-	-	-
[A; T; C]	Pakistan	-	-	-	-

different when we compared data from Pakistan with China (Zhang et al., 2006), Republic of Macedonia (Trajkov et al., 2009), Turkey (Ates et al., 2008) and Italy (Scassellatia et al., 2004) except [G; C; C/G; C; C] and [G; C; C/A; C; C] which showed no significant association between Pakistan and China (Zhang et al., 2006). As [G; T; A] haplotype was reported only from us, so no diplotype was present, containing [G; T; A] in any other including group. The distributions of the polymorphisms in local Pakistani population are in hardy Weinberg equilibrium.

DISCUSSION

Expression of IL-10 gene is tightly regulated and genetic factors contribute to 75% of differential inter-individual IL-10 secretion (Westendorp et al., 1997). Human IL-10 gene promoter is highly polymorphic with two CA repeats (IL G and IL-10 R) (Eskdale et al., 1995; Eskdale et al., 1996) and three SNPs at AF295024.1:c.-1082 G>A, AF295024.1:c.-819 C>T, AF295024.1:c.-592 C>A and results in three haplotypes {[G; C; C], [A; C; C], [A; T; A]} (Turner et al., 1997). The AF295024.1:c.-1082 G>A substitution contribute to IL-10 gene promoter activity (Kube et al., 1995). The disease in which outcomes are not identifiable with single polymorphism, study of multi site haplotypes and diplotypes is highly informative allelic markers. Haplotype indicates how single polymorphism interacts with each other to amplify or moderate their individual effect (Ouma et al., 2008). The allele A at

Table 7. Comparison of IL-10 diplotypes among different populations.

IL-10 polymorphism	Pak 419	F (%)	Chin 322	F (%)	Mac 374	F (%)	Tur 208	F (%)	Ita 368	F (%)
[G; C; C/G; C; C] (high)	1	0.2	2	0.6	27	7.2	31	14.9	61	16.6
[G; C; C/G; T; A] (high)	37	8.8	-	-	-	-	-	-	-	-
[G; T; A/G; T; A] (high)	8	1.9	-	-	-	-	-	-	-	-
[G; C; C/A; C; C] (intermediate)	14	3.3	12	3.7	135	36.1	46	22.1	107	29.1
[G; C; C/A; T; A] (intermediate)	308	73.5	27	8.4	116	31	53	25.5	66	17.9
[G; T; A/A; C; C] (intermediate)	-	-	-	-	-	-	-	-	-	-
G; T; A/A; T; A(intermediate)	30	7.2	-	-	-	-	-	-	-	-
[A; C; A/G; C; C] (intermediate)	-	-	-	-	3	0.8	-	-	-	-
[A; T; C/G; C; C] (intermediate)	-	-	-	-	6	1.6	-	-	-	-
[A; C; C/A; C; C] (low)	1	0.2	41	12.7	25	6.7	21	10.1	43	11.7
[A; C; C/A; T; A] (low)	18	4.3	132	41	29	7.8	43	20.7	60	16.3
[A; T; A/A; T; A] (low)	2	0.5	108	33.4	24	6.4	14	6.7	31	8.4
[A; C; A /A; T; A] (low)	-	-	-	-	9	2.4	-	-	-	-

Pak= Pakistan; Chi = China; Mac = Republic of Macedonia; Tur = Turkey; Ita = Italy; F = frequencies.

Table 8. P values of IL-10 polymorphic diplotypes from Pakistani population with different ethnic groups.

IL-10 Polymorphism	Pakistan	China	Macedonia	Turkey	Italy
[G; C; C/G; C; C]	Pakistan	0.416	0.00001	0.00001	0.00001
[G; C; C/G; T; A]	Pakistan	-	-	-	-
[G; T; A/G; T; A]	Pakistan	-	-	-	-
[G; C; C/A; C; C]	Pakistan	0.77745	0.00001	0.00001	0.00001
[G; C; C/A; T; A]	Pakistan	0.00001	0.00001	0.00001	0.00001
[G; T; A/A; C; C]	Pakistan	-	-	-	-
[G; T; A/A; T; A]	Pakistan	-	-	-	-
[A; C; A /G; C; C]	Pakistan	-	-	-	-
[A; T; C /G; C; C]	Pakistan	-	-	-	-
[A; C; C/A; C; C]	Pakistan	0.00001	0.00001	0.00001	0.00001
[A; C; C/A; T; A]	Pakistan	0.00001	0.0395	0.00001	0.00001
[A; T; A/A; T; A]	Pakistan	0.00001	0.00001	0.00001	0.00001
[A; C; A /A; T; A]	Pakistan	-	-	-	-

AF295024.1:c.-1082 and haplotypes [A; T; A] containing this allele has been associated with low IL-10 production while AF295024.1:c.–1082 G allele and haplotypes [G; C; C] containing this allele has the opposite effect (Crawley et al., 1999). [G; C; C], [A; C; C], [A; T; A] haplotypes are predominant in Caucasian population (Turner et al., 1997) and a fourth [G; T; A] haplotypes was identified in Chinese population (Mok et al., 1998) as IL-10 haplotypes distribution varies among populations. Considering the immunosuppressive and immuno-regulatory role of IL-10 and contribution of genetic contribution in its secretion, this study was conducted to determine the IL-10 alleles, genotypes, haplotypes and diplotypes ratios in which they are present in Pakistani population. According to our results, the allele frequencies of AF295024.1:c.-1082 G, and AF295024.1:c.-1082 A were 0.53 and 0.47. The AF295024.1:c.-819 C, AF295024.1:c.-819 T (AF295024.1:c.-592 C, AF295024.1:c.-592 A) alleles at AF295024.1:c.-819/ AF295024.1:c.-592 was almost similarly inherited in Pakistani population, that is, 49.4 and 50.6%, respectively. The genotype frequencies of AF295024.1:c.-1082 GG, AF295024.1:c.-1082 GA and AF295024.1:c.-1082 AA was 11, 84 and 5%, respectively. The frequencies for AF295024.1:c.-819 CC, AF295024.1:c.-819 TCb and AF295024.1:c.-819 TT (AF295024.1:c.-592 CC, AF295024.1:c.-592 CA and AF295024.1:c.-592 CA) at AF295024.1:c.-819 (AF295024.1:c.–592) were 0.04, 0.86 and 0.1, respectively. This study also showed that there were four haplotypes {[G; C; C], [A; C; C], [A; T; A], [G; T; A]} present in Pakistani population. The haplotype [G; T; A] was previously found only in Chinese population and now in Pakistan population. The frequencies of these

haplotypes [G; C; C], [G; T; A], [A; C; C] and [A; T; A] were 43.7, 9.9, 3.7 and 43%, respectively. We found the presence of nine different combinations of haplotype inheritance; [G; C; C/A; T; A] was the major diplotype (73.5%) while [A; T; A/A; T; A], [G; C; C/G; C; C] and [A; C; C/A; C; C] were the minor ones.

Results regarding investigations on IL-10 polymorphism suggest that frequency of promoter alleles, genotypes, haplotypes and diplotypes vary widely across different ethnic groups. These differences are due to different exertion of selective pressure on human genome; this pressure affects particularly the host immune responsive genes which mediate susceptibility and clinical outcomes of the diseases (Kwiatkowski, 2005). The genetic factors which contributes towards inter individual variation in IL-10 production results in susceptibility of a number of infectious, autoimmune, inflammatory, lymphomas and allergic diseases such as tuberculosis (T.B), hepatitis C virus (HCV), malaria, coronary artery disease (CAD), rheumatoid arthritis, psoriasis, Crohn's disease, and systemic lupus erythematosus. Our study shows that Pakistani (Asian) people have very few numbers of individuals with high IL-10 production genotype and diplotypes. The results are in accordance with Hoffman et al. (2000) which shows that Asian people are low producers of IL-10 as compared with the whites. This low IL-10 production promotes renal allograft tolerance in Asian as compared with whites and blacks (Hoffman et al., 2003).

As studies from different ethnic groups included for comparison with our results were already reported, the difference in pattern of inheritance of polymorphism reported here should be interpreted with appropriate caution. IL-10 plays significant role in number of disease conditions and its production varies from individual to individual. The potential effects of polymorphism on disease pathogenesis are not fully established. However it is cleared that ethnicity dramatically influenced the inheritance of polymorphic variants of IL-10. Results of the study, which is the first from Pakistan, may stimulate other studies for identifying the role of IL-10 polymorphism in different human diseases.

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