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DISTRIBUTION OF INTERLEUKIN-6 -174 (G/C) GENE POLYMORPHISM IN THE WESTERN ALGERIAN POPULATION

Nawal. LABIAD ^{a,*}, Moussa. MESSATFA^b; Youcef. BOUALI-YOUCF^c, Houria. KADIRI^d, Nardjess. OUIKHLEF^e

^aImmunology laboratory, EHU 1^{er} Novembre 1954, Oran, Algeria.

^bImmunology laboratory, Pasteur Institute of Algeria, Branch of Oran, Algeria Affiliation

^cImmunology laboratory, EHU 1^{er} Novembre 1954, Oran, Algeria.

^dImmunology laboratory, EHU 1^{er} Novembre 1954, Oran, Algeria.

^eImmunology laboratory, EHU 1^{er} Novembre 1954, Oran, Algeria.

KEYWORDS

Interleukin-6

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Abstract

Introduction: Interleukin-6 (IL-6) is a pleiotropic cytokine with a central role in host defense. Polymorphism in the promoter region of IL-6 gene at position -174(G/C) could affect both the transcription and serum levels of IL-6 and may be involved in the pathogenesis of various inflammatory and autoimmune diseases. The frequency of IL-6 -174 (G/C) polymorphism has been well studied and involved in disease susceptibility. It has also been shown to differ in various populations and among different ethnic groups.

Methods: The aim of this study was to investigate the genomic frequency of the C and G alleles of the IL-6 -174 (G/C) polymorphism in the Western Algerian population. Genomic DNA was extracted from blood samples by a magnetic bead technique. Cytokine genotyping was performed using real-time polymerase chain reaction-based assay.

Results: A total of 262 blood samples were available for analysis. The median age of the subjects was 43,61 years. The genotyping results showed a significant difference in the distribution of G and C allele frequency of the IL-6 -174 (G/C) polymorphism in the population. The total frequencies for the G and C alleles were 88,74% and 11,25% respectively. 79% of the subjects carried the GG genotypes and only 1,52% carried the CC genotype.

Conclusion: Polymorphisms within cytokine genes can serve as important immunologic markers for identifying individuals at risk for disease. Our findings are the first reported in the Western Algerian population and may be important to evaluate the value of IL-6 -174 (G/C) polymorphism in assessing genetic predispositions to locally prevalent diseases. Therefore, this study serves as an important reference study for the Algerian population and provides data for further studies.

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*** CORRESPONDING AUTHOR****Email address :** labiad.nawel.1993@gmail.com**Introduction :**

Identified in 1986 as a *B cell stimulation factor-2* (BSF-2) which promotes the differentiation of effector B cells to antibody-producing cells, Interleukin-6 (IL-6) is a 21-26 kDa single polypeptide chain consisting of 184 amino acids in human [1,2].

IL-6 is a pleiotropic cytokine, it induces synthesis of acute-phase proteins and plays an important role in mediating the humoral and cellular immune response related to inflammation and tissue damage. IL-6 can also promote differentiation or proliferation of several non-immune cells [3]. Because of this pleiotropic activity, a continual dysregulated production of IL-6 can lead to the onset or development of various diseases [4]. Furthermore, genome-wide association studies and analyses of single-nucleotide polymorphisms (SNPs) and microarray data have identified links between IL-6 and disease outcome. The bi-allelic polymorphism within the promoter region of the IL-6 gene at position -174G>C (*rs1800795*) has been described to be associated with coronary heart disease, idiopathic juvenile arthritis, cancer and many other inflammatory and auto-immune conditions [5-9]. This polymorphism affects IL-6 gene transcription and its circulating serum level. The common allele, G, exhibited higher transcriptional activity and was associated with higher production of IL-6 [10].

Alongside studies involving IL-6 gene polymorphisms in susceptibility to various diseases, there have been multiple studies showing significant variations in the frequencies of this polymorphism (*rs1800795*) among various populations and different ethnic groups.

Objective : Considering the functional implications of the IL-6 gene, we proposed to study the prevalence of IL-6 -174 (G/C) gene polymorphism in the Western Algerian population. The result of this study can be used as a reference point for further studies to determine the risk of diseases associated with this particular IL-6 gene polymorphism among patients in the Algerian population. The differences in the distribution of the polymorphism variants may explain the ethnic differences in susceptibility or severity of certain diseases.

Materials and methods**Population :**

Blood samples were obtained from healthy unrelated individuals residing in West Algeria on

EDTA (ethylenediaminetetraacetic) tubes, labeled and stored at -80°C until DNA extraction.

Genomic DNA was extracted from blood samples by a magnetic bead technique using MagNA Pure LC 2.0 instrument and MagNA Pure LC Total Nucleic Acid Isolation Kit. The quantity and quality of the extracted DNA were evaluated by spectrophotometry (NANO MAESTROGEN).

Cytokine Genotyping :

Cytokine genotyping was performed using polymerase chain reaction with sequence-specific primers assay "TaqMan® single nucleotide polymorphism (SNP) genotyping assay", a real-time PCR test for customised genotyping of single nucleotide polymorphisms (SNPs).

All the primers and probes used for the IL-6 *rs1800795* polymorphism study are shown in Table 1.

Table 2. IL-6 SNP (rs1800795) Primers and Probes

<i>SNP rs1800795</i>	
Primers	Forward: 5' -CGACCTAAGCTGCACTTTTCC- 3' Reverse: 5'-GGGCTGATTGGAAACCTTATTAAGATTG 3'
Probes	5' -CCTTTAGCATGGCAAGAC- 3' marked with VIC 5' -CCTTTAGCATCGCAAGAC- 3' marked with FAM

Preparation of the reaction mixture:

Using the 96 well Applied Biosystems PCR Plate, each well containing 20µl of reaction Mixture is used to study the polymorphism in question for each sample. The reaction Mixture without DNA is prepared according to the number of samples (n) and distributed per well using the volumes indicated in Table 2. The DNAs are then added, the optical plate sealed, centrifuged and introduced into the thermocycler (7500 Real Time PCR System, Applied Biosystems). Data processing was performed by Applied Biosystems ABI 7500 v2.0.4 software.

Table 2. Composition of the TaqMan PCR reaction Mixture

Component	Quantity/ sample	Quantity/ n sample
Deionized water	9,6 µl	9,6 µl
MgCl ₂	04 µl	04 µl
Primer F	01 µl	01 µl
Primer R	01 µl	01 µl
Probe / VIC	0,5 µl	0,5 µl
Probe / FAM	0,5 µl	0,5 µl
Taq polymerase + dNTP	0,4 µl	0,4 µl
TOTAL WITHOUT DNA	17 µl	17µl
DNA	03 µl	-
TOTAL	20 µl	-

The PCR program used for amplification :

- 1 cycle : 2 mins 50°C
- 1 cycle : 10 min 95°C (denaturation)
- 40 cycles : 15 sec 95°C (denaturation)
- 1 min 60°C (hybridization/extension)

Qualitative analysis “Allelic discrimination plot”:

Our study is based on a qualitative analysis to verify the association of variants (G>C) of the IL-6 polymorphism in the population. The first probe, labeled with the fluorescent dye FAM, is designed to detect the wild type allele sequence (G), while the second probe, labeled with the fluorescent dye VIC, detects the mutant type allele sequence (C).

- If the sample is homozygous for the G allele, the fluorescence reading or “Allelic discrimination plot” mainly shows VIC fluorescence, while for a sample homozygous for the C allele, the plot mainly shows the FAM dye signal.

- If the sample is heterozygous, however, there should be approximately equal signal for each dye. Allele and genotype frequencies were estimated by direct counting and then dividing by the number of subjects (to produce genotype frequency) or chromosomes (to produce allele frequency). The polymorphism was tested for Hardy-Weinberg equilibrium.

Results

A total of 262 blood samples were available for analysis. The median age of the subjects was 43,61 years (ranging from 14 to 87 years old) with a Sex ratio of F:M = 4:1

Among the 262 samples studied, 79% carried the wild type GG, 19,46% carried the genotype GC and only 1,52% carried the genotype CC (Table 3).

Table 3. Genotypic frequencies

Genotype Frequencies n (%)		
GG	GC	CC
207 (79 %)	51 (19,46 %)	4 (1,52 %)

The total allele frequencies for the G and C alleles were 88,74% and 11,25%, respectively. (Table 4).

Table 4. Allelic frequencies

Allele Frequencies n (%)	
G	C
265 (88,74 %)	59 (11,25 %)

Discussion

The gene encoding for IL-6 is located on the short arm of chromosome 7 at position 21 (Chr 7p21). It contains 5 exons separated by 4 introns. Polymorphisms in the IL-6 gene promoter can result in interindividual variations in the transcription and expression of the cytokine influencing individual's susceptibility to various diseases. For the IL-6 *rs1800795* polymorphism, a functional SNP located at position –174 is linked to the constitutive transcription rate of IL-6, which could control its blood levels. A single nucleotide change from G to C at this position corresponds to a negative regulatory domain and results in downregulation of IL-6 transcription. Indeed, the presence of the G allele at position –174 has been found to be associated with high production of IL-6 and with an increased inflammatory response (GG/GC genotype carriers), whereas low production is observed with the C allele (homozygous CC genotype carriers). (Fishman *et al.*, 1998) [10]. Thus, this polymorphism can be used as a functional variant to explore the causative role of elevated levels of IL-6 in many common diseases and can further explain the susceptibility of certain populations to different inflammatory and autoimmune diseases.

Furthermore, the distribution of IL-6 –174 (G/C) variants is found to be with significant differences among people from either European descent or Africans, Americans and Asians. In fact, it has been reported that the frequency of the –174C allele is much lower in Asians and Africans compared to European Caucasians. The majority of Asian populations carry the GG genotypes, ranging from 67 to 100% while it occurs at low frequencies in the European population, ranging from 19 to 67% (Table 5).

The most noticeable feature within IL-6 analysis is the complete absence of the C polymorphism within the following reported groups: Singapore Chinese group [11], Malaysian Chinese group [12], Japanese and Chinese [13]. Moreover, Thai and other Asian populations (Chinese Han, Malaysian Malays, Malaysian Indians, Indians and Omani) seem to have higher proportions of the allele associated with high levels of IL-6 (G). The -174G allele has also been reported to be monomorphic (100%) in Nigeria [13] and throughout all the populations in Taiwan [14].

Even though the high producer IL-6 -174G allele occurs at very high frequencies in most of the populations, a low occurrence has been reported in the Caucasian population. The Caucasian Northern Ireland group present a lower G allele frequency, with almost equal proportions of the G (54%) and C allele (46%) [11]. Same results found in Czech Caucasian group, G (55%) and C allele (45%) [15], Polish population group G (53,4%) and C allele (46,6%) [16] and Finnish population group, G (43,4%) and C (56,6%) [17].

Moreover, significant differences in the distribution of IL-6 -174 (G/C) variants are shown to be present in deferent ethnic groups. A study that included Malaysian three major ethnic groups, Malays, Chinese and Indians showed a complete absence of the -174C polymorphism in the Chinese subjects. In contrast, the C allele frequency was significantly higher (19%) in the Indians [12]. Data from Indian populations in India had also demonstrated a difference in the distribution of allelic variants among the North and South Indians (32,36% and 15,5%, respectively for the C allele) [18, 19]. On the other hand, *Cox et al., 2001* showed significant ethnic differences within the American population, Whites carried a G/C allelic distribution of 65%/35% whereas African-Americans had an allelic distribution of 91% / 9% ($P < 0.0001$) [20].

The data from our analysis are consistent with those reported in the literature, approving that non-Caucasian populations predominate in presentation of the high producer G allele. Indeed, the allele frequency of -174C was only 11,25 % in our study, lower than those reported in the European population. Furthermore, 79% of the subjects carried the GG genotypes and only 1,52% carried the CC genotype.

Note that, given it extended borders, the Algerian population is a mixture of several different ethnics. It would be interesting to determine distinct genetic profiles and compare the distribution of IL-6 -174 (G/C) polymorphism variants among its various ethnic groups. Future studies could assess whether or not this IL-6 -174 (G/C) polymorphism distribution in the Algerian population is related with genetic predisposition to certain diseases.

Conclusion

Polymorphisms within cytokine genes can serve as important immunologic markers for identifying individuals at risk for disease. The frequency of IL-6 -174 (G/C) gene polymorphism has been widely studied in different populations and ethnicities. Our study and its results can be particularly useful as a possible tool for anthropological analysis and research for correlations between ethnicity and either allelic distribution or genotype frequency. Moreover, our findings are the first reported in the Western Algerian population and may be important in determining the risk of diseases associated with IL-6 polymorphism in this population.

Declaration of interests

The authors declare that they have no conflicts of interest.

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Table 5. Genotypic and allelic distribution of IL-6 SNP (rs1800795) in different populations

Population	<i>n</i>	Genotype (%)			Alleles (%)		Reference
		GG	GC	CC	G	C	
Asian Population							
Taiwan	-	100	0	0	100	0	[14]
Japanese	-	100	0	0	100	0	[13]
Chinese	-	100	0	0	100	0	
Chinese Han	232	99,57	0,43	0	99,78	0,32	[21]
Singapore Chinese	83	100	0	0	100	0	[11]
Thai	-	97	3	0	99	01	[13]
Malaysian Chinese	122	100	0	0	100	0	[12]
Malaysian Malays	86	93	7	0	96	4	
Malaysian Indians	140	66	30	4	81	19	
Omani	80	76,2	22,5	1,3	87,5	12,5	[11]
North Indians	343	50,1	35,0	14,9	67,64	32,36	[19]
South Indians	210	71,9	25,2	2,9	84,6	15,5	[18]
European Population							
UK Caucasians	240	31,7	52,5	15,8	-	-	[22]
Italian Caucasians	362	49,7	39,3	11	71	29	[23]
Italian	140	41	50	9	66	34	[14]
English		59	37	04	77	23	
Greek Cypriot	100	67	29	04	81,5	18,5	[24]
Czech Olomouc	120	37	50	14	61	39	[25]
Czech Caucasian	103	29	51	19	55	45	[15]
Slovakia	140	37,86	47,14	15	61,43	38,57	[26]
Polish	205	28,8	49,3	21,9	53,4	46,6	[16]
Finnish	485	19,2	48,5	32,4	43,4	56,6	[17]
N. Ireland	100	30	48	22	54	46	[11]
African population							
Our Study	262	79	19,46	1,52	88,74	11,25	-
Algiers	100	74	25	1	86,5	13,5	[27]

Nigeria	-	100	0	0	100	0	[13]
South African, Zulu	86	98,8	1,2	0	99,4	0,6	[11]
Tunisia	406	73,2	25,1	1,7	85,7	14,2	[28]
American Population							
Americans	613	37.5	47.8	14.7	61.4	38.6	[29]
African Americans	43	81	19	0	91	9	[20]
White Americans	102	45	39	16	65	35	
Brazilians	214	49,3	40,8	9,9	69,7	30,3	[30]
Mexican Mestizos	40	90	7,5	2,5	93,8	6,2	[11]