Research Article

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Cytokine gene polymorphism frequencies in Turkish population living in Marmara region

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Abstract

Objectives: Sequence variants in cytokine genes are related to affect cytokine gene levels. In this study, it was aimed to examine eight single nucleotide polymorphisms (SNPs) in five cytokine genes (TNF-α, INF-γ, IL-6, IL10, TGF-β) for the Turkish population living in Marmara region and to reveal the genetic distance between the study group and other populations.

Methods: In this study, three-hundred unrelated healthy individuals were involved and all genotyping were performed by using sequence-specific primers PCR (PCR-SSP) method. The SNP data were analyzed for Hardy Weinberg equilibrium fit by calculating expected genotype frequencies and comparing them to the observed values using Arlequin software version 3.1. The genetic distances between the study group and other populations were calculated and a neighbor-joining tree was constructed by PHYLIP.

Results: The observed genotypes of TNF-α (–308), IFN-γ (+874), TGF-β (codon 10), and TGF-β (codon 25) of the subjects were found to be similar with other populations investigated in this study. However, there is a significant frequency difference for IL-6 and IL-10 genotypes between populations.

Conclusions: The current population study provided more reference values for these polymorphisms and generated a control group to be used in further association studies especially for transplantation, GVHD, autoimmune and malign disease.

Keywords: cytokine; disease; genetics; population; single nucleotide polymorphism.

Introduction

Cytokines are the soluble molecules which are produced by many cell types that regulate immunity, inflammation and hematopoiesis. They have different sequence variants and lead to different cytokine productions. The cytokines have both anti- and pro-inflammatory cytokine features. The third and widely accepted categorization is the production of cytokines in dependent on distinct lineages of T helper (Th) cells. The Th1 cells can produce IL-2, IL-5 and IFN-γ, where Th2 cells produce IL-4/-5/-6/-9/-13 [1]. Both Th1 and Th2 cells produce IL-10 [1]. In recent years, a third group, except for Th1 and Th2, has been formed and they include Th17 and T regulatory (Treg) cells, which also produce cytokines [1]. The cytokines have important roles in regulation of the systemic immune responses in the presence of infection, tissue damage or other stimuli [1]. Thus, cytokines are crucial in cellular communication and play a key role in controlling immune response. The analysis of cytokine gene polymorphisms and cytokine expressions could explain the different susceptibility to various diseases including autoimmune, infectious or cancer diseases observed among individual belonging to the same population [2, 3].

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Microsatellites, SNPs, insertions and deletions have been identified in cytokine genes [4]. It has been shown in some studies that cytokine gene polymorphisms affect gene transcription and are associated with sera cytokine production. It has been suggested that polymorphisms in the cytokine gene play a key role in the susceptibility, development and severity of infectious diseases [5]. SNPs were decided based on the literature of previous studies and in silico functional estimation from the National Institute of Environmental Health Sciences (NIEHS), SNPinfo web page (https://snpinfo.niehs.nih.gov/snpinfo/snpfunc.html) has been selected. In this study, only SNPs of cytokines with potential effects on function and important disease associations were selected. Most of which were found in non-coding regions containing regulatory sequences (promoter, intronic and 3' untranslated regions). Variations in these regulatory regions may affect the expression cytokines, leading to individual differences in cytokine production [6–8]. In addition to the change in cytokine production from person to person, cytokine level change is also associated with disease.

Cytokine expression levels have been shown to be related with the susceptibility, severity of autoimmune disorders or resistance to many diseases such as cancer, bacterial and viral infections, allergic or cardiovascular diseases and tissue transplantation [9–14]. The polymorphisms of the cytokine genes vary among different ethnic groups, which may contribute to the difference in disease incidences [15–18]. SNPs were genotyped in Greek patients with pulmonary sarcoidosis and in healthy Greek control subjects using multiplexed MassARRAY. They found TGF-β3 rs3917200*G variant was associated with sarcoidosis [19]. Barış S et al. showed the correlations between the articular involvement and IL-1RN, the ocular involvement and the IL-1β, and the age of disease onset and the IL-2 and IL-10 gene polymorphisms in patients with Behçet disease [20].

Cytokine storm is a general term that covers a variety of diseases. Cytokine storm is an extreme immune response to external stimuli. The pathogenesis of cytokine storm is complex. Cytokine storm syndromes of viral origin appear to have a common pathogenesis of unbalanced immune response with exaggerated inflammatory reaction with reduction and functional depletion of T cells [21]. Some evidence suggests, especially in COVID-19 patients who have severe acute respiratory tract problems which is closely related to the cytokine storm in their bodies [22, 23]. A systematic review and meta-analysis on the efficacy of anti-IL-6 receptor (anti-IL-6R) antibody in neutralizing IL-6 in patients with severe COVID-19 demonstrated that IL-6 –174C allele carrier status is associated with higher level of IL-6 production and more severe forms of pneumonia in general and also IL-6 plays a pivotal role in novel coronavirus pneumonia (NCP) progression [24]. The immune system can recognize mRNA in vaccines as an antigen and activate proinflammatory cascades and immunological pathways that may induce systemic reactions in certain individuals [25, 26].

The aim of this study is to form a basis for further disease association studies by evaluating the allele frequencies of eight polymorphisms in five cytokine genes: TNF-α (–308 G → A, rs1800629), IL-10 (–1082 G → A, rs1800896, –819 C → T, rs1800871 and –592 C → A, rs1800872), IL-6 (–174 G → C, rs1800795), TGF β1 (+10 T → C, rs1982073, 25 C → G, rs1800471) and IFN-γ (+874 A → T, rs2430561) in the population who lives in Marmara region of Turkey.

Materials and methods

Patient and DNA isolation

The study involved 300 unrelated healthy Turkish individuals (156 females and 144 males) from armara Region were examined. Average age of the subject group was 47.5 (range: 21–91) years. All subjects volunteered to participate in the study and informed consent was obtained from all subjects. Genomic DNA was isolated from peripheral blood leukocytes by three-methylamine bromide salts precipitation/denaturation method [27]. The concentration and purity of each sample was measured at an optical density 260/280 nm by spectrophotometer (NanoDrop 2000, Thermo Scientific, Wilmington, DE, USA). DNA samples were stored at –20 °C. The study was performed according to the amended declaration of Helsinki and performed approval of Halic University's Local Institutional Ethics Committee (17.03.2011, 2011–03/01).

SNPs genotyping

In this study, TNF-α (–308 G → A, rs1800629), IL-10 (–1082 G → A, rs1800896, –819 C → T, rs1800871 and –592 C → A, rs1800872), IL-6 (–174 G → C, rs1800795), TGF β1 (+10 T → C, rs1982073, 25 C → G, rs1800471) and IFN-γ (+874 A → T, rs2430561) were genotyped by PCR-SSP. Amplification of TNF-α, TGF-β, IL-10, IL-6, and IFN-γ alleles and internal control, the human β-globin gene, were carried out according to the manufacturer's recommendations (Cytokine Genotyping Tray, One Lambda, Canoga Park, CA). Briefly, after addition of the appropriate primer pairs, salts, buffer, and Taq polymerase, the samples were subjected to PCR. The amplified products were analyzed by agarose gel electrophoresis. Interpretation of PCR results was based on the presence or the absence of a specific amplified fragment.

Table 1: Genotype frequencies of the study group and their comparison with the frequencies of different ethnic groups.

Cytokine genotype	Present study	Turk[a]	Slo[b]	Irl[c]	Pol[d]	Eng[e]	Gre[f]
TNF-α (–308)	n=300%	n=50	n=130	n=100	n=205	n=145	n=100
(A, A)	1.3	6	2.14	7	3.9	1	–
(G, A)	13.7	28	22.14	32	22	23	15
(G, G)	85	66	75.72	61	74.1	76	85
IL-6 (–174)	n=300	n=49	n=140	n=100	n=205	n=145	n=100
(C, C)	5.7	18.4	15	22	21.9	4	4
(G, C)	35.3	26.5	47.14	48	49.3	37	29
(G, G)	59	55.1	37.86	30	28.8	59	67
IFN-γ (+874)	n=300	n=50	n=140				
(A, A)	34.7	42	29.28	–	–	–	–
(T, A)	46	42	47.86	–	–	–	–
(T, T)	19.3	16	22.86	–	–	–	–
TGF-β (codon 10)	n=300	n=50	n=139			n=107	n=100
(C, C)	18.3	18	19.42	–	–	11	19
(T, C)	51.7	46	51.08	–	–	48	47
(T, T)	30	36	29.5	–	–	41	34
TGF-β (codon 25)	n=300	n=50	n=139			n=107	n=100
(C, C)	1.3	6	–	–	–	1	1
(G, C)	17.3	8	15.83	–	–	18	12
(G, G)	81.3	86	84.17	–	–	81	87
IL-10 (–1,082)	n=300	n=50	n=140	n=100	n=205	n=145	n=100
(A, A)	40.7	64	35	19.4	19	34	40
(G, A)	43.7	26	43.57	46.2	50.3	35	44
(G, G)	15.7	10	21.43	34.4	30.7	34	16
IL-10 (–819)	n=300	n=50	n=140	n=100	n=205	n=145	n=100
(C, C)	51	48	55	64.5	60,5	59	61
(T, C)	42	38	36.43	34.4	31.2	38	31
(T, T)	7	14	8.57	1.1	8.3	3	8
IL-10 (–592)	n=300	n=50	n=140	n=100	n=205	n=145	n=100
(A, A)	7	14	8.57	1.1	8.3	3	8
(C, A)	42	38	36.43	34.4	31.2	38	31
(C, C)	51	48	55	64.5	60.5	59	61

Cytokine genotype	Ita[g]	Tai[h]	Sin[i]	Bra[j]	AmC[k]	AmA[l]	Mex[m]
TNF-α (–308)	n=140	n=50	n=83	n=210	n=102	n=43	n=40
(A, A)	2	2	1	–	2	2.3	–
(G, A)	14	12	22	26.2	25.5	20.9	5
(G, G)	84	86	77	73.8	72.5	76.8	95
IL-6 (–174)	n=140	n=50	n=83	n=213	n=102	n=43	n=40
(C, C)	9	–	–	9.9	15.7	–	2.5
(G, C)	50	–	–	40.8	39.2	18.6	7.5
(G, G)	41	99.99	99.99	49.3	45.1	81.4	90
IFN-γ (+874)	n=140			n=211	n=102	n=43	
(A, A)	30	–	–	31.8	25.5	37.2	–
(T, A)	47	–	–	54	53.9	55.8	–
(T, T)	23	–	–	14.2	20.6	7	–
TGF-β (codon 10)	n=140	n=50					
(C, C)	23	38	–	–	–	–	–
(T, C)	45	40	–	–	–	–	–
(T, T)	32	22	–	–	–	–	–
TGF-β (codon 25)	n=140	n=50					
(C, C)	3	–	–	–	–	–	–
(G, C)	10	–	–	–	–	–	–
(G, G)	87	99.99	–	–	–	–	–
IL-10 (–1,082)	n=140	n=50	n=83	n=211	n=101	n=41	n=40
(A, A)	34	94	95	39.3	28.7	36.6	46.2

Table 1: (continued)

Cytokine genotype	Ita[g]	Tai[h]	Sin[i]	Bra[j]	AmC[k]	AmA[l]	Mex[m]
(G, A)	54	6	5	44.1	54.5	61	35.9
(G, G)	12	–	–	16.6	16.8	2.4	17.9
IL-10 (–819)	n=140	n=50	n=83	n=211	n=101	n=41	n=40
(C, C)	50	4	11	46.4	52.5	26.8	35.9
(T, C)	44	36	43	43.6	41.6	63.4	46.2
(T, T)	6	60	46	10	5.9	9.8	17.9
IL-10 (–592)	n=140	n=50	n=83	n=211	n=101	n=41	n=40
(A, A)	6	60	46	10	5.9	9.8	17.9
(C, A)	44	36	43	43.6	41.6	63.4	46.2
(C, C)	50	4	11	46.4	52.5	26.8	35.9

[a]Turkish (Budak et al. 2007). [b]Slovak (Javor et al., 2007). [c]Irish (Meenagh et al. 2002). [d]Polish (Kurzawski et al., 2005). [e]English (Uboldi et al., 2003). [f]Greek Cypriots (Costeas et al. 2003). [g]Italian (Uboldi et al., 2003). [h]Taiwanese (Trejaut et al., 2004). [i]Singaporean (Meenagh et al. 2002). [j]Brazilian (Visentainer et al. 2008). [k]American Caucausian (Visentainer et al. 2008). [l]American African (Visentainer et al. 2008). [m]Mexican (Meenagh et al. 2002).

Statistical analysis

The SNP data were tested for Hardy–Weinberg equilibrium fit by calculating expected genotype frequencies and comparing them to the observed values using Arlequin software version 3.1 [28]. Genotype frequencies found in our populations were compared with published data for other populations. Genetic relationships among these populations were analyzed by non-metric multidimensional scaling (MDS) analysis of genotype frequencies. The genetic distances between the study group and other populations were calculated and a neighbor-joining tree was constructed by PHYLIP [29].

Results

The cytokine genotype frequencies of the study group were found to fit Hardy-Weinberg equilibrium. The observed genotypes of TNF-α (–308) (AA: 1.3%; GA: 13.7%; GG: 85%), IFN-γ (+874) (AA: 30%; TA: 47%; TT: 23%), TGF-β (codon 10) (CC: 18.3%; TC: 51.7%; TT: 30%), and TGF-β (codon 25) (CC: 1.3%; GC: 17.3%; GG: 81.3%) of the study group were found to be similar with other populations investigated in this study. The observed differences were statistically not significant (Table 1). There is a significant difference between the IL-6 (–174) genotypes of the European populations and the genotypes of non-Europeans. The study group is found much closer to the European populations compared to non-Europeans and the observed genotypes were CC: 5.7%; GC: 35.3%; GG: 59%. Significant differences between European and Asian populations were also observed in the genotype frequencies of all IL-10 genes investigated, IL-10 (–1,082, –819, and –592). The genotypes of the study group are also match the European populations in all IL-10 loci analyzed. The genetic distances of the investigated populations according to TNF-α (–308), IL-6 (–174), IL-10 (–1,082, –819, and –592) data are given in (Table 2).

Discussion

The cytokines are the one of the most effective and important player of immune system regulation. There are various studies of cytokines, which play very important roles for several pathological processes with pro- and anti-inflammatory effects. Some certain cytokine gene polymorphisms may affect disease susceptibility and specific population studies give valuable information such as disease and cancer susceptibility. In this report, we summarize cytokine polymorphism and variation with the samples from Turkish population in Marmara Region. It forms a passage between the Balkan Peninsula and Anatolia. The lands of Europe and Asia are connected to each other in this region. It has been an important trade center since ancient times, is located on the intercontinental transport routes and it makes the region superior throughout the country. This region heavily urbanized after Turkish Republic establishment. Because of these reasons, we selected Marmara Region to collect samples.

The phylogenetic tree based derived from the genetic distances clearly shows the different positions of two Turkish populations studied (Figure 1). Budak et al. have studied small group of 40 patients whereas we studied 300 participants [30]. Different number of participants might be the reason of this discrepancy. Modern humans have inhabited Anatolia since upper Paleolithic [31]. Being a crossroad between Europe, Asia and Middle East, Anatolia has become the major pathway of population migrations in the history and because of the nomadic

Table 2: Genetic distances among investigated populations.

	Current study	Turkish[a]	SK	IRL	PL	GB	GR-CY	I	BR	Am. Caucaus	Am. African	TWN	SG	MX
Current study	0.0000	0.0456	0.0429	0.1116	0.0725	0.0345	0.0056	0.0143	0.0105	0.0198	0.0679	0.1935	0.1970	0.0375
Turkish[a]	0.0456	0.0000	0.0697	0.1584	0.1427	0.0883	0.0549	0.0710	0.0346	0.0595	0.0914	0.1302	0.1160	0.0785
SK	0.0429	0.0697	0.0000	0.0467	0.0349	0.0493	0.0521	0.0336	0.0302	0.0229	0.1546	0.3466	0.3401	0.1389
IRL	0.1116	0.1584	0.0467	0.0000	0.0145	0.0535	0.1062	0.0977	0.0849	0.0566	0.2959	0.6178	0.5911	0.2546
PL	0.0725	0.1427	0.0349	0.0145	0.0000	0.0499	0.0757	0.0536	0.0617	0.0345	0.2453	0.5302	0.5304	0.1918
GB	0.0345	0.0883	0.0492	0.0535	0.0499	0.0000	0.0240	0.0619	0.0387	0.0427	0.1605	0.3291	0.3150	0.0939
GR-CY	0.0056	0.0549	0.0520	0.1062	0.0757	0.0240	0.0000	0.0309	0.0218	0.0277	0.0841	0.2207	0.2182	0.0399
I	0.0143	0.0710	0.0336	0.0977	0.0536	0.0619	0.0309	0.0000	0.0135	0.0130	0.0899	0.2662	0.2756	0.0878
BR	0.0105	0.0346	0.0302	0.0849	0.0617	0.0387	0.0218	0.0135	0.0000	0.0088	0.0694	0.2133	0.2077	0.0704
Am. Caucaus	0.0198	0.0595	0.0229	0.0566	0.0345	0.0427	0.0277	0.0130	0.0088	0.0000	0.0927	0.2975	0.2934	0.0956
Am. African	0.0679	0.0914	0.1546	0.2959	0.2453	0.1605	0.0841	0.0899	0.0694	0.0927	0.0000	0.1075	0.1088	0.0539
TWN	0.1935	0.1302	0.3466	0.6178	0.5302	0.3291	0.2207	0.2662	0.2133	0.2975	0.1076	0.0000	0.0046	0.0996
SGN	0.1970	0.1160	0.3401	0.5911	0.5304	0.3150	0.2182	0.2756	0.2077	0.2934	0.1088	0.0046	0.0000	0.1107
MX	0.0375	0.0785	0.1389	0.2546	0.1918	0.0939	0.0399	0.0878	0.0704	0.0956	0.0539	0.0996	0.1107	0.0000

SK, Slovak-Slovakia; IRL, Irish-Ireland; PL, Polish-Poland; GB, English-Great Britain; GR-CY, Greek Cypriot-Cyprus; I, Italian-Italy; BR, Brazilian-Brazil; Am. Caucaus, American Caucasian-USA; Am. African, American African-USA; TWN, Taiwanese-Taiwan; SG, Singapore; MX, Mexican-Mexico. [a]The data belongs to the previous study of Budak's et al., 2007.

invasion occurred in the beginning of 11th century, Central Asian Turkic populations are historically considered to be the origin of the modern population living in Turkey. However, Turkish population is an intermediate between Europe, Middle East and Central Asia and carries the genetic traces of all the historical human movements occurred in this geography, leading to a very heterogeneous population. Karaca et al. analyzed four immunogenetic loci (IL13-IL4, IL4R, and ADAM33) in five different regions of Turkey and they did not find significant difference among Turkish groups. However, the population level comparisons brought more details regarding the genetic structure of the loci. Turkish population significantly differed with respect to Northern/Western Europeans whereas no significant difference with Central Asian samples. Thus, the genotypes of the populations may locally differ according to the genetic background [32]. To the best of our knowledge, there have been two studies reporting the allelic distributions of several cytokine genes in the Mexican population in South America. When compared with the data of the Mexican population, differences are observed in the Turkish population [33].

The study population is located on Marmara Region, the western part of Turkey, which is also geographically closer to European populations rather than the Asians investigated. The genotypes of the study group were found to be different from the control group of a previous study conducted on a Turkish population [31]. The genetic distance of our study group is closer to Europeans, whereas the control group of the previous study is closer to Asian groups. The difference observed between the results in this study group and a previously reported Turkish population study indicates that the population living in Turkey is heterogeneous. In a study conducted in Italy, they presented findings similar to our study.

On the other hand, Santovito et al. In their study, they compared the population of northern and southern Italy. Santovito et al. found differences for all SNPs of cytokine polymorphisms in their study [34]. It is not surprising to found the smallest genetic distance between the study group and Greek Cypriots, who had similar historical background [35]. Cytokine genetic polymorphisms and their functional expression can be very different in various populations of the same ethnicity, and extensive cohort studies are required to assess the true relevance of certain cytokine alleles or haplotypes to disease [36–40]. In this study, cytokine study, which is the genotypes of the Turkish population, showed that for most of the SNPs, Hardy Weinberg equilibrium was compatible. These results showed that it can be used for anthropological

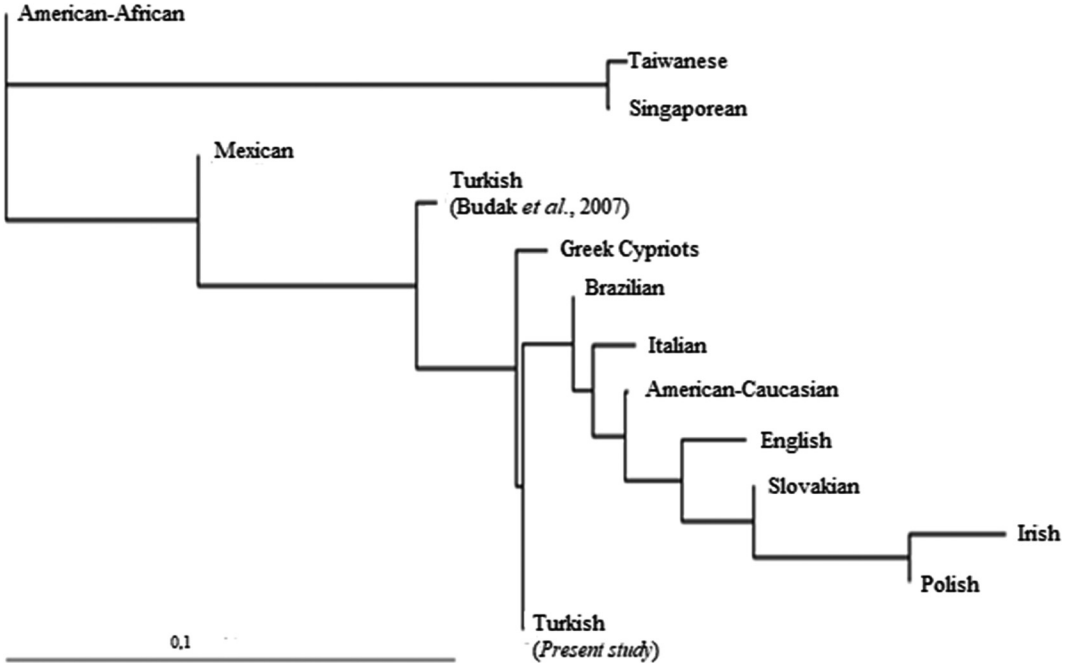

Figure 1: Phylogenetic tree of the investigated populations based on TNFA, IL6, IL10 genotypes.

comparisons and association studies with diseases. The protective role of cytokine polymorphism investigation is well known in literature, but anthropological evolution for the current region has not been investigated or not explained either.

Conclusions

Cytokine polymorphism studies have an important place in the understanding of various diseases. The cytokine genotypes can also have implications for immune responses generated in populations due to environmental factors. We hope that the data of our study will be of use for the prospective researches that will reveal the relationship between transplantation, hematological malignancy and cytokine polymorphism. Further studies should be performed in larger population groups in Turkey to confirm the biological significance of our results.

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