Incidence of Genetic Polymorphism of IL-1Ra and IL-4 in Egyptian and other Populations

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Abstract: Cytokines play a key role in immune response and inflammation. IL-1 receptor antagonist (Ra) is a naturally occurring structural variant of IL-1 that competitively inhibits receptor binding of IL-1 induced pro-inflammatory activity. IL-4 an anti-inflammatory cytokine plays a key role in activation and differentiation of B-cells, mast cells.IL-4 is also known to inhibit macrophage activation and therefore may be involve in cancer. The two important cytokines genes IL-1Ra and IL-4 of 124 healthy individuals from the Nile Delta region of Egypt were compared with the published polymorphism of other populations. Genomic DNA was isolated from the blood of all subjects and the variable number of tandem repeat (VNTR) polymorphisms of IL-1Ra and IL-4 genes was identified by polymerase chain reaction. It was seen that our population differs from Mediterranean, European, African and Asian populations at IL-1Ra (VNTR) and IL-4 (VNTR) genes.
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Key words: VNTR, IL-1Ra, IL-4, gene polymorphism.

1. Introduction

DNA sequences of the human genome reveal that many genes are polymorphic. In coding or non-coding regions of a specific gene, there may be either a single base pair substitution or a variable number of repeats of a short repetitive DNA sequence (VNTR). The susceptibility or severity of a number of disorders will be influenced by possession of specific alleles of polymorphic genes ***(Bid et al., 2004)***. Cytokines are small molecules secreted by cells in response to specific stimuli and alter the behavior of the same or other cells ***(Kesarwani et al., 2008)***. Interleukin-1 (IL-1) is one of the most pro-inflammatory agents, and has a central role in inflammation and destruction ***(Hutyrova et al., 2002).*** The most important members of the IL-1 family are the IL-1α, IL-1β and interleukin-1 receptor antagonists (IL-1Ra). IL-1Ra is an anti-inflammatory cytokine competes with IL-1α and IL-1β in binding to IL-1 receptors without intrinsic effects ***(Arend, 1990 and Dinarello, 1998).*** Genes encoding IL-1 are located on the 430 kb region of chromosome 2q13-21 ***(Nemetz et al., 1999).*** In intron 2 of the IL-1Ra gene, a polymorphism due to the presence of variable numbers of an 86-bp tandem repeat (VNTR) has been described .This polymorphism leads to the existence of five alleles, each corresponding to a different number of repeats. The most common allele has been termed allele A1 has 4 repeats=410 bp, allele A2 has 2 repeats=240 bp, allele A3 has 5 repeats=500bp, allele A4 has 3 repeats=325 bp, allele A5 has 6 repeats=595bp ***(Tarlow et al., 1993)***.IL-4 an anti-inflammatory cytokine plays a key role in activation and differentiation of B-cells ***(Sosroseno et al., 1994)***. IL-4 is also known to inhibit macrophage activation and therefore may be involve in cancer. The IL-4 gene has been mapped to the q arm (q23-31) of chromosome 5. A variable number of tandem repeat (VNTR) of 70 base pair repeat is situated in third intronic region of the IL-4 gene. Three repeat allele (at 342 bp) is most common (A1) and two repeat allele (at 272 bp) is rare (A2). There is another much rare allele of four repeat (at 412 bp) (A5), which is reported only in few populations ***(Mout et al., 1991)***.In this study we found another two rare alleles one (at 200 bp) (A3) and another one at (180 bp) (A4)**.** The present study is an attempt to investigate the polymorphism of IL-1Ra and IL-4 genes in healthy individuals from Egypt and compared it with other populations.

2. Subjects and Methods

The study was performed on 124 healthy Egyptian blood donors. They were 110 males and 14 females with an age ranging between 17.0-42.0 years with the mean age 25.98±5.7 years. An informed consent was obtained from all individuals who participate in the study and they were fully informed of the nature of the study and the diagnostic procedures involved. This work has been done in laboratories of Genetics Unit of Mansoura University Childern Hospital.

DNA was extracted from peripheral blood which were collected in a tube containing EDTA solution, pH 8.0 as an anticoagulant, and it was purified using the generation DNA purification capture column kits (Gentra system,USA).

Amplification of DNA by PCR technique was carried out to intron 2 of IL-1Ra which contained 86 bp VNTR zone. Each PCR was carried out in 25 µl reaction volume, mixture containing 10 µl PCR Master Mix (2x) (Fermentase, Germany), 8 µl PCR distilled water, 2 µl of primer IL-1Ra forward (5'-CTCAGCAACACTCCTAT-3') , 2 µl of primer IL-1Ra reverse (5'-TCCTGGTCTGCAGGTAA-3') (Bio Basic Inc., Canada) *(Kanemoto et al.,2000)* and 3 µl extracted DNA. PCR conditions were as follows: initial denaturation cycle of 95°C for 5min followed by 35 cycles in the form of 94°C for 30 seconds (denaturation), 55°C for 30 seconds (annealing) and 72°C for1min(extension) with a final extension cycle of 5min at 72°C *(Zhang et al.,2004)*.

Genotyping of IL-4 (VNTR):

Amplification of DNA by PCR technique was carried out for IL-4 intron 3 contained the 70 bp VNTR zone. Each PCR was carried out in 25 µl reaction volume, mixture containing 10 µl PCR Master Mix (2x) (Fermentase, Germany), 8 µl PCR distilled water, 2 µl of primer IL-4 forward (5'-GTAAATAGGCTGAAAGGGGGAAA-3'), 2 µl of primer IL-4 reverse (5'-CATCTTTTCCTCCCCTGTATCTT-3') (Metabion, Germany) *(Arai et al.,1989 and Mout et al.,1991)* and 3 µl extracted DNA. PCR conditions were as follows: initial denaturation cycle of 95°C for 2min followed by 40 cycles in the form of 95°C for 1min (denaturation), 56°C for 1min (annealing) and 72°C for 30 seconds (extension) with a final extension period of 5min at 72°C *(Buchs et al., 2000)*.

PCR product was detected by using agarose gel (3%) electrophoresis and photographed on ultraviolet transilluminator) for detection IL-1Ra (VNTR) (Figure 3) and IL-4 (VNTR) gene polymorphism (Figure 4).

Statistical Analysis:

Statistical analysis was done using the statistical package of social sciences (SPSS) software version 11.5 *(SPSS, 1999)*.

3. Results

The present study shows our population was screened and 4 different genotypes were identified for IL-1Ra (VNTR) and 8 different genotypes were identified for IL-4 (VNTR). The distribution of IL-1Ra (VNTR) alleles and genotypes frequencies in Egyptian population is shown in Tables 1, 2. The most frequent allele observed was A1 (56.0%) followed by A2 (43.5%) and A3 (0.40%). A4 and A5 alleles were absent in our population. The distribution of IL-4(VNTR) alleles and genotypes frequencies in Egyptian population is shown in Tables 3,4. The most frequent allele observed was A1 (71.7%) followed by A2 (20.96%) and A3 (3.6%). A4 and A5 alleles were rare alleles in our population (0.81% and 2.8% respectively.). Genotype distributions for both the genes were in agreement with Hardy- Weinberg equilibrium. In comparison between our population and other population we have found significant difference in genotyping frequency and allelic frequency in case of IL-1 Ra (VNTR) and IL-4 (VNTR) genes. The table (1) showed that the most frequent alleles observed were A1 and A2 alleles in our Egyptian population and other populations (Figure 1). Statistical analysis showed that IL-1 Ra (VNTR) allelic frequencies among studied Egyptian population were significantly different from that of Turkish, Flemish, Berlin, Russian, Scottish, Caucasian, African American, Chinese, Taiwan Chinese where ($P<0.0001$), German ($P =0.002$) and North Indian population ($P=0.0003$).

The table (2) showed that the most frequent genotypes observed were A1/A1, A1/A2 and A2/A2 in our Egyptian population (Figure 2) and other populations. Statistical analysis showed that IL-1Ra (VNTR) genotype frequencies among studied Egyptian population were significantly different from that of Turkish, German, Flemish, Berlin population, Scottish, Caucasian, African American, North Indian, Chinese and Taiwan Chinese population where ($P<0.0001$).

The table (3) showed that the most frequent alleles observed were A1 and A2 alleles in our Egyptian population and other population (Figure 3). Statistical analysis showed that IL-4 (VNTR) allelic frequencies among studied Egyptian population were significantly different from that of North Indian, Japanese, Koreans where ($P<0.0001$), Taiwanese ($P=0.003$) and French population ($P=0.0002$).

The table (4) showed that the most frequent genotypes observed were A1/A1and A1/A2 in our Egyptian population (Figure 4) and other populations then A1/A3 in our Egyptian population while A2/A2 in the other populations. Statistical analysis showed that the IL-4 (VNTR) genotypes frequencies among studied Egyptian population were significantly different from that of North Indian, Japanese, Koreans where ($P<0.0001$) and French population ($P=0.011$), while genotype frequency of Taiwanese population ($P=0.096$) was not significantly different from our Egyptian population.

Table (1). Frequency distribution of alleles related to IL-1Ra (VNTR) gene polymorphism among Egyptian studied subjects compared to other populations.

Population	N	References	A1 (%)	A2 (%)	A3 (%)	A4 (%)	A5 (%)	P
Mediterranean								
Egyptian	124	*The present study.*	56.0	43.5	0.40	0.0	0.0	
Turkish	170	*Arman et al., 2008.*	75.0	21.8	3.2	0.0	0.0	<0.0001**
European								
German	145	*Glas et al.,2004.*	68.0	29.0	3.0	0.0	0.0	0.002*
Flemish	401	*Vijgen et al.,2002.*	74.3	23.8	1.9	0.0	0.0	<0.0001**
Berlin	112	*Sehouli et al.,2003.*	73.2	7.1	0.0	0.0	0.0	<0.0001**
Russian	93	*Chistyakov et al.,2000.*	61.8	28.0	3.8	3.2	3.2	<0.0001**
Scottish	115	*McGarry et al.,2001.*	92.0	7.0	1.0	0.0	0.0	<0.0001**
Caucasian	295	*Rider et al.,2000.*	90.2	46.4	2.7	0.7	0.0	0.0174*
African								
African African American	176	*Rider et al.,2000.*	96.6	16.5	1.1	2.8	0.0	<0.0001**
Asian								
North Indian	165	*Bid et al.,2004.*	63.9	30.6	4.6	0.9	0.0	0.0003**
Chinese	249	*Zhang et al.,2004.*	90.4	8.8	0.0	0.8	0.0	<0.0001**
Taiwan Chinese	103	*Chou et al.,2003.*	0.95	0.04	0.0	0.01	0.0	<0.0001**

N= number of subjects, (%) = percentage, alleles A1, A2, A3, A4, A5 are expressed in percentages, *P*= probability test to study the statistical difference between Egyptian and other populations where *P<0.05 significant **P<0.001 extremely significant.

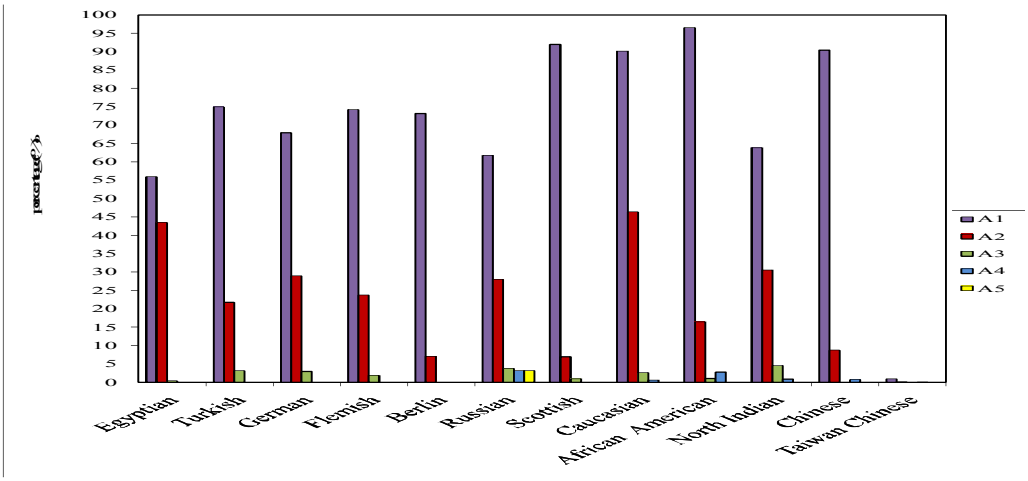


Figure (1): Comparison of IL-1Ra (VNTR) allele frequency between Egyptian population and other populations.

Table(2). Frequency distribution of genotypes related to IL-1 Ra (VNTR) gene polymorphism among Egyptian studied subjects compared to other populations.

Population	N	References	A1/A1 (%)	A1/A2 (%)	A2/A2 (%)	A1/A3 (%)	Others (%)	P
Mediterranean								
Egyptian	124	*The present study.*	20.2	71.0	8.1	0.8	0.0	
Turkish	170	*Arman et al.,2008.*	55.3	32.9	5.3	6.5	0.0	<0.0001**
European								
German	145	*Glas et al., 2004.*	45.0	41.0	8.0	5.0	1.0	<0.0001**
Flemish	401	*Vijgen et al.,2002.*	58.1	30.4	8.0	2.0	1.5	<0.0001**
Berlin	112	*Sehouli et al.,2003.*	62.0	15.2	5.4	2.7	1.8	<0.0001**
Russian	93	*Chistyakov et al., 2000.*	most frequent	most frequent				
Scottish	115	*McGarry et al.,2001.*	90.0	1.0	7.0	2.0	0.0	<0.0001**
Caucasian	295	*Rider et al., 2000.*	50.8	37.3	8.5	2.0	1.4	<0.0001**
African								
African American	176	*Rider et al., 2000.*	79.6	13.1	3.4	1.1	2.8	<0.0001**
Asian								
North Indian	165	*Bid et al.,2004*	49.7	24.2	18.2	3.6	4.2	<0.0001**
Chinese	249	*Zhang et al., 2004.*	81.1	16.9	0.4	0.0	1.6	<0.0001**
Taiwan Chinese	103	*Chou et al., 2003.*	92.0	6.0	1.0	0.0	1.0	<0.0001**

N= number of subjects, (%) = percentage, genotypes A1/A1, A1/A2, A2/A2, A1/A3 are expressed in percentages, P= probability test to study the statistical difference between Egyptian and other populations where **P<0.001 extremely significant. Others (other genotypes like A1/A4,A2/A3,A2/A4,A3/A3,A4/A4 which found rarely in populations).

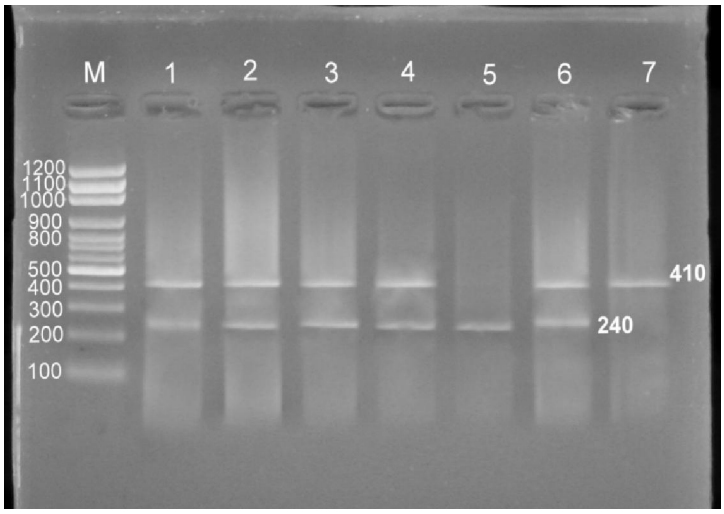


Figure (2): Agarose gel shows PCR product for IL-1Ra gene intron 2 polymorphism in healthy people.

- Lane (M): shows DNA ladder (100-1200 bp).
- Lanes (1, 2, 3, 4, and 6): show A1/A2 heterozygote polymorphism of IL-1Ra where A1 at 410 bp and A2 at 240 bp.
- Lane (5): shows homozygote polymorphism of IL-1Ra A2/A2 genotype where A2 at 240 bp.
- Lane (7): shows homozygote polymorphism of IL-1Ra A1/A1 genotype where A1 at 410 bp.

Table (3). Frequency distribution of alleles related to IL-4 (VNTR) gene polymorphism among Egyptian studied subjects compared to other populations.

Population	N	References	A1 (%)	A2 (%)	A3 (%)	A4 (%)	A5 (%)	P
Mediterranean								
Egyptian	124	*The present study.*	71.7	20.96	3.6	0.81	2.8	
Asian								
North Indian	343	*Kesarwani et al., 2008.*	76.7	23.3	0.0	0.0	0.0	<0.0001**
Taiwanese	100	*Su et al., 2007.*	80.9	19.1	0.0	0.0	0.0	0.003*
Japanese	60	*Hegab et al., 2004.*	31.7	68.3	0.0	0.0	0.0	<0.0001**
Koreans	481	*Um and Kim, 2009.*	21.0	78.5	0.5	0.0	0.0	<0.0001**
European								
French	104	*Buchs et al., 2000.*	86.5	13.5	0.0	0.0	0.0	0.0002**

N= number of subjects, (%) = percentage, alleles A1, A2, A3, A4, A5 are expressed in percentages, *P*= probability test to study the statistical difference between Egyptian and other populations where *P<0.05 significant, **P<0.001 extremely significant.

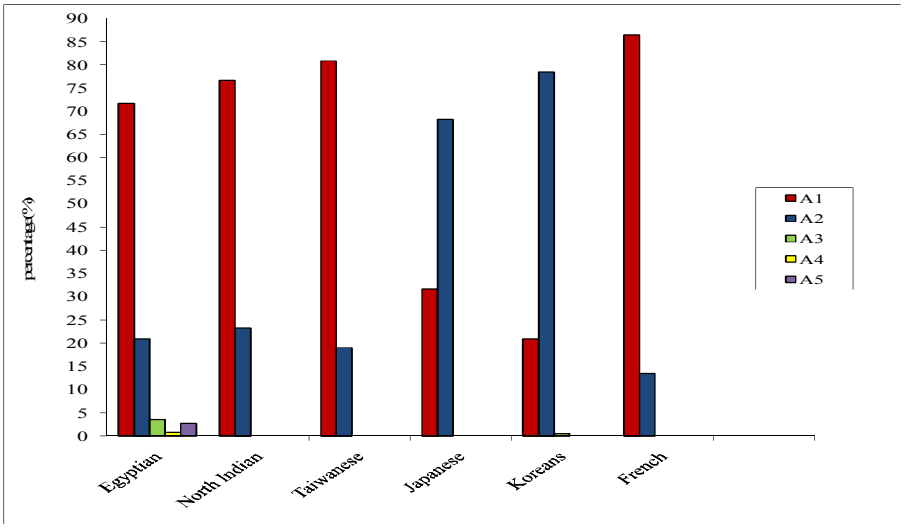
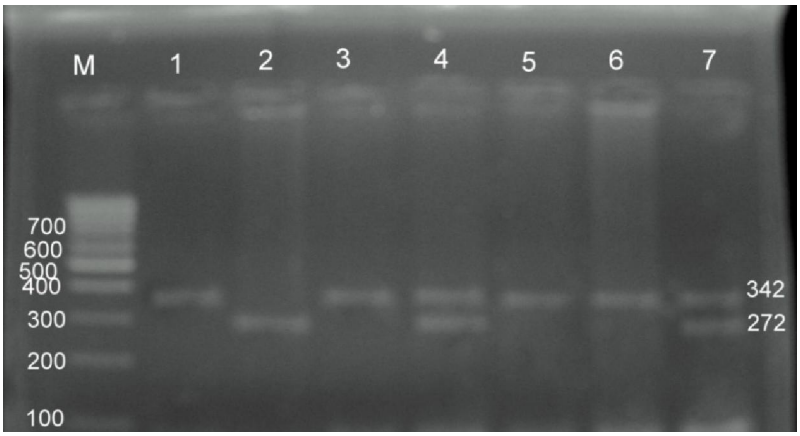


Figure (3): Comparison of IL-4 (VNTR) allele frequency between Egyptian population and other populations.

Table(4). Frequency distribution of genotypes related to IL-4 (VNTR) gene polymorphism among Egyptian studied subjects compared to other populations.

Population	N	References	A1/A1 (%)	A1/A2 (%)	A2/A2 (%)	A1/A3 (%)	Others	*P*
Mediterranean								
Egyptian	124	*The present study.*	51.6	35.5	2.4	3.2	7.2	
Asian								
North Indian	343	*Kesarwani et al.,2008.*	54.8	43.7	1.5	0.0	0.0	<0.0001**
Taiwanese	100	*Su et al., 2007.*	64.0	33.0	3.0	0.0	0.0	0.096
Japanese	60	*Hegab et al.,2004.*	8.3	46.7	45.0	0.0	0.0	<0.0001**
Koreans	481	*Um and Kim, 2009.*	4.6	32.6	61.7	0.2	0.8	<0.0001**
European								
French	104	*Buchs et al.,2000.*	74.0	25.0	1.0	0.0	0.0	0.011*

N= number of subjects, (%) = percentage, genotypes A1/A1, A1/A2, A2/A2, A1/A3 are expressed in percentages, *P*= probability test to study the statistical difference between Egyptian and other populations where *P<0.05 significant **P<0.001 extremely significant. Others (other genotypes like A1/A4, A2/A3, A5/A5, A5/A3 which found rarely in populations).



Figure(4): Agarose gel shows PCR products for IL-4 VNTR gene intron 3 polymorphism in healthy people.

- Lane (M): Shows DNA ladder (100-1200 bp).
- Lanes (1, 3, 5, 6): Show A1/A1 homozygote polymorphism of IL-4 VNTR where A1 at 342 bp.
- Lane (2): Shows A2/A2 homozygote polymorphism of IL-4 VNTR where A2 at 272 bp.
- Lanes (4, 7): Shows A1/A2 hetrozygote polymorphism of IL-4 VNTR where A1 at 342 bp and A2 at 272 bp.

4. Discussion

Identification of single nucleotide polymorphisms (SNPs) in human genome has great implications in the study of disease susceptibility. The SNP in IL-1Ra and IL-4 gene VNTRs have been found to be associated with different immunological diseases. Our basic objective of this study was to provide population genetic characterizations of VNTR polymorphism. A worldwide comparison of the distribution of IL-4 (VNTR) and IL-1Ra (VNTR) polymorphism in our population revealed certain key variations. It has been reported that the frequency of the individual alleles varies among different ethnic or geographic populations.

In IL-1Ra (VNTR) and IL-4 (VNTR) genes, five alleles were described suggesting possible functional significance. Allele A1 is more common than allele A2 except in Japanese and Koreans allele A2 is more common than allele A1 in case of IL-4 VNTR ***(Hegab et al., 2004 and Um and Kim, 2009)***. Our observations are in agreement to the studies of different populations reported, though the frequencies of genotypes differ, whereas the remaining alleles, representing A3, A4 and A5 were less common in other populations ***(Arman et al., 2008 and Kesarwani et al., 2008).***

It was noted from our study that the distribution of alleles related to IL-1Ra (VNTR) gene polymorphism among healthy Egyptian were significantly different from that of other populations as Turkish population (A1 56.0% vs 75.0% and A2 43.5% vs 21.8% respectively , P<0.0001) ***(Arman et al. ,2008)***, German population (A1 56.0 vs. 68.0% while A2 43.5% vs. 29.0% respectively, P=0.002) (***Glas et al., 2004)***, Flemish population (A1 56.0% vs. 74.3% while A2 43.5% vs. 23.8% respectively, P<0.0001) ***(Vijgen et al., 2002)***, Berlin population (A1 56.0% vs. 73.2% while A2 43.5% vs. 7.1% respectively, P<0.0001) ***(Sehouli et al., 2003)***, Russian population (A1 56.0% vs. 61.8% while A2 43.5% vs. 28.0% respectively, P<0.0001) ***(Chistyakov et al., 2000)***, Scottish population (A1 56.0% vs. 92.0% while A2 43.5% vs. 7.0% respectively, P<0.0001) ***(McGarry et al., 2001)***, Caucasian population (A1 56.0% vs. 90.2% and A2 43.5% vs. 46.4% respectively, P=0.0174) ***(Rider et al. ,2000)***, African American population (A1 56.0% vs. 96.6% while A2 43.5% vs. 16.5% respectively, P<0.0001) ***(Rider et al., 2000)***, North Indian population (A1 56.0% vs. 63.94% while A2 43.5 vs. 30.61% respectively, P=0.0003***)*** ***(Bid et al., 2004)*** , Chinese population (A1 56.0% vs. 90.4% while A2 43.5% vs. 8.8% respectively, P<0.0001) ***(Zhang et al.,2004)*** and Taiwan Chinese population (A156.0% vs. 0.95% and A2 43.5% vs. 0.04% respectively, P<0.0001) ***(Chou et al., 2003)***.

Our study revealed that the distribution of genotypes related to IL-1Ra (VNTR) polymorphisms among healthy Egyptian were significantly different from that of other populations as Turkish population

(A1/A1 20.2% vs. 55.3% while A1/A2 71.0% vs. 32.9% respectively, P<0.0001) ***(Arman et al. ,2008)***, German population (A1/A1 20.2% vs. 45.0% while A1/A2 71.0% vs. 41.0% respectively, P<0.0001) ***(Glas et al., 2004)*** , Flemish population (A1/A1 20.2% vs. 58.1% while A1/A2 was 71.0% vs. 30.4% respectively, P<0.0001) ***(Vijgen et al., 2002)***, Berlin population (A1/A1 20.2% vs. 62.0% while A1/A2 was 71.0% vs. 15.2% respectively, P<0.0001) ***(Sehouli et al., 2003)***, Scottish population (A1/A1 20.2% vs. 90.0% while A1/A2 was 71.0% vs. 1.0% respectively, P<0.0001) ***(McGarry et al., 2001)***, Caucasian population (A1/A1 20.2% vs. 50.8% while A1/A2 was 71.0% vs. 37.3% respectively, P<0.0001) ***(Rider et al., 2000)***, African American population (A1/A1 20.2% vs. 79.6% while A1/A2 was 71.0% vs. 13.1% respectively, P<0.0001) ***(Rider et al., 2000),*** North Indian population (A1/A1 20.2% vs. 49.7% while A1/A2 was 71.0% vs. 24.2% respectively, P<0.0001) ***(Bid et al., 2004)***, Chinese population (A1/A1 20.2% vs. 81.1% while A1/A2 was 71.0% vs. 16.9% respectively, P<0.0001) ***(Zhang et al., 2004)*** and Taiwan Chinese population (A1/A1 20.2% vs. 92.0% while A1/A2 was 71.0% vs. 6.0% respectively, P<0.0001) ***(Chou et al., 2003)***.

This study showed that the distribution of alleles related to IL-4 (VNTR) gene polymorphism among healthy Egyptian were significantly different from that of other populations as North Indian population (A1 71.7% vs. 76.7% and A2 20.96% vs. 23.3% respectively, P<0.0001) ***(Kesarwani et al., 2008),*** Taiwanese population (A1 71.7% vs. 80.9% while A2 20.96% vs. 19.1% respectively, P=0.003) ***(Su et al., 2007),*** Japanese population (A1 71.7% vs. 31.7% while A2 20.96% vs. 68.3% respectively, P<0.0001) ***(Hegab et al., 2004),*** Koreans population (A1 71.7 % vs. 21.0% while A2 20.96% vs. 78.5% respectively, P<0.0001) ***(Um and Kim, 2009)*** and French population (A1 71.7 % vs. 86.5% while A2 20.96% vs. 13.5% respectively, P=0.0002***) (Buchs et al., 2000).***

It was noted that the distribution of genotypes related to IL-4 (VNTR) polymorphisms among healthy Egyptian were significantly different from that of other populations as North Indian population (A1/A1 51.6% vs. 54.8% while A1/A2 was 35.5% vs. 43.7% respectively, P<0.0001) ***(Kesarwani et al., 2008)***, Japanese population (A1/A1 51.6% vs. 8.3% while A1/A2 was 35.5% vs. 46.7% respectively, P<0.0001) ***(Hegab et al., 2004)***, Koreans population (A1/A1 51.6% vs. 4.6% while A1/A2 was 35.5% vs. 32.6% respectively, P<0.0001) ***(Um and Kim, 2009)*** and French population (A1/A1 51.6% vs. 74.0% while A1/A2 was 35.5% vs. 25.0% respectively, P<0.0001) ***(Buchs et al., 2000)*** but were not significantly different from that of Taiwanese population (A1/A1 51.6% vs. 64.0% and A1/A2 was 35.5% vs. 33.0%, respectively, P=0.096) ***(Su et al., 2007)***.

Conclusion

The IL-1Ra (VNTR) and IL-4 (VNTR) have highly polymorphic content. Thus, VNTRs constitute useful tools in population genetic studies in understanding population and ethnic variations. Allelic association studies are in progress with several chronic inflammatory and degenerative diseases in which IL-1 Ra (VNTR) and IL-4 (VNTR) may be involved. In the long run, these studies may help in determining disease susceptibility and clinical management of patients.

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