Cytokine gene polymorphisms of TNFα, IL-6, IL-10, TGFβ and IFNγ in the Saudi population

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ABSTRACT

Studies have demonstrated associations between cytokine gene polymorphisms and ethnicity. In the present work the authors examine polymorphisms in the genes encoding interleukin-6 (IL-6), IL-10, interferon-γ (IFNγ), tumour necrosis factor-α (TNFα) and transforming growth factor-β (TGFβ1) using the polymerase chain reaction sequence-specific primer (PCR-SSP) method in 150 healthy unrelated Saudis, and results compared with those from other studied populations. The genotype distributions were consistent with the Hardy-Weinberg equilibrium, and the genotype frequencies observed among Saudis showed both similarity and difference to other populations. The most notable difference was in the distribution of IL-6, where the Saudi population showed a lower CG genotype frequency compared with White American (22% *vs*. 39.2%, $P=0.004$), Italian (22% *vs*. 50%, $P<0.0001$) and Brazilian (22% *vs*. 40.8%, $P<0.0001$) populations. The study population also showed a higher frequency of the IL-6 GG genotype compared with White Americans (72% *vs*. 45.1%, $P<0.0001$), Italians (72% *vs*. 41%, $P<0.0001$) and Brazilians (72% *vs*. 49.3%, $P<0.0001$). These results may have significant clinical relevance to the understanding of prevalent diseases in Saudi Arabia.

KEY WORDS: Cytokines.
Genotype.
Polymorphism, genetic.

Introduction

Cytokines are signalling molecules that play important roles in regulating the immune and inflammatory responses. They are key components in the pathogenesis of many diseases, including cancer, metabolic disorders, autoimmune diseases and inflammatory conditions.[1–3] Differences in cytokine gene polymorphisms are associated with disease predisposition,[4] such as that for systemic lupus erythematosus (SLE),[5] inflammatory bowel disease,[6,7] rheumatoid arthritis,[8] juvenile arthritis,[9] type I diabetes,[10,11] and in patients presenting with sepsis.[12] Although most studies are limited by their study design and sample size, it is noteworthy that such associations have been observed.

Polymorphisms in several cytokine genes have been identified and associations between cytokine genotype distribution and ethnicity have been demonstrated.[13–15] As an anthropological tool, cytokine genotypes have been useful in predicting genetic susceptibility to disease. This is evident in a study demonstrating that patients requiring renal transplantation differed from the general population in terms of their cytokine genotype.[14]

The present study aims to identify polymorphisms in the cytokine genes encoding interleukin-6 (IL-6), IL-10, interferon-γ (IFNγ), tumour necrosis factor-α (TNFα) and transforming growth factor beta-1 (TGFβ1) among healthy Saudi volunteers, and compare the results with those from other populations and identify significant differences.

Materials and methods

Unrelated normal healthy subjects ($n=150$) were included in this study, which was performed between July 2009 and May 2010. The study was approved by the Institutional Review Board/Ethics Committee of the College of Medicine, King Saud University, Riyadh, Saudi Arabia. Written, informed consent was obtained from each individual included in the study. Healthy controls were selected randomly and had no associated medical illness.

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DNA extraction from peripheral blood

Peripheral blood (8 mL) was drawn into a tube containing an acid-citrate-dextrose (ACD) anticoagulant and subsequently centrifuged. Extraction of DNA was performed using a QIAamp DNA mini kit (Qiagen, Valencia CA, USA) in accordance with the manufacturer's instructions. The concentration and purity of the recovered DNA was assessed by spectrophotometry (GeneQuant II, Pharmacia Biotech, Sweden), and the sample was stored in elution buffer at –20°C until use.

Cytokine genotyping

The investigated gene polymorphisms are listed in Table 1. Cytokine genotyping was performed using the polymerase chain reaction sequence-specific primer (PCR-SSP) method, as applied in a cytokine-genotyping tray (One Lambda, Canoga Park CA, USA). Briefly, DNA samples were thawed at room temperature and mixed with D-mix and recombinant *Thermus aquaticus* (*Taq*) polymerase, and the

mixture dispensed to a Micro SSP primer set tray containing specific primers. The target DNA fragments were amplified in a thermocycler (Perkin Elmer 9700, Perkin Elmer, Foster City CA, USA) using the following amplification times: denaturation at 96°C for 2 min, nine cycles of 96°C for 10 sec and 63°C for 50 sec, and then 20 cycles of 96°C for 10 sec, 59°C for 50 sec and 72°C for 30 sec. The amplified DNA products were electrophoresed and identified using a gel-documentation system (Alpha Inotech, Santa Clara CA, USA).

Statistical analysis

Genotype frequencies were calculated by direct counting. Observed and expected frequencies were compared using the χ^2 test to check for Hardy-Weinberg equilibrium. Genotypic frequencies were compared across the six populations using the χ^2 or Fisher's exact tests. $P<0.05$ was considered significant. All analyses were performed using the Statistical Software Package for the Social Sciences (SPSS, version 18.0; SPSS, Chicago IL, USA).

Results

The distributions of the observed genotypes were not significantly different from the expected distribution according to Hardy-Weinberg equilibrium (Table 1). When the genotype frequencies in the Saudi population were compared with those of White American and African-American,[14] Italian,[16] Greek,[17] Brazilian[18] and Omani[19] populations, similarities and differences were apparent (Table 2).

The frequencies of the IL-6 (–174) genotypes in the Saudi population were similar to data published for the African-American, Greek and Omani populations. However, significant differences were observed in the CG genotype distributions of the other populations, with the Saudi population showing a lower frequency compared with those in the White American (22% *vs.* 39.2%, $P=0.004$), Italian (22% *vs.* 50%, $P<0.0001$) and Brazilian (22% *vs.* 40.8%, $P<0.0001$) populations. The Saudi population showed a higher frequency of the IL-6 GG genotype compared with White Americans (72% *vs.* 45.1%, $P<0.0001$), Italians (72% *vs.* 41%, $P<0.0001$) and Brazilians (72% *vs.* 49.3%, $P<0.0001$). The Saudis also showed a lower frequency of the CC genotype compared to White Americans (6% *vs.* 15.7%, $P=0.012$).

The frequencies of the IL-10 (–1082, –819, –592) genotypes among Saudis differed from those of the White American, African-American, Italian and Greek populations. The Saudi population had a higher frequency of the ATA ATA genotype compared to the White American (14% *vs.* 5.9%, $P=0.043$) and Italian (14% *vs.* 5%, $P=0.01$) populations. There were more IL-10 GCC GCC genotypes (18.7% and 2.4%, respectively, $P=0.01$) but fewer GCC ATA genotypes (22% and 43.9%, respectively, $P=0.005$) when Saudis were compared to African-Americans, whereas the frequency of the GCC ATA genotype was higher among Saudis versus the Greek population (22% *vs.* 10.7%, $P=0.025$).

The frequencies of TNFα (–308) genotypes in the Saudi population were similar to those in the White American and African-American populations. The Saudi population showed a higher AA genotype frequency compared to the Italian (6% *vs.* 2%, $P=0.042$) population, whereas this genotype was not seen among the Greek, Brazilian and Omani populations. The AG genotype was also more frequent among Saudis (25.3%) compared to Italians (25.3% *vs.* 14%, $P=0.019$). Similarly, the Saudis had a lower GG genotype frequency (68.7%) compared to the Italian (68.7% *vs.* 84%, $P=0.002$), Greek (68.7% *vs.* 85%, $P=0.003$) and Omani (68.7% *vs.* 83.8%, $P=0.013$) populations.

The frequencies of IFNγ (+874) genotypes in the Saudi population were similar to those found in the White American, Italian and Greek populations. The African-American (7% *vs.* 23.3%, $P=0.017$) and Brazilian (14.2% *vs.* 23.3%, $P=0.037$) populations showed lower frequencies for the TT genotype compared to that in the Saudi population.

Regarding TGFβ1 (codon 10, codon 25) genotypes, the Saudi population had a distribution similar to that found in the Italian population. The Saudis showed a lower frequency for the TC GG genotype compared to White Americans (35.3 % *vs.* 49%, $P=0.037$), and did not harbour the TT CC genotype found in the African-American (4.4%, $P=0.009$) population.

Table 1. Hardy-Weinberg equilibrium tests for the investigated cytokine gene polymorphisms.

Cytokine	Observed genotype (%)	Expected genotype (%)	*P* value
TNFα (–308)			
AA	6.0	3.5	0.274
AG	25.3	30.3	0.304
GG	68.7	66.2	0.622
IFNγ (+874)			
TT	23.3	22.7	0.891
AT	48.7	49.9	0.817
AA	28.0	27.4	0.897
IL-6 (–174)			
CC	6.0	2.9	0.156
CG	22.0	28.2	0.230
GG	72.0	68.9	0.612
IL-10 (–1082, –819, –592)			
GCC GCC	18.7	16.0	0.542
GCC ACC	20.7	22.4	0.674
GCC ATA	22.0	25.6	0.497
ACC ACC	10.7	7.8	0.472
ACC ATA	14.0	17.9	0.345
ATA ATA	14.0	10.2	0.286
TGFβ1 (codons 10 and 25)			
TT GG	31.3	26.3	0.373
TC GG	35.3	37.6	0.719
TC GC	10.0	13.5	0.368
CC GG	18.0	12.3	0.199
TT GC	0	0	–
CC GC	4.7	8.8	0.164
CC CC	0.7	1.4	0.562
TT CC	0	0	–
TC CC	0	0	–

Discussion

In the present study, unrelated healthy Saudi individuals were genotyped for TNFα, IFNγ, IL-6, IL-10, and TGFβ gene polymorphisms and the results compared with those published for other populations. To date, this is the first study describing cytokine genotypes among Saudis, and the results showed similarities and significant differences compared with other populations.

Studies have identified cytokine gene polymorphisms and demonstrated associations with ethnicity.[13–15] Comparison of different populations has identified similarities in cytokine genotypes as well as certain population-specific distributions.[14,16,18,19] Polymorphisms in the regulatory regions of the cytokine genes may influence their expression[20–22] and can have an impact on disease susceptibility and the severity of the disease process. It is therefore relevant to investigate the distributions of cytokine genotypes in different ethnic groups.

Interleukin-6, which is a multifunctional cytokine that mediates inflammatory and stress-induced responses,[23] is associated with many diseases, including diabetes,[24] atherosclerosis,[25] depression,[26] Alzheimer's disease,[27] SLE,[28] prostate cancer[29] and rheumatoid arthritis.[30] Notably, the G allele is associated with higher plasma IL-6 levels compared with the C allele.[9] In the present study, the IL-6 (–174) genotype distribution in the Saudi population was found to be similar to those previously published for the African-American, Greek and Omani populations. However, the Saudi population showed a lower frequency of CG genotypes and a higher frequency of GG genotypes compared with those of the White American, Italian and Brazilian populations. In addition, Saudis showed a lower frequency of the CC genotype compared to White Americans. This observation may be significant, as associations have been reported between the GG genotype and asymptomatic carotid artery atherosclerosis,[31,32] the risk of coronary heart disease,[33] peripheral arterial occlusive

Table 2. Genotype frequencies in Saudis compared with other populations.

Cytokine	Saudi	White American[14]	African-American[14]	Italian[16]	Greek[17]	Brazilian[18]	Omani[19]
TNFα (–308)	*n*=150	*n*=102	*n*=43	*n*=140	*n*=100	*n*=210	*n*=80
AA	9 (6.0)	2 (2.0)	1 (2.3	2 (2.0)[a]	0 (0)[b]	0 (0)[c]	0 (0)[d]
AG	38 (25.3)	26 (25.5)	9 (20.9)	20 (14.0)[e]	15 (15.0)	55 (26.2)	13 (16.2)
GG	103 (68.7)	74 (72.5)	33 (76.8)	118 (84.0)[f]	85 (85.0)[g]	155 (73.8)	67 (83.8)[b]
IFNγ (+874)	*n*=150	*n*=102	*n*=43	*n*=140	*n*=100	*n*=211	–
TT	35 (23.3)	21 (20.6)	3 (7.0)[h]	32 (22.9)	31 (31.0)	30 (14.2)[i]	–
AT	73 (48.7)	55 (53.9	24 (55.8)	66 (47.1)	43 (43.0)	114 (54.0)	–
AA	42 (28.0)	26 (25.5)	16 (37.2)	42 (30.0)	26 (26.0)	67 (31.8)	–
IL-6 (–174)	*n*=150	*n*=102	*n*=43	*n*=140	*n*=100	*n*=213	*n*=80
CC	9 (6.0)	16 (15.7)[j]	0 (0)	13 (9.0)	4 (4.0)	21 (9.9)	1 (1.3)
CG	33 (22.0)	40 (39.2)[k]	8 (18.6)	70 (50.0)[c]	29 (29.0)	87 (40.8)[c]	18 (22.5)
GG	108 (72.0)	46 (45.1)[c]	35 (81.4)	57 (41.0)[c]	67 (67.0)	105 (49.3)[c]	61 (76.2)
IL-10 (–1082, –819, –592)	*n*=150	*n*=101	*n*=41	*n*=140	*n*=100	–	–
GCC GCC	28 (18.7)	17 (16.8)	1 (2.4)[l]	17 (12.1)	14 (14.0)	–	–
GCC ACC	31 (20.7)	30 (29.7)	7 (17.1)	43 (30.7)	31 (31.4)	–	–
GCC ATA	33 (22.0)	25 (24.8)	18 (43.9)[m]	34 (24.3)	11 (10.7)[d]	–	–
ACC ACC	16 (10.7)	6 (5.9)	3 (7.3)	10 (7.1)	16 (15.7)	–	–
ACC ATA	21 (14.0)	17 (16.8)	8 (19.5)	29 (20.7)	20 (19.8)	–	–
ATA ATA	21 (14.0)	6 (5.9)[n]	4 (9.8)	7 (5.0)[l]	8 (8.3)	–	–
TGFβ1 (codon 10, 25)	*n*=150	*n*=102	*n*=45	*n*=140	–	–	–
TT GG	47 (31.3)	27 (26.5)	14 (31.1)	45 (32.1)	–	–	–
TC GG	53 (35.3)	50 (49.0)[l]	15 (33.3)	52 (37.1)	–	–	–
TC GC	15 (10.0)	9 (8.8)	3 (6.7)	10 (7.1)	–	–	–
CC GG	27 (18.0)	10 (9.8)	6 (13.3)	24 (17.1)	–	–	–
TT GC	0 (0)	0 (0)	0 (0)	1 (0.7)	–	–	–
CC GC	7 (4.7)	5 (4.9)	5 (11.1)	3 (2.1)	–	–	–
CC CC	1 (0.7)	1 (1.0)	0 (0)	3 (2.1)	–	–	–
TT CC	0 (0)	0 (0)	2 (4.4)o	1 (0.7)	–	–	–
TC CC	0 (0)	0 (0)	0 (0)	1 (0.7)	–	–	–

Data presented as number (%).

[a]*P*=0.042; [b]*P*=0.013; [c]*P*=<0.0001; [d]*P*=0.025; [e]*P*=0.019; [f]*P*=0.002; [g]*P*=0.003; [h]*P*=0.017; [i]*P*=0.037; [j]*P*=0.012; [k]*P*=0.004; [l]*P*=0.01; [m]*P*=0.005; [n]*P*=0.043; and [o]*P*=0.009.

χ^2 or Fisher's exact tests were used to compare genotype frequencies among populations.

disease,[34] multi-infarct dementia[35] and longer hospital and intensive care unit stay after coronary artery bypass graft surgery.[36]

Coronary heart disease is the major cause of mortality among Saudis. According to the World Health Organization,[7] coronary heart disease deaths in Saudi Arabia reached 20,877 (23.98%) of total deaths in 2010.[37] The age-adjusted death rate of 180.58 per 100,000 individuals ranks Saudi Arabia at number 32 in the world. It would be interesting to examine the association of this cytokine genotype with coronary heart disease among Saudis. Notably, the frequency of the CC genotype, which has been shown to protect against systemic juvenile-onset chronic arthritis in other populations,[9] was low in the Saudi population. A future large-scale study could assess whether or not the low frequency of the IL-6 CC genotype confers a genetic predisposition to this disease among Saudis.

Interleukin-10, which may play a major role in inducing and maintaining the anergic state,[38,39] can suppress IFNγ, TNFα, granulocyte-macrophage colony-stimulating factor (GM-CSF) and the lymphotoxin production and proliferation of human T cells.[40] The Saudi population showed a higher frequency of the ATA ATA genotype in IL-10 compared to the White American and Italian populations. Saudis also showed a higher frequency of the GCC GCC genotype, but a lower frequency of the GCC ATA genotype compared to African-Americans. Compared to the Greek population, the frequency of the GCC ATA genotype was higher among the Saudi population. Polymorphisms in the IL-10 gene are associated with rheumatoid arthritis[41] and SLE.[42,43] The GCC GCC genotype is associated with higher production of IL-10, which, when combined with low TNFα, is associated with a higher prevalence of discoid lupus erythematosus (DLE).[44]

Tumour necrosis factor-α, which is involved in systemic inflammation and contributes to stimulating the acute-phase reaction, is produced chiefly by activated macrophages and is primarily involved in regulating immune cells. Dysregulation of TNFα production has been implicated in various human diseases, including Alzheimer's disease,[27] cancer,[45] major depression,[26] SLE[46] and inflammatory bowel disease.[47] The frequencies of the TNFα (–308) genotypes in the Saudi population were similar to those found in the White American and African-American populations. The Saudi population showed a higher AA/AG genotype frequency and a lower GG genotype frequency compared to those found in the Italian, Greek, Brazilian and Omani populations.

Cytokine polymorphisms in IL-10 and TNFα have been associated with SLE,[48] which has a prevalence of 19.28 per 100,000 individuals among Saudis.[49] The present study showed that Saudis have a relatively low TNFα (–308) GG genotype frequency and a relatively high frequency of the IL-10 GCC GCC genotype. High-frequency TNFα genotypes have been associated with SLE independent of IL-10 alleles, whereas the risk of developing DLE was shown to be raised in the high IL-10/low TNFα producer group.[44]

Regarding response to treatment, TNFα antagonists are reported to be more effective in genetically low TNFα producers,[50] and in patients with combined high IL-10/low TNFα genotypes.[51] In contrast, those with combined low IL-10/high TNFα genotypes were found to respond better to antimalarial therapy.[52] The prevalence of the high IL-10/low TNFα genotype found among Saudis in the present study suggests the need for further investigation to evaluate the therapeutic response to these agents in the local population.

Interferon-γ is a Th1 cytokine that plays important roles in modulating almost all immune responses, including haematopoiesis, T-cell differentiation, antiproliferative, antitumour and antiviral activities.[53,54] The IFNγ (+874) genotypes in the Saudi population were similar to those in the White American, Italian and Greek populations.

Transforming growth factor-β1, which is a multifunctional peptide that controls proliferation, differentiation and other functions in many cell types, plays important roles in controlling the immune system, and has various effects on cells of different types at different developmental stages. The TGFβ1 (codon 10, codon 25) genotypes in the Saudi population showed similar distributions to those of the Italian population. However, the Saudi population showed a lower frequency for the TC GG genotype compared to White Americans, and Saudis did not harbour the TT CC genotype found in the African-American population.

In conclusion, this study confirms that there are differences in the distributions of some cytokine genotypes between Saudis and other populations, and that these differences may be clinically relevant. However, the study was limited by the relatively small number of participants, and the findings may not be applicable to the general Saudi population. Therefore, large-scale studies are recommended to validate current findings and evaluate the value of cytokine gene polymorphisms in assessing genetic predispositions to locally prevalent diseases. □

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