Cytokine single nucleotide polymorphisms in Iranian populations

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ABSTRACT. Cytokines are important immunomodulatory molecules involved in immune responses against microorganisms; they also have an important role in the setting of immune system disorders. Cytokine single nucleotide polymorphisms have been extensively studied in different, normal populations as well as in association with disease. Cytokine gene polymorphisms are potentially important as genetic predictors of disease susceptibility, clinical outcome, and as a tool for anthropological studies. In this study, samples have been collected from 455 healthy individuals located in different regions of Iran (Tehran, Yazd, Sistan and Balochistan). Allele and genotype frequencies of cytokine SNP, including: IL-1α, IL-1β, IL-1R, IL-1RA, IL-2, IL-4, IL-4RA, IL-6, IL-10, IL-12, TNF-α, TGF-β and IFN-γ were investigated, using the PCR-SSP method. Allele frequencies in Tehran and Yazd populations were similar, except for TGF-β. Allele frequencies in Sistani & Baloch populations were similar at all positions, except for IL-1β at position of -511 and IFN-γ genes at position UTR5644; there were some differences in allele frequencies comparing these populations with the Yazd population, including: IL-4, IL-6, IL-10, TGF-β and TNF-α. Although some significant differences were observed for some cytokines, it seems that the cytokine gene polymorphism profile of the Iranian population is similar to that of Caucasians, particularly the Italian population.

Keywords: cytokine gene, Iran, polymorphisms

Cytokines are potent immunomodulatory molecules with important roles in immune responses to foreign elements, such as microorganisms or transplants. Several studies have reported the role of cytokine gene polymorphisms in transplant rejection [1-3], and autoimmune [4, 5] and malignant diseases [6-8]. Cytokines act as factors for immunocyte activation, differentiation and function. There are also many reports about cytokine gene polymorphisms and cytokines production influencing the balance of the immune response. Most polymorphisms have been reported to be of the single nucleotide polymorphism (SNP) type or microsatellite polymorphism, but other types such as insertion and deletion have also been observed [9]. The study of cytokine gene polymorphisms is a useful tool for anthropological analysis and for the prediction of genetic susceptibility to diseases in certain populations [10].
Recent studies indicate significant ethnic and racial differences [11-17]. We have previously investigated Th1 and Th2 cytokine gene polymorphisms in two indigenous Iranian populations [18]. In the present study, we extend our research to the determination of cytokine single nucleotide polymorphisms in four, indigenous, Iranian populations. This investigation provides data from a normal Iranian population for future anthropological and disease association studies.

METHODS AND DONORS

Study region

Iran is a middle-eastern country, situated to the east of Turkey and Iraq; and to the west of Afghanistan and Pakistan. It is limited by the Caspian Sea to the north and the Persian Gulf to the south. Four indigenous populations from three provinces of Iran (Tehran, Yazd, and Sistan & Balochistan) were randomly selected for this study *(figure 1)*.
Tehran is the capital of Iran with more that 11 million people, which includes a mixed population from all of the different Iranian ethnic groups resulting frommigrations. Yazd is a province in the center of Iran with approximately 1 million people, which includes a pure Iranian population. It is regarded as the second most ancient and historic site in the world, as the centre of the Zoroastrian culture, with a

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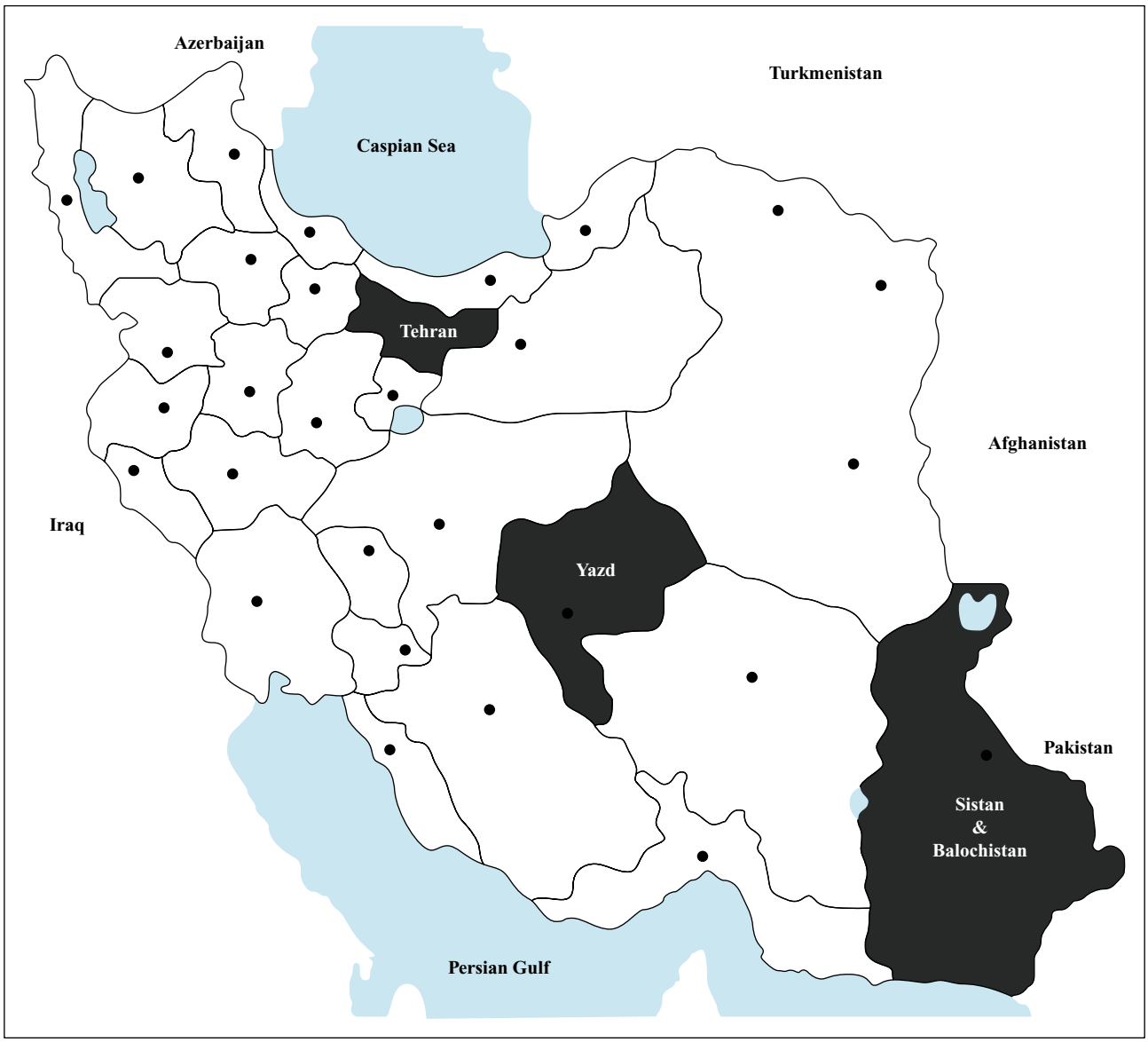

Figure 1
The location of the regions investigated in this study (Tehran, Yazd, and Sistan & Balochistan).

3 000 year-long history. They have been little affected by the different invasions of Iran and they are regarded as the original Iranian population. Sistan & Balochistan, as the largest province in Iran with more than 2 million people, is situated in the south-east of Iran, and includes two ethnic groups, the Sistani and Baloch. Sistan is one of the eastern territories of Darius the Great, and an indigenous Sistani population currently lives there. The history of the Baloch is unclear. Baloch people could have belonged to the part of northern Iran, now inhabited by Ashkanis, who were originally Aryans. Although some scholars believe that they came from Halab and are Semites, others think that the Baloch are the remnants of the indigenous population of the area, possibly locally mixed with various peoples such as the Scythians, Ashkanis, Turks, and many others. The Baloch population is distributed in this area of Iran as well as in areas of Pakistan, Afghanistan and, although present in smaller numbers, in Turkmenistan, India, East Africa, and Oman [19]. According to previous HLA-typing studies, the Baloch population of Iran is genetically very close to the Baloch and Brahui populations of Pakistan [19].

Subjects

Four hundred and fifty five healthy individuals were investigated in this study, including 140 subjects from Tehran (70 male and 70 female), 121 subjects from Yazd (61 male and 60 female), 98 subjects from Sistan (49 male and 49 female), and 96 subjects from Balochistan (48 male and 48 female). Data for cytokine gene polymorphisms in Tehran and Yazd was previously reported in our first report [18]. The ethnic origin of each volunteer was determined by official documents and family history of three generations (nationality, place of birth and language spoken by parents and grandparents). All individuals were healthy, unrelated,

and randomly selected from these regions, and blood samples were collected after obtaining informed consent from the individuals.

DNA sampling and genotyping

DNA was isolated from whole blood collected with EDTA as anticoagulant, using a "salting out" method [20]. All cytokine typing was performed by polymerase chain reaction with a sequence-specific primers (PCR-SSP) assay, which uses identical amplification and detection conditions, enabling rapid and cost-efficient analysis of polymorphisms. The PCR-SSP kit used was the Heidelberg cytokine gene polymorphism SSP kit (Heidelberg University, Heidelberg, Germany). Amplification was carried out using a PCR Techne Flexigene apparatus (Rosche, Cambridge, UK) under the following conditions: initial denaturation 94°C, 2 min; denaturation 94°C, 10 sec; annealing + extension 65°C, 1 min (10 cycles); denaturation 94°C, 10 sec; annealing 61°C, 50 sec; extension 72°C, 30 sec (20 cycles). The presence or absence of PCR products was visualized by 2% agarose gel electrophoresis. After electrophoresis, the gel was placed on a UV transilluminator and then a picture for interpretation and documentation was taken. Each of the primer mixes contained a control primer pair that amplified either a part of the β-globin gene or a part of the C-reactive protein (CRP) gene. The β-globin control primers produce an 89-bp fragment, while the primer pairs amplifying the CRP gene produced a 440-bp amplicon.

The allele and genotype frequencies of the following cytokine genes were determined: interleukin (IL)-1α (T/C -889), IL-1β (C/T -511, T/C +3962), IL-1R (C/T pst-I 1970), IL-1RA (T/C Mspa-I 11100), IL-2 (T/G -330, G/T +166), IL-4 (T/G -1098, T/C -590, T/C -33), IL-4RA (G/A +1902), IL-6 (G/C -174, G/A nt565), IL-10 (G/A -1082, C/T -819, C/A -592), IL-12 (C/A -1188), TNF-α (G/A -308, G/A -238), IFN-γ[A/T untranslated region (UTR) 5644] and transforming growth factor (TGF)-β(C/T codon 10, G/C codon 25).

Statistical analysis

The differences in allele and haplotype frequencies for every cytokine gene among the four indigenous Iranian populations were analyzed using the Chi-square test after Yates correction [21]. The odds ratio (OR) and its 95% confidence intervals (CI) were calculated and a p-value of 0.05 or less was considered to be significant using InStat version 3.06 (GraphPad Software Inc, CA, USA 2003).

RESULTS

We analyzed the distribution of some cytokine gene polymorphisms in four indigenous population from Iran; Tehran, Yazd, and Sistan & Balochistan provinces *(figure 1)*. The results for cytokine gene polymorphisms in the four indigenous Iranian populations are shown in *tables 1-4*.

The allele frequencies in the Tehran and Yazd populations were similar, except for the TGF-β gene at two positions including: codon 10 (C/T) and codon 25 (C/G).

The allele frequencies in the Sistani and Baloch populations were similar, except for IL-1β at position -511 and IFN-γ genes at position UTR5644. The IL-1β C allele was significantly more frequent in the Sistani than the Baloch population [p = 0.015, 95% CI: 1.68 (1.1-2.56)], while the T allele was significantly more frequent in the Baloch population (p = 0.015); the CC genotype being significantly more frequent in the Sistani population [p = 0.004, 95% CI: 2.70 (1.32-5.55)]. The AA genotype for IFN-γ (UTR5644) was significantly more frequent in the Baloch than the Sistani population [p = 0.02, 95% CI: 0.48 (0.26-0.90)], while the AT genotype was also significantly more frequent in the Sistani population [p = 0.03, 95% CI: 1.93 (1.04-3.61)] *tables 1* and *2)*.

Despite differences between the origins of the Sistani and Baloch populations, allele frequencies were similar at all positions, probably as a result of living in one location for centuries and marriages between these two populations. However, there were some differences in allele frequencies when comparing these populations with other ethnic groups in Iran; *i.e.* those in Yazd and Tehran, including: IL-4, IL-6, IL-10, TGF-β and TNF-α *(table 4)*.

DISCUSSION

Cytokines as one of the important elements of immune system regulation and play a critical role in several pathogenic processes in this system. There are various reports of the collective influences of some cytokines, especially those with pro-inflammatory or anti-inflammatory effects. As a result of the 13th International Histocompatibility Workshop and Congress (IHWC), 22 SNPs have been identified for 14 cytokine genes [22].

Cytokine gene polymorphisms may affect inter-individual differences in serum levels of each cytokine by influencing on the transcriptional regulation. Different levels of several cytokines have been reported in investigations, involving graft response [23], gastric [24], breast [25], and prostate cancers [26]. Also, there are recent reports of the influence of cytokine gene polymorphism on infection by *Helicobacter pylori* [27, 28]. Because of the important role of cytokine gene polymorphisms in susceptibility to autoimmune diseases, cancers and graft rejection, it would be of value to conduct extensive investigations into the distribution of these genes in different ethnic groups. Several studies have been reported involving specific ethnic groups [14-18]. In this study, we have genotyped a sample of healthy Iranian subjects for a number of cytokine genes (IL-1, IL-1R, IL-1RA, IL-2, IL-4, IL-4RA, IL-6, IL-10, IL-12, IFN-γ, TNF-α, and TGF-β). We have selected our samples from four different ethnic groups in Iran (from Tehran, Yazd, Sistan & Baloch). The Tehran population represents a mixed population resulting from migration, while the Yazd population is as a relatively pure Iranian population. Sistan is a Farsi population in south eastern Iran and Baloch is a population that may be regarded as an original Iranian population.

Comparison of allele frequencies for IL-1α, IL-1β, IL-1R and IL-1RA, indicates no significant differences between the four indigenous Iranian populations. Compared to other populations of the world, allele and genotype frequencies for IL-1α (-889) were similar to other populations studied, while the C allele was significantly less frequent in Iranian than the Korean population [11]. For IL-1β, the frequency of the -511 C allele was significantly lower in our population than those from England [29] and

Table 1
Inflammatory cytokine gene polymorphisms (alleles/genotypes) in four indigenous Iranian populations and some other Caucasian populations

Cytokine	Position	Alleles/ Genotypes	Sistani (n = 98) N (%)	Baloch (n = 96) N (%)	Tehran[1] (n = 140) N (%)	Yazd[1] (n = 121) N (%)	Italian[2] (n = 140) (%)	Greek[3] (n = 100) (%)	English[4] (n = 145) (%)
IL-1α	-889	C	141 (71.9)	126 (65.6)	186 (68.4)	162 (66.9)	71	-	73
		T	55 (28.1)	66 (34.4)	86 (31.6)	80 (33.1)	29	-	27
		CC	52 (53.1)	43 (44.8)	62 (45.6)	55 (45.4)	52	-	59
		TC	37 (37.7)	40 (41.7)	62 (45.6)	52 (43)	39	-	28
		TT	9 (9.2)	13 (13.5)	12 (8.8)	14 (11.6)	9	-	13
IL-1β	-511	C	117 (59.7)*	90 (46.9)*	154 (55.4)	130 (53.7)	71	-	71
		T	79 (40.3)*	102 (53.1)*	124 (44.6)	112 (46.3)	29	-	29
		CC	36 (36.7)*	17 (17.7)*	36 (25.8)	27 (22.3)	50	-	49
		TC	45 (45.9)	56 (58.3)	82 (59)	76 (62.8)	41	-	44
		TT	17 (17.4)	23 (24)	21 (15.2)	18 (14.9)	9	-	7
IL-1β	3962	C	138 (70.4)	137 (71.4)	198 (70.7)	168 (69.4)	74	-	79
		T	58 (29.6)	55 (28.6)	82 (29.3)	74 (30.6)	26	-	21
		CC	54 (55.1)	47 (49)	70 (50)	51 (42.1)	54	-	66
		TC	30 (30.6)	43 (44.8)	58 (41.4)	66 (54.6)	40	-	27
		TT	14 (14.3)	6 (6.2)	12 (8.6)	4 (3.3)	6	-	7
IL-1R	Pst-I	C	113 (57.7)	125 (65.1)	174 (62.1)	160 (66.1)	67	-	71
	1970	T	83 (42.3)	67 (34.9)	106 (44.2)	82 (33.9)	33	-	29
		CC	31 (31.6)	41 (42.7)	54 (38.6)	47 (38.8)	43	-	46
		TC	50 (51)	43 (44.8)	66 (47.1)	66 (54.6)	48	-	50
		TT	17 (17.4)	12 (12.5)	20 (14.3)	8 (6.6)	9	-	4
IL-1RA	Mspa-I	C	24 (12.2)	27 (14.1)	64 (22.9)	58 (24.2)	23	-	25
	11100	T	172 (87.8)	165 (85.9)	216 (77.1)	182 (75.8)	77	-	75
		CC	3 (3.1)	1 (1)	4 (2.9)	0 (0)	3	-	2
		CT	17 (17.4)	25 (26)	56 (40)	58 (48.3)	41	-	47
		TT	78 (79.5)	70 (73)	80 (57.1)	62 (51.7)	56	-	51
TNF-α	-308	A	19 (9.7)	18 (9.4)	39 (14.2)	47 (20)	9	7.5	12
		G	177 (90.3)	174 (90.6)	235 (85.8)	189 (80)	91	92.5	88
		AA	1 (1)	0 (0)	0 (0)	1 (0.9)	2	0	1
		AG	17 (17.4)	18 (18.8)	39 (28.5)	45 (38.1)	14	15	23
		GG	80 (81.6)	78 (81.2)	98 (71.5)	72 (61)	84	85	76
TNF-α	-238	A	17 (8.7)	8 (4.2)	59 (21.5)	59 (25)	7	-	7
		G	179 (91.3)	184 (95.8)	215 (78.5)	177 (75)	93	-	93
		AA	0 (0)	0 (0)	1 (0.7)	0 (0)	0	-	<1
		GA	17 (17.3)	8 (8.3)	57 (41.6)	59 (50)	14	-	13
		GG	81 (82.7)	88 (91.7)	79 (57.7)	59 (50)	86	-	87
IL-12	-1188	A	141 (71.9)	138 (71.9)	204 (72.9)	172 (71.7)	-	-	-
		C	55 (28.1)	54 (28.1)	76 (27.1)	68 (28.3)	-	-	-
		AA	49 (50)	48 (50)	72 (51.4)	57 (47.5)	-	-	-
		CA	43 (43.9)	42 (43.8)	60 (42.9)	58 (48.3)	-	-	-
		CC	6 (6.1)	6 (6.2)	8 (5.7)	5 (4.2)	-	-	-
IL-6	-174	C	39 (19.9)	29 (15.1)	101 (36.3)	97 (40.4)	34	18.5	23
		G	157 (80.1)	163 (84.9)	177 (63.7)	143 (59.6)	66	81.5	77
		CC	2 (2)	1 (1)	4 (2.9)	6 (5)	9	4	4
		CG	36 (36.7)	27 (28.1)	93 (66.9)	85 (70.8)	50	29	37
		GG	60 (61.3)	68 (70.8)	42 (30.2)	29 (24.2)	41	67	59
IL-6	nt565	A	42 (21.4)	32 (16.7)	50 (18)	54 (22.5)	33	-	-
		G	154 (78.6)	160 (83.3)	228 (82)	186 (77.5)	67	-	-
		AA	1 (1)	2 (2.1)	4 (2.9)	0 (0)	9	-	-
		GA	40 (40.8)	28 (29.2)	42 (30.2)	54 (45)	48	-	-
		GG	57 (58.2)	66 (68.7)	93 (66.9)	66 (55)	43	-	-

* p-value < 0.05 between two groups of Sistani and Baloch.
[1] Iran (18); [2] Italy (13); [3] Greece (12); [4] England (29).

Italy [13], while the frequency of the +3962 C allele was lower in the Iranian than the Korean population [11]. The +3962 C/C and T/C genotype frequencies in our population were significantly lower and higher than a population from England, respectively. The alleles and genotypes were similarly distributed in the populations studied. For IL-1RA, there was a significant difference in comparison with the Korean population; the C allele at the Mspa-I 11100 position was significantly less frequent in the Iranian than the Korean population [11].

IL-2, which is mainly expressed in Th1 cells, acts as an autocrine growth factor. Two single base substitutions have

Table 2
T-helper 1-derived cytokine gene polymorphisms (alleles/genotypes) in four indigenous Iranian populations and two other Caucasian populations

Cytokine	Position	Alleles/ Genotypes	Sistani (n = 98) N (%)	Baloch (n = 96) N (%)	Tehran[1] (n = 140) N (%)	Yazd[1] (n = 121) N (%)	Italian[2] (n = 140) (%)	Greek[3] (n = 100) (%)	English[3] (n = 79) (%)
IL-2	-330	G	91 (46.4)	86 (44.8)	110 (39.6)	91 (37.6)	34	-	27
		T	105 (53.6)	106 (55.2)	168 (60.4)	151 (62.4)	66	-	73
		GG	19 (19.4)	17 (17.7)	8 (5.8)	1 (0.8)	12	-	8
		GT	54 (54.6)	52 (54.2)	94 (67.6)	89 (73.6)	44	-	39
		TT	25 (26)	27 (28.1)	37 (26.6)	31 (25.6)	44	-	53
IL-2	166	G	150 (76.5)	142 (74)	219 (78.8)	191 (78.9)	78	-	-
		T	46 (23.5)	50 (26)	59 (21.2)	51 (21.1)	22	-	-
		GG	64 (65.3)	55 (57.3)	82 (59)	72 (59.5)	61	-	-
		GT	22 (22.4)	32 (33.3)	55 (39.6)	47 (38.8)	32	-	-
		TT	12 (12.3)	9 (9.4)	2 (1.4)	2 (1.7)	7	-	-
IFN-γ	UTR5644	A	108 (55.1)	123 (64.1)	140 (50.7)	133 (55.9)	-	-	-
		T	88 (44.9)	69 (35.9)	136 (49.3)	105 (44.1)	-	-	-
		AA	30 (30.6)*	46 (47.9)*	43 (31.2)	50 (42)	-	-	-
		AT	47 (48)*	31 (32.3)*	54 (39.1)	33 (27.8)	-	-	-
		TT	20 (20.4)	19 (19.8)	41 (29.7)	36 (30.2)	-	-	-

* p-value < 0.05 between two groups of Sistani and Baloch.
[1] Iran (18); [2] Italy (13); [3] England (31).

been identified at -330 G/T and +166 G/T positions from the transcription start point [30]. No significant differences in allele frequencies were observed in the four different populations from Iran. Compared to other studies, a significantly higher frequency of the -330 G allele was observed in our population in comparison with English [31] and African-American [10] populations. This allele is associated with increased levels of cytokine production. The -330 G/G genotype, reported as a polymorphism with a high level of cytokine expression after anti-CD3/CD28 stimulation of lymphocytes [32], was similar in all populations studied, except in the African-American population there is a lower frequency [10].

Also, the increased frequency of the -330 T/T that has been reported in the African-American population is the main genotype causing low IL-2 levels, while -330 G/T was the main genotype in our population, with an intermediate level of IL-2 gene expression. IL-2 seems to play a critical role in the development of self-tolerance, predisposition to autoimmunity, and allograft tolerance induction [33].

IL-4 is the major cytokine in isotype switching of B cells to IgE-producing cells by activating pre-T helper cells to Th2 cells [11, 34], leading to humoral responses, helminthic immunity and allergies [35]. The allele frequencies were similar in the Tehran and Yazd samples at -1098, -590 and -33 positions. The -1098 G, -590 C and -33 C alleles are significantly less frequent in Tehran and Yazd in comparison with the Sistani and Baloch populations. As regards the promoter -590 polymorphism, the frequency of the C allele, as the main allele in Iranian populations was 62.8%, which was lower than for populations from England (87%) [29] and Italy (89%) [13], while the T allele has been reported to be the main allele in Orientals [22, 36]. The -590 T allele is associated with increased IL-4 gene expression *in vitro* and increased total serum IgE levels *in vivo* [37]. The highest frequency genotype in the Iranian population was T/C (73.6%), but the main genotype in populations from England and Italy was C/C (frequencies of 76% and 79%, respectively). For the IL-4RA gene, an analysis of SNP at position +1902 showed no significant differences in allele frequencies in our indigenous populations. Also, compared with other countries such as Korea, England and Italy, similar results were obtained.

IL-6 mediates T-cell activation, growth and B and T cell differentiation. IL-6 serves as an important pro-inflammatory cytokine in processes, such as pyrexia and induction of acute phase proteins production in hepatocytes. However, IL-6 mediates several anti-inflammatory effects by reducing IL-1 and TNF, and stimulating IL-1RA production. In the SNP of IL-6 gene at the -174 position, the G allele is associated with increased levels of IL-6 production [38]. Also, a greater level of IL-6 production in acute graft rejection and autoimmune rheumatoid diseases has been reported [29, 38]. In our indigenous populations, the C allele at position -174 was significantly more frequent in Yazd than in the Sistani and Baloch populations. Although the -174 G allele is the most frequent allele in the general population in almost all studied ethnic groups, there are remarkable differences in Orientals compared with other ethnic groups. We found that the allele frequencies at this position were very similar to Caucasians, such as English and Italian populations.

IL-10 inhibits production of IFN-γ and IL-2 (Th1 cytokines), IL-4 and IL-5 (Th2 cytokines), IL-1β, IL-6, IL-8, IL-12, and TNF-α (by mononuclear phagocytes), and IFN-γ and TNF-α (by NK cells). In addition, IL-10 inhibits monocyte MHC class II molecule, CD23, ICAM-1, and B7 expression [39]. IL-10 has been suggested to induce and maintain the anergic state [40]. The -819 C and -592 C alleles were more and less frequent in the Yazd than in the Sistani population, respectively. The -1082 G allele is associated with increased levels of IL-10 production [2]. This allele was significantly lower in Orientals, such as Korean and Chinese populations than other world populations. The allele frequencies in our population were similar to Caucasians, especially Greek [12] and Italian [13] populations. The main genotype in the Chinese population [41] differs greatly from other ethnic groups (A/A in Chinese,

Table 3
T-helper 2- and T-regulatory cell-derived cytokine gene polymorphisms (alleles/genotypes) in four indigenous Iranian populations and some other Caucasian populations

Cytokine	Position	Alleles/ Genotypes	Sistani (n = 98) N (%)	Baloch (n = 96) N (%)	Tehran[1] (n = 140) N (%)	Yazd[1] (n = 121) N (%)	Italian[2] (n = 140) (%)	Greek[3] (n = 100) (%)	English[4] (n = 145) (%)
IL-4	-1098	G	94 (48)	91 (47.4)	84 (30.2)	95 (39.3)	9	-	-
		T	102 (52)	101 (52.6)	194 (69.8)	147 (60.7)	91	-	-
		GG	2 (2)	3 (3.1)	1 (0.7)	0 (0)	1	-	-
		GT	90 (91.8)	85 (88.6)	82 (59)	95 (78.5)	16	-	-
		TT	6 (6.2)	8 (8.3)	56 (40.3)	26 (21.5)	83	-	-
IL-4	-590	C	146 (74.5)	146 (76)	149 (53.6)	129 (53.3)	89	-	87
		T	50 (25.5)	46 (24)	129 (46.4)	113 (46.7)	11	-	13
		CC	48 (49)	52 (54.1)	10 (7.2)	8 (6.6)	79	-	76
		TC	50 (51)	42 (43.8)	129 (92.8)	113 (93.4)	20	-	21
		TT	0 (0)	2 (2.1)	0 (0)	0 (0)	1	-	3
IL-4	-33	C	168 (85.7)	172 (89.6)	200 (71.9)	153 (63.2)	86	-	-
		T	28 (14.3)	20 (10.4)	78 (28.1)	89 (36.8)	14	-	-
		CC	72 (73.5)	77 (80.2)	61 (43.9)	32 (26.4)	75	-	-
		TC	24 (24.5)	18 (18.8)	78 (56.1)	89 (73.6)	23	-	-
		TT	2 (2)	1 (1)	0 (0)	0 (0)	2	-	-
IL-4RA	1902	A	167 (85.2)	163 (84.9)	242 (87.7)	193 (82.5)	81	-	79
		G	29 (14.7)	29 (15.1)	34 (12.3)	41 (17.5)	19	-	21
		AA	70 (71.4)	67 (69.8)	106 (76.8)	87 (74.4)	68	-	61
		GA	27 (27.6)	29 (30.2)	30 (21.7)	19 (16.2)	27	-	35
		GG	1 (1)	0 (0)	2 (1.5)	11 (9.4)	5	-	4
IL-10	-1082	A	126 (64.3)	127 (66.1)	181 (64.6)	147 (60.7)	61	62	49
		G	70 (36.1)	65 (33.9)	99 (35.4)	95 (39.3)	39	38	51
		AA	34 (34.7)	37 (38.5)	53 (37.8)	30 (24.8)	34	40	34
		GA	58 (59.2)	53 (55.2)	75 (53.6)	87 (71.9)	54	44	35
		GG	6 (6.1)	6 (6.3)	12 (8.6)	4 (3.3)	12	16	34
IL-10	-819	C	123 (62.8)	123 (64.1)	199 (71.1)	187 (77.3)	72	76.5	78
		T	73 (37.2)	69 (35.9)	81 (28.9)	55 (22.7)	28	23.5	22
		CC	35 (35.7)	40 (41.7)	71 (50.7)	69 (57)	50	61	59
		CT	52 (53.1)	43 (44.8)	57 (40.7)	49 (40.5)	44	31	38
		TT	10 (10.2)	13 (13.5)	12 (8.6)	3 (2.5)	6	8	3
IL-10	-592	A	74 (37.8)	69 (35.9)	81 (28.9)	55 (22.7)	28	23.5	22
		C	122 (62.2)	123 (64.1)	199 (71.1)	187 (77.3)	72	76.5	78
		AA	10 (10.2)	13 (13.5)	12 (8.6)	3 (2.5)	6	8	3
		CA	54 (54.7)	43 (44.8)	57 (40.7)	49 (40.5)	44	31	38
		CC	34 (35.1)	40 (41.7)	71 (50.7)	69 (57)	50	61	59
TGF-β	Codon 10	C	104 (53.1)	100 (52.1)	131 (47.5)	151 (64)	-	42.5	35[5]
		T	92 (46.9)	92 (47.9)	145 (52.5)	85 (36)	-	57.5	65
		CC	24 (24.5)	24 (25)	20 (14.5)	42 (35.6)	-	19	11
		CT	56 (57.1)	52 (54.2)	91 (65.9)	67 (56.8)	-	47	48
		TT	18 (18.4)	20 (20.8)	27 (19.6)	9 (7.6)	-	34	41
TGF-β	Codon 25	C	13 (6.6)	14 (7.3)	21 (7.6)	44 (18.6)	-	7	10[5]
		G	183 (93.4)	178 (92.7)	255 (92.4)	192 (81.4)	-	93	90
		CC	0 (0)	2 (2.1)	2 (1.5)	2 (1.7)	-	1	1
		CG	13 (13.3)	10 (10.4)	17 (12.3)	40 (33.9)	-	12	18
		GG	85 (86.7)	84 (87.5)	119 (86.2)	76 (64.4)	-	87	81

* p-value < 0.05 between two groups of Sistani and Baloch.
[1] Iran (18); [2] Italy (13); [3] Greece (12); [4] England (29); [5] England (n = 107) (9).

while G/A in other ethnics). There are reports of the association of the -1082 A allele with diseases such as joint destruction, recurrence of hepatitis C in liver transplant recipients, and cutaneous malignant melanoma [42-44].

At the IL-12 -1188 position, no significant differences were found between our four indigenous populations, but there were marked and significant differences in comparison with the Korean population. The C allele was significantly less frequent in the Iranian than the Korean population. The C/A genotype frequency was significantly higher than in a population from Ireland [45].

IFN-γ plays a pivotal role in defense against viruses and intracellular agents, and in the inhibition of allergic responses through an inhibitory effect on IL-4 production. The IFN-γ UTR5644 alleles is similarly distributed in our indigenous populations compared with studies in other countries showing an increased frequency of the T allele in our population compared to Korean and African-American

Table 4
Cytokine gene haplotype distribution in four indigenous Iranian populations

Cytokine	Haplotype	Tehran (140 subjects) N (%)	Yazd (121 subjects) N (%)	Sistani (98 subjects) N (%)	Baloch (96 subjects) N (%)
TGF-β (codon10, codon25)	CG	110 (39.9)	110 (46.6)	82 (41.8)	83 (46.1)
	TG	145 (52.5)	81 (34.3)	101 (51.5)	81 (45)
	CC	21 (7.6)	41 (17.4)	13 (6.7)	15 (8.3)
	TC	0 (0)	4 (1.7)	0 (0)	1 (0.6)
TNF-α (-308, -238)	GG	176 (64.2)	138 (58.5)	158 (84)	162 (88.1)
	AG	39 (14.2)	41 (17.4)	17 (9)	14 (7.6)
	GA	59 (21.5)	51 (21.6)	13 (7)	7 (3.8)
	AA	0 (0)	6 (2.5)	0 (0)	1 (0.5)
IL-2 (-330, +166)	GG	107 (38.8)	78 (32.2)	89 (46.8)	79 (43.4)
	TG	112 (40.6)	113 (46.7)	52 (27.4)	58 (31.9)
	TT	56 (20.3)	37 (15.3)	46 (24.2)	43 (23.6)
	GT	1 (0.3)	14 (5.8)	3 (1.6)	2 (1.1)
IL-4 (-1098, -590, -33)	TTC	51 (18.3)	27 (11.2)	17 (8.9)	27 (14.8)
	GCC	83 (30)	91 (37.6)	89 (46.8)	83 (45.6)
	TTT	76 (27.3)	85 (35.1)	26 (13.7)	13 (7.1)
	TCC	65 (23.4)	34 (14.1)	55 (29)	50 (27.5)
	TCT	2 (0.7)	1 (0.4)	0 (0)	4 (2.2)
	GTT	1 (0.3)	0 (0)	0 (0)	0 (0)
	GCT	0 (0)	3 (1.2)	2 (1.1)	1 (0.6)
	GTC	0 (0)	1 (0.4)	1 (0.5)	4 (2.2)
IL-6 (-174, nt565)	GG	173 (62.2)	141 (58.8)	144 (76.6)	146 (81.1)
	CG	55 (19.8)	45 (18.8)	3 (1.6)	2 (1.1)
	CA	46 (16.6)	52 (21.7)	39 (20.7)	28 (15.6)
	GA	4 (1.4)	2 (0.8)	2 (1.1)	4 (2.2)
IL-10 (-1082, -819, -592)	GCC	99 (35.4)	95 (39.3)	67 (37.2)	62 (34.8)
	ACC	100 (35.7)	92 (38)	47 (26.1)	54 (30.4)
	ATA	81 (28.9)	55 (23)	65 (36.1)	62 (34.8)

populations. This allele is associated with high levels of expression. The allele and genotype frequencies in our population were similar to the Italian population [13].

TNF-α induces antitumor immunity through direct cytotoxic effects on cancerous cells and by stimulating antitumor immune responses. However, the severe side effects restrict its potential therapeutic value in the treatment of tumors. TNF-α also acts as a pro-inflammatory cytokine by neutrophil recruitment, degranulation and respiratory burst [39]. Several studies suggest the role of the -308 A allele as an allele of high activity in susceptibility to diseases, such as obstructive sleep apnoea–hypopnoea syndrome (OSAHS), asthma and chronic lymphocytic leukemia [46-48]. The allele frequencies were similar in our four indigenous populations at the -308 position. The allele and genotype frequencies at this position were similar in the general population of Iran, Caucasians and the Korean population. In contrast, there were significant differences in comparison with data from Japan [49] and China [24]. The frequency of the -308 A allele was significantly higher in Iranian than Japanese and Chinese populations. At position -238 (A/G), the A allele was more frequent in the Yazd than Sistani and Baloch populations. Comparing with results of other countries, the -238 A allele was significantly more frequent in Iranian than Chinese populations. The -238 G/A and -238 G/G genotype frequencies in the Iranian population were significantly higher and lower than populations from China, England and Italy, respectively.

TGF-β is a cytokine with both stimulatory and inhibitory effects on different cell types [50]. T cells producing TGF-β is a distinct type of Th3 cells. TGF-β induces several actions such as fibrosis, scar formation and macrophage chemoattraction. Although TGF-β is constitutively produced in the healthy lung, it is associated with fibrosis in allergic asthma. The TGF-β codon 10 C allele was significantly more frequent in the Yazd than in the Tehran population; and the frequency of the codon 25 C allele was significantly higher than in the three other indigenous populations.

At the TGF-β codon 10 (C/T) position, the C allele was significantly more frequent in the Iranian population than populations from England [9] and Finland [51]. The C/C genotype in Iran was significantly more frequent than in the English population, while the T/T genotype was significantly less frequent than in English, Greek and African-American populations. Analysis of codon 25 SNP, showed that alleles and genotypes were similar in Iranian, Greek [12], English [9], Finnish [51] and African-

American [10] populations. The C allele at codon 25 is a rare allele in all reports, but it was never observed in the Korean population [11].

In conclusion, we analyzed the frequencies of different cytokine gene polymorphisms in four indigenous Iranian populations and compared them with other ethnic groups. Some significant differences were observed in some cytokines, demonstrating ethnic differences as well as the impact of migration on the genetic profile of a population. It seems that allele and genotype frequencies the Iranian population is similar to those of Caucasians, especially the Italian population. Analysis of cytokine gene polymorphisms in different ethnic groups may be helpful in anthropological studies and the study of the origin of populations, and for investigating the role of cytokines in different diseases.

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