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Original article

Interleukin-6 G-174C gene polymorphism and susceptibility to upper respiratory tract infection among endurance athletes

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Abstract

The aim of this study was to investigate the influence of interleukin (IL)-6 gene polymorphisms on upper respiratory tract infection (URTI) incidence. To this end, 100 healthy elite male athletes participating in the study were classified as either healthy or prone to frequent URTI. Blood samples and DNA isolation, multiplex polymerase chain reaction, and Taqman real-time polymerase chain reaction were carried out. Genomic DNA was extracted from peripheral leukocytes of whole blood samples using the QIAmp DNA Blood Mini Kit according to the manufacturer's protocols. For comparison of the distribution of genotypes between the two groups and for estimating odds ratios for URTI susceptibility in relation to the IL-6 polymorphism, Pearson's χ^2 and logistic regression methods were used, respectively. The IL-6-174 genotype distribution differed between athletes with URTI and healthy athletes ($\chi^2 = 11.68$, $p = 0.003$). The IL-6 low-expression genotype (CC), relative to the other two genotypes combined (GC + GG), was associated with a tendency for an increased likelihood of frequent URTI (odds ratio: 3.33, 95% confidence interval: 1.40–7.92; $p = 0.006$). In conclusion, findings from this study have identified a potential role of genetic variation in influencing the risk for URTI in athletic populations and single nucleotide polymorphisms in the IL-6 genes were associated with an altered risk profile. These measures may have a predictive value in the identification of individuals who are more likely to experience recurrent infections when exposed to high physical stress in the areas of athletic endeavor.

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Keywords: Cytokine; Elite athletes; Gene expression; GG genotype; Single nucleotide polymorphisms

Introduction

There is incontrovertible evidence that regular physical activity contributes to the primary and secondary prevention of several chronic diseases and is associated with a reduced risk of premature death. There appears to be a graded linear relation between the volume of physical activity and health status, such that the most physically active people are at the lowest risk.¹

However, epidemiological evidence shows that high volume and/or high intensity of training, particularly in endurance

athletes, can be associated with an increased risk of developing respiratory tract syndromes, including upper respiratory tract infection (URTI).² The incidence of URTI in both highly trained and healthy untrained individuals is known to increase in response to increases in activity. This risk of illness in response to exercise has been modeled by the J Curve. This model suggests that individuals engaging in moderate physical activity are at lower risk of illness compared with sedentary individuals. Conversely, excessive volumes of strenuous endurance exercise may suppress immune function, thereby increasing the risk of illness.³

Specific differences in the expression of cytokine genes may account for differences in susceptibility to URTI (and other infectious diseases) by influencing the function of immune cells or the cytokine response to pathogens.^{4,5} In addition, pro-

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and anti-inflammatory cytokine responses to exercise can differ between healthy and illness-prone athletes, and may account in part for differences in the history of upper respiratory illness between individual athletes.⁶

Some investigations found that heavy physical activity produces a rapid, transient increase in cytokine production and entails increases in both proinflammatory [interleukin (IL)-2, IL-5, IL-6, IL-8, and tumor necrosis factor (TNF)- α] and anti-inflammatory (IL-1 receptor antagonist and IL-10) cytokines. They mediate communication within and between cells, organs, and organ systems throughout the body in immune, inflammatory, and several other responses.^{7,8} Upregulation of inflammatory cytokines, such as TNF- α , IL-1 β , IL-6, and IL-10, and a cytokine-mediated inflammatory response has also been documented as being responsible for the severity of respiratory tract infections.⁹

IL-6 is a typical pleiotropic cytokine that plays roles in the immune, endocrine, nervous, and hematopoietic systems, and on bone metabolism.¹⁰ It has the ability to stimulate B-cell differentiation, activate thymocytes and T cells for differentiation, activate macrophages, stimulate hepatocytes to produce acute-phase proteins, and activate natural killer cells. IL-6 also possesses anti-inflammatory properties. In T cells, IL-6 confers significant effects on proliferation, survival, and T helper 1/2 responses.¹⁰

Cytokines play a pivotal role in the regulation of the type and magnitude of the immune response, and the polymorphic nature of the cytokine genes may confer flexibility on the immune response.¹¹ All genes encoding cytokines involved in the modulation of inflammatory responses are candidate genes for determination of the human genetic background that is responsible for interindividual differences in susceptibility and outcome of sepsis.^{11,12} Examination of the regulation of cytokine expression at the genetic level may prove important in understanding the substantial interindividual differences in cytokine responses to exercise, as well as individual differences in susceptibility to URTI.⁶

The influence of cytokine gene polymorphisms on gene expression and disease has been addressed at two levels of research: studies using *in vitro* gene expression, and those involving *in vivo* disease associations.¹³ These studies attempt to identify immunogenetic markers for a given disease. Association is sought between specific cytokine gene polymorphisms and clinical outcome by direct comparison of individual cytokine genotypes and the clinical features of the disease (e.g., susceptibility, duration, and severity). The *a priori* involvement of dysregulation of a specific cytokine or receptor in the disease is usually, though not always, the rationale for selecting a cytokine or cytokine receptor gene for analysis. Using these and other clues, many studies have identified statistically significant associations between cytokine alleles and disease.¹³

Several investigations have examined the influence of cytokine gene single nucleotide polymorphisms (SNPs) on the incidence of infectious illness and patient prognosis. For example, Doyle et al¹⁴ reported that individuals with IL-6-174 C/C genotype had more days with URTI and higher symptom scores, compared with carriers of the G allele. In other studies, there was an association between IL-6-174 G/C SNP and a risk

of URTI.^{15,16} The IL-6 gene is located at chromosome 7p21. Common polymorphisms have been described and the most studied is the promoter polymorphism -174 G/C (rs1800795), which has an influence on the transcription of the IL-6 gene and plasma levels of IL-6.¹⁷ The C allele has been found to be associated with lower levels of plasma IL-6 in healthy individuals and lower expression after lipopolysaccharide or IL-1 stimulation in HeLa cells.¹⁷

In spite of the increasing studies affirming potential clinical implications of functional polymorphisms, investigators are not aware of any efforts to assess the role of genetic variation being responsible for differences in incidence of URTI in athletes, or individual differences in cytokine responses to exercise. The primary purpose of this study was to investigate comparison of the frequency of SNPs in IL-6 cytokine gene between healthy athletes and athletes prone to frequent URTI.

Methods

Participants

One hundred healthy elite male athletes who were engaged in regular sports training (predominantly endurance-based activities such as running, cycling, swimming, triathlon, and other sports) volunteered to participate in the study. Participants ranged from state active to national and Asian athletes. Participants were needed to complete a comprehensive health-screening questionnaire prior to starting the study and had not taken any medication in the 8 weeks prior to the study. All participants were wholly informed about the rationale for the study. Participants provided written consent to take part in the study, which had earlier received the approval of Tabriz Medical University Ethical Advisory Committee (Tabriz, Iran; No. 91134 date: November 13, 2012).

Participants could be included if they were currently healthy, had been involved in endurance training for at least 2 years, engaged in at least three sessions, and at least 3 hours of total moderate/high-intensity training time per week,⁴ and were between 18 years and 35 years of age. One hundred participants completed the study with baseline characteristics (mean \pm standard deviation) as follows: age 23.97 ± 5.93 years, body weight 71.85 ± 7.12 kg, body height 177.70 ± 7.80 cm, body mass index 22.77 ± 2.09 kg/m², and self-reported professional training experience 5.11 ± 3.52 years.

Study design

The study was an evaluation of the influence of IL-6 gene polymorphisms on URTI incidence. As a result of the difficulty in interpreting the biological influences of genetic differences in small populations, we used a genotype-specific clinical model and participants were selected based on exercise experience. They were requested to continue with their normal training programs during the 12-month subsequent study period and they completed a health (URTIs symptoms) questionnaire on a weekly basis. The illness symptoms listed on the questionnaire were: sore throat, catarrh in the throat,

runny nose, cough, repetitive sneezing, fever, persistent muscle soreness, joint aches and pains, weakness, headache, and loss of sleep. The non-numerical ratings of light, moderate, or severe symptoms were scored as 1, 2, or 3, respectively, to provide a quantitative means of data analysis and the total symptom score for every participant each week was calculated by multiplying the total number of days each symptom was experienced by the numerical ratings symptom severity. In any given week, a total symptom score ≥ 12 was taken to indicate that a URTI was present. This score was chosen because to achieve it, a participant would have to record at least three moderate symptoms lasting for 2 days or two moderate symptoms lasting for at least 3 days in a given week. A single URTI episode was defined as a period during which the weekly total symptom score was ≥ 12 and separated by at least 1 week from another week with a total symptom score ≥ 12 .⁴

Therefore, the participants were classified as either healthy or prone to frequent URTI (notated as illness-prone) based on information collected via the questionnaire. In keeping with the average annual incidence of upper respiratory illness previously reported in the general population (2.4 episodes per year),⁶ athletes reporting two or fewer episodes of URTI in the previous 12-month period were grouped as healthy ($n = 53$), and athletes reporting three or more episodes of URTI in the previous 12-month period were grouped as illness-prone ($n = 47$). Participants with lower respiratory tract symptoms (cough, wheeze, or chest pain) were excluded from participation. Characteristics of the two classification groups are shown in Table 1.

Genotyping analyses

Blood samples and DNA isolation, multiplex polymerase chain reaction (PCR), and conventional allele-specific PCR were

Table 1
Characteristics of healthy and illness-prone groups including mean (\pm standard deviation) number of self-reported episodes of URTI in the preceding 12-month period and the proportion of each group and athletes participating in different sports.^a

Characteristics	Groups	
	Healthy (≤ 2 URTI)	Illness-prone (≥ 3 URTI)
<i>n</i>	53 (53)	47 (47)
Age (y)	23.85 \pm 3.24	24.11 \pm 3.88
Body weight (kg)	72.92 \pm 6.91	70.64 \pm 6.73
Height (cm)	178.15 \pm 6.11	177.19 \pm 5.62
BMI (kg/m ²)	22.98 \pm 1.96	22.53 \pm 2.23
Professional training experience (y)	5.64 \pm 3.34	4.51 \pm 2.91
URTIs episodes/y	1.00 \pm 0.73	3.06 \pm 0.25
Duration of the symptoms of URTI in every episode (d)	3.45 \pm 2.15	5.38 \pm 0.90
Sports type		
Runners	11 (20.75%)	11 (23.40%)
Swimmers	17 (32.08%)	12 (25.53%)
Cyclists	1 (1.89%)	7 (14.89%)
Triathlon	13 (24.53%)	8 (17.02%)
Other endurance sports	11 (20.75%)	9 (19.16%)

Data are presented as *n* (%) or mean \pm SD.

URTIs = upper respiratory tract infection.

^a Number of athletes participating in different sports is shown.

performed. Genomic DNA was extracted from peripheral leukocytes of whole blood samples using the QIAmp DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocols.¹⁸ The polymorphism of the IL-6 promoter region at -174 was studied by PCR–restriction fragment length polymorphism. The region of interest was amplified by PCR using primers 5-TGACTTCAGCTTTACTTTGT-3 and 5-AATCTTAATAAGGTTTCCA-3. The reaction was carried out in a final volume of 25 μ L containing 1.5 mmol/L MgCl₂, 0.2 mmol/L each dNTP, 0.2 mmol/L each primer, and 1 U Taq polymerase (Cinagene, Tehran, Iran). DNA was amplified during 30 cycles with an initial denaturation of 10 minutes at 94°C and a final extension of 10 minutes at 72°C. The cycle program consisted of 1 minute denaturation at 94°C, 1 minute and 35 seconds annealing at 62°C, and 1 minute extension at 72°C.¹⁹ The PCR product was digested by adding 10 U *Nla*III restriction enzyme at 37°C overnight, the digested fragments were separated by agarose gel electrophoresis, and detected by ethidium bromide staining. The polymorphism was due to a replacement of G by C at position -174. The identified genotypes were named according to the presence or absence of the enzyme restriction sites, so *Nla*III (GG), (GC), and (CC) were homozygote for the presence of the site (153/40 bp), and heterozygote for the presence/absence of the site (193/153/40), and homozygote for the absence of the site (198 bp), respectively.

Statistical analysis

Data were expressed as percentages or mean \pm standard deviation. Prior to statistical analysis to eliminate the feasibility of any population bias, Hardy–Weinberg equilibrium of the allele distribution was tested. Pearson's χ^2 was used for comparison of the distribution of genotypes between the two groups. For each polymorphism, genotypes were classified as high, moderate, or low based on documented effects on cytokine gene expression. Logistic regression method was used to estimate odds ratios, expressed with their 95% confidence intervals for URTI susceptibility in relation to the IL-6 polymorphism. Statistical significance was set at $p \leq 0.05$. The statistical analyses were performed using SPSS version 19.0 (SPSS Inc., Chicago, IL, USA).

Results

Genotype distribution of the IL-6-174 polymorphism in URTI-prone and healthy athletes is shown in Table 2. Two groups were in Hardy–Weinberg equilibrium, with significant χ^2 values for the observed and expected genotype frequencies. The IL-6-174 genotype distribution differed between athletes with URTI and healthy athletes ($\chi^2 = 11.68$, $p = 0.003$). The IL-6 high-expression genotype was observed at a greater frequency in the healthy group compared with the illness-prone group (64.15% vs. 14.89%).

The predictive value of the polymorphism in assessing the classification as illness-prone athletes demonstrated that the IL-6 low-expression genotype (CC), relative to the other two genotypes combined (GC + GG), was associated with a

Table 2
Distribution of interleukin (IL)-6-174 C/G single nucleotide polymorphisms in athletes with upper respiratory tract infection and healthy athletes and results of χ^2 analysis.

Genotype		Groups		χ^2	p
		Healthy	Illness-prone		
Genotype	Level of gene expression	n = 53	n = 47		
CC	Low	3.77	31.92	11.68	0.003
GC	Moderate	32.08	53.19		
GG	High	64.15	14.89		

Data are presented as %.

tendency for an increased likelihood of frequent URTI (odds ratio: 3.33, 95% confidence interval: 1.40–7.92; $p = 0.006$).

Discussion

This study examined the role of an IL-6 promoter SNP at -174C/G in URTI susceptibility. Our findings suggest an association between common SNPs at IL-6 genes and URTI in well-trained athletes. Other studies have demonstrated that the expression of IL-6 is related to its allelic variant. It is known that the IL-6 gene has about 50 SNPs in its promoter region, like the variants -597 G/A, -572 G/C, -373 A/T, and -174 G/C, among others. The -174 G/C SNP in the promoter region of the IL-6 gene has gained considerable interest because it has been associated with a variety of disease states.²⁰ Several studies have questioned the functional role of the -174 G/C SNP in the production of IL-6 both *in vivo* and *in vitro*. The change of guanine bases to cytosine (G → C) at position -174 seems to affect the transcription of the IL-6 gene and therefore the plasma levels of this cytokine in young, elderly, and centenarian individuals.²¹ Functional SNPs in the proinflammatory cytokine genes including IL-6 (174G/C) and TNF- α (308G/A), that affect the production of anti-inflammatory cytokines, such as IL-10 have been associated with both susceptibility to outcomes of infectious diseases and sepsis.^{22–24}

We observed that IL-6-174 polymorphism CC was associated with susceptibility to URTI, and participants with the GG genotype had a lower incidence of URTI than those with the CC genotypes. The IL-6-174 GG homozygous carrier state has already been associated with diminished susceptibility to acute and chronic inflammatory diseases.²⁵ This finding is in agreement with a report that showed that the -174 GG genotype was associated with improved survival in sepsis.²⁶ Mahdavian et al²⁷ found that GG and GC genotypes of IL-6 at position -174 lead to high production of IL-6, whereas CC genotype leads to low production of this cytokine. Higher IL-6 levels were observed in the group of elderly women with the GG genotype, as also observed in a recent Brazilian study.²¹ Another small study reported that high IL-6 expression genotype was associated with reduced symptom severity in response to experimentally induced respiratory syncytial virus infection.²⁸ As reported in other studies, higher IL-6 concentrations were positively correlated in both phase and magnitude with the expressed viral symptom/sign complex leading to the expectations that the high production IL-6

(-174) phenotype would predict higher IL-6 concentrations and consequently greater illness magnitudes.¹⁴

In interpreting these results, IL-6 G/C promoter polymorphism has functional significance. The -174G/C polymorphism is contained in a sequence bearing partial nucleotide homology with the Sma- and Mad-related protein (Smad)4 binding element. Smad4 is a transcription factor that participates in the signal transduction cascade of transforming growth factor- β and activin to inhibit the expression of proinflammatory molecules. The C allele at the variant position in the consensus-binding element binds Smad4 more effectively, and hence represses IL-6 transcription, whereas substitution by a G allele at this position decreases the binding efficiency by 90% and therefore increases transcription of the IL-6 gene.²⁹

These results are in contrast to data presented by Jones et al.³⁰ who have reported that high plasma IL-6 concentrations in patients and healthy individuals were associated with the C allele and CC genotype rather than with the G allele and the GG or GC genotype. Another recent report showed an association of the IL-6 high expression genotype and an increased risk for frequent URTI.⁶ Some studies have also indicated that IL-6-174G/C polymorphism does not significantly affect plasma IL-6 concentrations.^{31,32} Contradictory reports about the role of IL-6 polymorphisms in URTI susceptibility may not be surprising. Those discrepancies might be explained either by the diverse ethnic background of the two studied populations or by the different mechanisms possibly involved in the development of URTI.

We found that the GG homozygous genotype at the -174 locus of the IL-6-174 gene was the dominant genotype in healthy athletes (64.15%). Also, these data suggest that the IL-6 CC genotype affects URTI susceptibility, whereas the GG genotype may have a protective effect. It may be possible to identify those athletes who have a high susceptibility to infections with future genetic testing. The initial strategy for athletes suffering fatigue should be to reduce or cease training for a short period of time to allow recovery. It is important during this reduction in training to ensure appropriate nutrition, and avoid energy, carbohydrate, or protein deficits. If fatigue persists or infections recur despite rest and nutrition then further medical investigation is warranted.²⁵

The findings from this study have identified a potential role of genetic variation in influencing the risk for URTI in athletic populations, and SNPs in the IL-6 genes were associated with an altered risk profile. These measures may have a predictive value in the identification of individuals who are more likely to experience recurrent infections when exposed to high physical stress in the areas of athletic and military endeavor.⁴ Hence, the cause of the increased incidence of infection in athletes is likely to be multifactorial: a variety of stressors (physical, psychological, environmental, or nutritional) can suppress immune function,³³ and these effects, together with increased exposure to pathogens, can make the athlete more susceptible to infection.

This possibility is further supported by evidence demonstrating that a combination anti-inflammatory/antibacterial throat spray used after a marathon was able to reduce the post-race incidence and severity of URTI in distance runners.⁶

Preventive infection-control measures should be taken to avoid infection. These include regular hand washing, minimal contact with sick persons, and the avoidance of sharing personal items. Adequate rest and proper nutrition are vital to a healthy immune system.^{34,35}

In conclusion, in this study we demonstrated the functional significance of -174G/C of IL-6 in association of the IL-6 (-174) CC genotype with URTI susceptibility. Uncovering and understanding the genetic determination of the susceptibility to infection offers the chance of developing valuable diagnostic tools and new therapeutic approaches in URTI.

Conflicts of interest

All authors declare no conflicts of interest.

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