Cytokine gene polymorphisms and risk for Upper Respiratory Symptoms in highly-trained athletes

Amanda J Cox[1, 2], Maree Gleeson[1], David B Pyne[3], Robin Callister[1], Peter A Fricker[3], Rodney J Scott[1, 2]

[1] School of Biomedical Sciences and Pharmacy, Faculty of Health, University of Newcastle, Callaghan, NSW, AUSTRALIA
[2] Information-based Medicine Research Program, Hunter Medical Research Institute, Newcastle, NSW, AUSTRALIA
[3] Australian Institute of Sport, Canberra, ACT, AUSTRALIA

ABSTRACT

Physiological and immunological factors contributing to risk for upper respiratory symptoms (URS) in athletic populations remain under investigation. Single nucleotide changes (polymorphisms) in cytokine genes and alterations in associated gene expression may influence risk for URS in some athletes. The aim of this study was to compare the frequency of cytokine gene polymorphisms in athletes with or without a history of frequent URS.

Cytokine gene polymorphisms were determined in samples from five previous investigations of immune function in highly-trained athletes (n=170). Participants were classified into two groups based on their self-reported number of episodes of URS in the preceding 12 months. Athletes were classified as healthy (n=82) if they reported ≤2 episodes of URS in the preceding 12 months. Athletes were classified as illness-prone (n=88) if reporting ≥3 episodes of URS. Polymorphisms in Interleukin(IL)-6, IL-8, IL-10, IL-1 receptor antagonist (IL-1ra), IL-2, IL-4 and Interferon(IFN)-γ were determined using real-time polymerase chain reaction allelic discrimination assays. The distribution of genotype frequencies between the two groups was compared using a Chi-square test and logistic regression was used to model risk for URS as a function of cytokine gene polymorphisms.

There was a tendency for IL-6 (χ^2=5.0, p=0.08) and IL-4 (χ^2=4.8, p=0.09) genotype frequencies to differ between the groups. The IL-6 high-expression genotype was associated with an increased likelihood of ≥3 URS episodes in a 12 month period (odds ratio (OR): 2.87, 95% confidence interval (CI): 1.10-7.53; p=0.03). The IL-2 high-expression genotype was associated with a tendency for a decreased likelihood of ≥3 URS episodes in a 12 month period (OR: 0.361, 95% CI: 0.124-1.06; p=0.06).

Correspondence:
Dr Amanda Cox, School of Biomedical Sciences and Pharmacy
University of Newcastle, Callaghan NSW 2308, AUSTRALIA
amanda.cox@uon.edu.au, Ph: +61 2 4985 5893, Fax: +61 2 4985 5895

These data suggest cytokine gene polymorphisms may account in part for differences in risk for URS in highly-trained athletes.

Keywords: URS, cytokines, genetics, polymorphism, athletes

INTRODUCTION

The risk for upper respiratory symptoms (URS) and potential disruptions in training and competition performance is an ongoing concern for athletes and coaches. Post-exercise disturbance in immune and inflammatory markers has been well characterised and is accepted as contributing to the increased risk for respiratory symptoms experienced by some athletes (18, 20, 22). However, not all episodes of URS in athletes have an infective cause and in some instances an inflammatory aetiology should be considered (24, 30, 31). In addition, pro- 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 (9). Examination of the regulation of cytokine expression at the genetic level may prove important in understanding the substantial inter-individual differences in cytokine responses to exercise, as well as individual differences in susceptibility to URS.

Single nucleotide polymorphisms (SNPs) are single base changes that occur in both the coding and non-coding regions of the human genome and are one form of genetic variation. The majority of SNPs occur in non-coding regions of the genome and have no direct consequences at the phenotypic level (34). In contrast, the presence of SNPs in regulatory sequences adjacent to coding regions can have significant implications on rates of gene transcription and subsequent protein production (4). An increasing number of SNPs are being identified in the regulatory

Table 1: Selected single nucleotide polymorphisms (SNPs) in key pro-inflammatory, anti-inflammatory and immune regulatory cytokine genes. Polymorphisms have been named here to indicate the DNA bases involved in the substitution and the position of the polymorphism relative to the transcription start site. For example, the G-174C polymorphism in IL-6 indicates the substitution of a cytosine (C) residue for a guanine (G) at a position 174 bases upstream of the transcription start site. The Reference SNP accession number (rs #) and the impact of the substitution on cytokine production is also indicated.

Cytokine	Polymorphism	rs #	Impact on Cytokine Production	Reference
IL-6	G-174C	1800795	decreased IL-6 expression	(3, 13)
IL-8	A-251T	4073	increased IL-8 expression	(17)
IL-10	G-1082A	1800896	decreased IL-10 expression	(28, 32)
IL-10	C-592A	1800872	decreased IL-10 expression	(6, 19)
IL-1ra	T+2018C	419598	decreased IL-1ra expression	(10, 27)
IL-2	T-330G	2069762	increased IL-2 production	(16)
IL-4	C-589T	2243250	increased IL-4 production	(25)
IFN-γ	T+874A	2430561	decreased IFN-γ production	(23, 26)

regions of genes encoding cytokines and cytokine receptors (4). Further, polymorphisms in a range of key pro-inflammatory, anti-inflammatory and immune-regulatory cytokines have been associated with changes in cytokine production (Table 1), confirming the potential for genetic regulation of cytokine expression.

The potential for functional SNPs in cytokine genes to influence clinical outcomes has been recognised previously, however, the establishment of clear and consistent associations with disease risk and prognosis remains an ongoing task. A number of investigations have examined the influence of cytokine gene SNPs on the incidence of infectious illness and patient prognosis. For example, a polymorphism upstream of the transcription start site in the IL-6 gene (G-174C), which causes increased IL-6 expression, has been associated with increased survival in sepsis (29). Other studies have observed an association between a polymorphism in the IL-10 gene (G-1082A), which causes increased IL-10 expression, and poorer prognosis in sepsis, suggesting the possibility of IL-10 mediated suppression of inflammation and impaired bacterial clearance as potential underlying mechanisms (19, 32). The role of cytokine SNPs in less severe infections has also been recently investigated. One study examining the association between cytokine gene polymorphisms and the acute sickness response to viral infection found polymorphisms in the IL-10 (C-592A) and IFN-γ (T+874A) genes to be associated with the duration and severity of illness symptoms (35). Increased illness severity was associated with both the high expression IFN-γ and the low expression IL-10 genotype. These findings further support a potential role of genetic variation in influencing individual risk for, and response to, common infectious illnesses.

Despite the growing evidence supporting potential clinical implications of functional polymorphisms, we are unaware of any attempts to examine the role of genetic variation in accounting for differences in rates of URS in athletic populations, or individual differences in cytokine responses to exercise. The aim of this study was to compare the frequency of SNPs in a series of cytokine genes between healthy high-performance athletes and athletes prone to frequent upper respiratory symptoms.

METHODS

Subjects and Study Design

This project involved the laboratory analysis of stored biological specimens (blood and saliva) collected from 170 high-performance athletes (n=129 males and 41 females; age 25.4 ± 8.6 years (mean± SD)). Specimens were collected during five previous research projects investigating various aspects of immune function in high-performance athletes undertaken at both the Australian Institute of Sport (Canberra, Australia) and the University of Newcastle (Newcastle, Australia). Details of each study design and the number of biological specimens included in this investigation are listed in Table 2. The current study was undertaken with approval from the Ethics Committees of the Australian Institute of Sport and the University of Newcastle.

Table 2
Details of previous studies from which material and participant information were accessed for the current investigation. Numbers of subjects, allocations to healthy (H) and illness-prone (IP) groups, age, distribution of gender, maximal oxygen consumption (VO_{2max}), training load ($h.wk^{-1}$) at the time of recruitment to the initial investigation and number of episodes of upper respiratory symptoms (URS) reported for the previous 12 months are shown. Data are mean ± standard deviation unless otherwise indicated. URS = upper respiratory symptoms.

Study Design	**Subjects (n)**	**H : IP (n)**	**Age (years)**	**Gender %M : F**	**VO_{2max} ($ml.kg.min^{-1}$)**	**Training ($h.wk^{-1}$)**	**URS/yr**	**Reference**
Characterisation of cytokine responses to exercise of various intensities and durations in healthy and illness-prone runners	18	10 : 8	24.0 ± 6.7	100 : 0 %	64.2 ± 7.6	8.8 ± 4.9	2.9 ± 2.2	(9)
Clinical assessment and investigation of underlying causes of URS in a cohort of elite athletes presenting for treatment of current respiratory illness	64	19 : 45	19.6 ± 2.6	72 : 28 %	no data available	22.4 ± 5.8	3.6 ± 1.7	(7)
Randomised controlled trial examining effects of anti-inflammatory throat spray on incidence of URS in trained distance runners prior to and following completion of a half-marathon event	44	31 : 13	35.3 ± 8.0	80 : 20%	no data available	10.2 ± 4.6	1.5 ± 1.3	(8)
Randomised controlled trials examining effects of probiotic supplementation on immune and inflammatory responses to exercise in training athletes	31	16 : 15	26.0 ± 6.5	68 : 32 %	51.3 ± 9.2	13.8 ± 6.6	2.7 ± 2.2	(5)
Clinical assessment of high performance athletes presenting with persistent fatigue and decrements in performance	13	6 : 7	21.8 ± 6.9	69: 31 %	47.0 ± 9.2	no data available	6.9 ± 7.9	(24)

Classification of Participants

Athletes were classified as either healthy or prone to frequent URS (notated as illness-prone) based on information collected via questionnaire and interview prior to their participation in the respective original investigations. Classification was based on the self-reported incidence of URS in the 12 months preceding their participation in the respective study. In keeping with the average annual incidence of upper respiratory illness previously reported in the general Australian population (2.4 episodes per year) (11), athletes reporting two or fewer episodes of URS in the previous 12 month period were grouped as healthy (n=82 from the five studies); athletes reporting three or more episodes of URS in the previous 12 month period were grouped as illness-prone (n=88 from the five studies). Characteristics of the two classification groups are shown in Table 3.

Table 3
Characteristics of healthy and illness-prone groups including mean (± standard deviation) number of self-reported episodes of upper respiratory symptoms (URS) in the preceding 12 month period and the proportion of each group with a self-reported history of asthma and/or allergy. Numbers of athletes participating in different sports is also shown.

	Healthy (≤2 URS)	**Illness-prone (≥3 URS)**
n	82	88
Age (years)	27.7 ± 9.5	23.3 ± 7.0
URS episodes/year	1.3 ± 0.8	4.2 ± 1.6
%Male : Female	76 : 24 %	75 : 25 %
% Asthma and/or allergy*	44 %	42 %
Sports		
Distance running	37 (45%)	16 (18%)
Triathlon	10 (12%)	10 (11%)
Swimming	4 (5%)	16 (18%)
Cycling	2 (2%)	7 (8%)
Rowing	1 (1%)	5 (6%)
Athletics (sprints/field)	10 (12%)	2 (2%)
Team sports†	12 (15%)	25 (28%)
Other‡	6 (7%)	7 (8%)

* data on the self-reported history of asthma and/or allergy was available from four of the five studies only (data was not available for the probiotic supplementation study). For the healthy group, this data was available for 66 athletes, and for the illness-prone group, for 73 athletes.
† team sports included basketball, netball, volleyball, soccer and rugby league
‡ other sports included waterpolo, gymnastics, boxing and archery

Identification of Cytokine Gene Polymorphisms

Nucleic acids were extracted from stored saliva samples using a commercially available QIAamp DNA Mini Kit (QIAGEN GmbH, Hilden, Germany). Nucleic acids were extracted from stored whole blood samples using an in-house standard salt extraction technique. Briefly, this process involved the combination of each sample with 40 mL of a prepared red cell lysis solution and centrifugation at 2500 rpm for 10 minutes. The supernatant was discarded and this process repeated twice before cell pellets were resuspended with 2 mL of a prepared nuclei lysis solution and 100 uL of Proteinase K (20 $mg.mL^{-1}$) (Promega Corporation, Madison, Wisconsin) and 100 uL of 20% SDS immediately added. Samples were mixed and then incubated for 4 hrs in a 55°C water bath with gentle shaking. The digested cell pellets were transferred to microcentrifuge tubes and 333 uL of a prepared 6M saturated salt solution added for each millilitre

transferred. Samples were then vortexed for 30 sec before centrifugation at 15000 rpm and 4°C for 30 min. Following centrifugation, the supernatant was added to 5 mL ice cold absolute ethanol (Ajax Finechem Pty Ltd, Taren Point, Australia) and tubes mixed by inversion. The precipitation/spooling of DNA was visualised where possible. Samples were centrifuged at 2500 rpm for 10 min, the supernatant discarded and the DNA pellet thoroughly resuspended in 1 mL of 70% ethanol before being transferred to a microcentrifuge tube. Samples were re-centrifuged at 13000 rpm before the supernatant was again discarded and the pellet resuspended in 70% ethanol. This process was repeated a second time with the supernatant discarded and the DNA pellet allowed to air dry. A volume of 200 uL of prepared Tris-EDTA (TE) buffer was added to each sample and reconstitution of DNA allowed to take place overnight at 55 °C.

Following extraction, DNA concentrations were determined using an Implen Nanophotometer (Implen GmbH, Munich, Germany). Extracted DNA was diluted in TE buffer to a concentration of 10 $ng.uL^{-1}$ for subsequent use. Commercially available real-time (RT) polymerase chain reaction (PCR) allelic discrimination assays (PE Applied Biosystems, Foster City, USA) were used for identification of the cytokine gene polymorphisms IL-6 G-174C (rs# 1800795), IL-8 A-251T (rs# 4073), IL-10 G-1082A (rs# 1800896), IL-10 C-592A (rs# 1800872), IL-1ra T+2018C (rs# 419598), IL-2 T-330G (rs# 2069762), IL-4 C-589T (rs# 2243250), IFN-γ T+874A (rs# 2430561).

Assays were completed in accordance with manufacturer's guidelines using a 7500 Real Time PCR System (PE Applied Biosystems, Foster City, USA). Automatic classification of samples as homozygous (for either allele) or heterozygous was undertaken using the SDS 7500 System Software Version 1.4 (PE Applied Biosystems).

Statistical Analysis

Allelic frequencies were confirmed to be in Hardy-Weinberg equilibrium prior to statistical analysis to eliminate the possibility of any population bias. For each polymorphism, genotypes were classified as high, moderate or low based on documented effects on cytokine gene expression (see Table 1). The distribution of genotypes between the two groups was compared using a Pearson's Chi-squared test. Logistic regression was used to model the relative risk for URS as a function of cytokine gene polymorphisms. Odds ratios (OR) were calculated to reflect the predictive value of the high-expression genotype (relative to the moderate- and low-expression genotypes combined) for frequent URS (i.e. classification as illness-prone) and 95% confidence intervals (95% CI) determined to reflect the precision of estimation. Statistical analysis was completed using the SPSS Statistics (release 17.0) analysis package (SPSS Inc, Chicago, USA). Statistical significance was accepted at $p<0.05$.

Table 4
Distribution of genotypes in healthy and illness-prone groups for each of the eight cytokine SNPs examined. The unique identifier (rs #) for each polymorphisms is listed and results from chi-squared (χ^2) analysis.

	Level of Gene Expression	Healthy (n=82)	Illness-prone (n=88)	χ^2	p-value
IL-6 G-174C (rs# 1800795)					
GG	High	9 %	20 %		
GC	Moderate	50 %	41 %	4.96	0.08
CC	Low	41 %	39 %		
IL-8 A-251T (rs# 4073)					
TT	High	24 %	29 %		
AT	Moderate	59 %	52 %	0.68	0.71
AA	Low	17 %	19 %		
IL-10 G-1082A (rs# 1800896)					
GG	High	27 %	30 %		
GA	Moderate	52 %	50 %	0.16	0.92
AA	Low	21 %	20 %		
IL-10 C-592A (rs# 1800872)					
CC	High	61 %	63 %		
CA	Moderate	33 %	34 %	0.69	0.71
AA	Low	6 %	3 %		
IL-1ra T+2018C (rs# 419598)					
TT	High	50 %	50 %		
TC	Moderate	43 %	41 %	0.19	0.91
CC	Low	7 %	9 %		
IL-2 T-330G (rs# 2069762)					
GG	High	14 %	7 %		
GA	Moderate	40 %	47 %	2.24	0.33
TT	Low	46 %	46 %		
IL-4 C-589T (rs# 2243250)					
TT	High	5%	1%		
CT	Moderate	30 %	20 %	4.83	0.09
CC	Low	65 %	78 %		
IFN-γ T+874A (rs# 2430561)					
TT	High	23 %	19 %		
TA	Moderate	45 %	55 %	1.51	0.47
AA	Low	32 %	26 %		

RESULTS

Distribution of Genotype Frequencies

For each of the polymorphisms examined, genotype frequencies for the healthy and illness-prone groups are shown in Table 4. The distribution of the IL-8, IL-10, IL-1ra, IL-2 and IFN-γ genotypes were not significantly different between the two groups. In contrast, there was a tendency for the IL-6 genotype frequencies to differ between the two groups (χ^2=5.0, p=0.08); the IL-6 high-expression genotype was present in the illness-prone group at more than double the frequency observed for the healthy group (20% v 9%). A trend for the IL-4 genotype frequencies to differ between the two groups was also evident (χ^2=4.8, p=0.09); the IL-4 low-expression genotype was observed at a greater frequency in the illness-prone group compared with the healthy group (78% v 65%).

Association between Cytokine Polymorphisms and Risk for URS

The predictive value of each polymorphism in assessing the risk for frequent URS (i.e. classification as illness-prone) is shown in Table 5. The IL-2 high-expression genotype (CC), relative to the other two genotypes combined (CA + AA), was associated with a tendency for a decreased likelihood of frequent URS (OR: 0.36, 95% CI: 0.12-1.06; p=0.06). In contrast, the IL-6 high-expression genotype (GG), relative to the other two genotypes combined (GC + CC), was associated with an increased likelihood of ≥3 URS episodes in a 12 month period (OR: 2.87, 95%

Table 5
The relative risk for frequent upper respiratory symptoms (i.e. classification as illness-prone) associated with the high-expression genotype for each of the selected single nucleotide polymorphisms (SNP). The 95% confidence intervals (CI) and p-values associated with each odds ratio (OR) are also shown.

Gene	Genotype (high-expression)	OR	95% CI	p-value
IL-6	GG	2.874	1.096 – 7.533	**0.03**
IL-8	TT	1.193	0.587 – 2.426	0.63
IL-10	GG	1.117	0.558 – 2.235	0.75
IL-10	CC	0.972	0.509 – 1.853	0.93
IL-1ra	TT	0.841	0.446 – 1.586	0.59
IL-2	GG	0.361	0.124 – 1.055	0.06
IL-4	TT	0.227	0.023 – 2.209	0.20
IFN-γ	TT	0.918	0.426 – 1.981	0.83

CI: 1.10-7.53; p=0.03). The distribution of the IL-6 genotypes associated with varying numbers of episodes of upper respiratory symptoms is included in Table 6.

Table 6
Distribution of genotypes for the IL-6 C-174G polymorphism (rs# 1800795) associated with numbers of episodes of self-reported upper respiratory symptoms over a 12 month period

		IL-6 G-174C Genotypes (level of expression)		
# reported URS episodes	**n**	**CC (low)**	**CG (moderate)**	**GG (high)**
0	19	37%	58%	5%
1	27	26%	59%	15%
2	36	56%	39%	6%
3	34	41%	38%	21%
4	24	42%	38%	21%
5	11	36%	55%	9%
6	7	43%	29%	29%
≥7	12	25%	50%	25%

DISCUSSION

The findings from this study support an association between the IL-6 high-expression genotype and increased risk for frequent upper respiratory symptoms in this cohort of highly trained athletes. A tendency for a reduced risk of frequent URS in association with the IL-2 high-expression genotype was also observed. Collectively these data support the possibility that the presence of functional SNPs in the genes encoding key inflammatory and immune-regulatory cytokines may account in part for individual differences in risk for URS in highly-trained athletes.

Based on the established roles of IL-6 in the inflammatory response, the association of an IL-6 high-expression genotype and an increased risk for frequent URS (i.e. membership in the illness-prone group) supports rationale for a potential inflammatory basis of some episodes of URS in athletic populations. However, this observation could be seen to contradict findings from clinical settings involving more severe infections where the IL-6 high expression genotype has been associated with improved prognosis (29). Further, another small study reported the high IL-6 expression genotype to be associated with reduced symptom severity in response to experimentally induced respiratory syncytial virus infection (15). The authors of this previous study acknowledged that the findings were in

conflict with the study hypothesis that the low-expression genotype would be associated with reduced symptom severity as a result of a less potent inflammatory response to infection. It is possible that in the absence of infection, a potent IL-6 driven inflammatory response in the upper airways (influenced by IL-6 high-expression genotype) may actually favour the development of non-infectious URS. This sequence of events could account for the association of the IL-6 high-expression genotype with an increased risk for URS observed in this cohort, particularly as our previous research has suggested that up to 40% of episodes of URS in an athletic population do not result from an infectious etiology (7). However, it should also be acknowledged that differences in training volume may act as an additional factor confounding risk for URS in athletic populations and that it was not possible to control for this factor in the current investigation due to the retrospective nature of the demographic data available to include in the analysis.

Findings from the current study also support a tendency for the IL-2 high-expression genotype to be associated with a decreased likelihood of frequent URS. The IL-2 polymorphism has been less frequently studied in association with infectious illness in humans, with little information available in the literature to either support or refute our observation. Considering the roles of IL-2 in inducing T cell activation and clonal expansion, enhancing cytolytic activity and stimulating further cytokine production (14), all important events in response to viral infection, the association between the IL-2 high-expression genotype and a reduced risk for URS is biologically plausible. The implication that IL-2 is protective against URS in athletic populations lends further support to the theory that a shift towards Th2-type immune responses in response to chronic exercise loads may increase an individual's risk for upper respiratory symptoms (12, 33).

A potential role of the polymorphism in the IL-4 gene in contributing to risk for upper respiratory symptoms could not be identified in the current investigation. Although the higher IL-4 expression genotype was noted at a greater frequency in the healthy group, the low absolute numbers of each genotype, particularly the high-expression genotype, meant that calculations of relative risk were not definitive. An association between the IL-4 high-expression genotype and predisposition for asthma (21) and allergy (1) has been reported previously, however it remains unclear if this association is based on the actions of IL-4 in promoting IgE isotype switching (2) or other biological actions of IL-4. In this cohort, the higher IL-4 expression genotype was not observed at a greater frequency in the illness-prone group, nor was the self-reported history of asthma and/or allergy significantly greater suggesting that asthma and allergy may only account for a relatively small proportion of self-reported episodes of URS in this cohort of highly-trained athletes. That is not to discount a potential association between the IL-4 high expression genotype and risk for asthma and/or allergy. However, given the incomplete nature of the data pertaining to the self-reported history of asthma/and or allergy, coupled with the low absolute frequency of the IL-4 high-expression genotype, this association could not be confirmed in the current investigation.

Overall, the genotype frequencies for the polymorphisms examined in IL-8, IL-10, IL-1ra and IFN-γ did not suggest differences between the two groups. Further,

the risk ratios did not implicate these polymorphisms in altered risk for URS in athletic populations. However, the inability to identify a substantial positive or negative association between the IL-4 polymorphism and risk for URS highlights the necessity for large sample sizes in these kinds of studies.

The work reported here is an initial exploration of the potential impacts of genetic variation on risk for URS in an athletic population. Additional studies utilizing larger sample sizes are clearly required to confirm these findings and to identify any additional relationships between cytokine gene polymorphisms and risk for upper respiratory symptoms in high-performance athletes. The influence of cytokine gene polymorphisms on the regulation of inflammatory disturbance post-exercise also warrants investigation. However, completion of further studies in highly-trained athletes may prove difficult in terms of accessing the large numbers of athletes required with specific characteristics. The need to standardise the collection of clinical and participant information, including training status, current physical fitness and history of asthma and allergy, becomes an important consideration in all future investigations if data from multiple studies is to be pooled, as was the case here. The potential for rates of respiratory illness to vary year to year within a single individual should also be recognised when deciding how to classify athletes according to phenotype.

In conclusion, findings from this study have identified a potential role of genetic variation in influencing the risk for URS in athletic populations. Specifically, SNPs in the IL-6 and IL-2 genes were associated with an altered risk profile. Further work is required to confirm these relationships and determine whether this form of genetic variation can also account for the substantial between-individual differences observed in cytokine responses to exercise.

ACKNOWLEDGEMENTS

The contribution of the participants in the original investigations is gratefully appreciated. This study was funded by a research grant from the Australian Institute of Sport.

REFERENCES

1. Adjers K, Karjalainen J, Pessi T, Eklund C and Hurme M. epistatis effect of TLR4 and IL4 genes on the risk of asthma in females. Int Arch Allergy Immunol 138: 251-256, 2005.
2. Basehore MJ, Howard TD, Lange LA, Moore WC, Hawkins GA, Marshik PL, Harkins MS, Meyers DA and Bleecker ER. A comprehensive evaluation of IL4 variants in ethnically diverse population: Association of total serum IgE levels and asthma in white subjects. J Allergy Clin Immunol 114: 80-87, 2004.

3. Bennermo M, Held C, Stemme S, Ericsson C, Silveira A, Green F and Tornvall P. Genetic predisposition of the interleukin-6 response to inflammation: Implications for a variety of major diseases? Clin Chem 50: 2136-2140, 2004.
4. Bidwell J, Keen L, Gallagher G, Kimberly R, Huizinga T, McDermott MF, Oksenberg J, McNicholl J, Pociot F, Hardt C and DAlfonso S. Cytokine gene polymorphism in human disease: On-line databases. Genes Immunity 1: 3-19, 1999.
5. Clancy RL, Gleeson M, Cox A, Callister R, Dorrington M, D'Este C, Pang G, Pyne D, Fricker P and Hendrickson A. Reversal in fatigued athletes of a deficit in interferon secretion after administration of Lactobacillus Acidophilus. Br J Sports Med 40: 351-354, 2006.
6. Claudino M, Trombone AP, Cardoso CR, Ferreira SB, Martins W, Assis GF, Santos CF, Trevilatto PG, Campanelli AP, Silva JS and Garlet GP. The broad effects of the functional IL-10 promoter 592 polymorphism: modulation of IL-10, TIMP-3 and OPG expression and their association with peridontal disease outcome. J Leukoc Biol 84: 1565-1573, 2008.
7. Cox AJ, Gleeson M, Pyne DB, Callister R, Hopkins WG and Fricker PA. Clinical and laboratory evaluation of upper respiratory symptoms in elite athletes. Clin J Sport Med 18: 438-445, 2008.
8. Cox AJ, Gleeson M, Pyne DB, Saunders PU, Callister R and Fricker PA. Respiratory symptoms and inflammatory responses to Difflam throat-spray intervention in half-marathon runners: a randomised controlled trial. Br J Sports Med in press, 2009.
9. Cox AJ, Pyne DB, Saunders PU, Callister R and Gleeson M. Cytokine responses to treadmill running in healthy and illness-prone athletes. Med Sci Sports Exerc 39: 1918-1926, 2007.
10. Dahmer MK, Randolph A, Vitali S and Quasney MW. Genetic polymorphisms in sepsis. Pediatr Crit Care Med 6: S61-S73, 2005.
11. Douglas RM and Muirhead TC. A Study of the social, psychological and environmental factors associated with respiratory infections amongst Australians. University of Adelaide, Department of Community Medicine, Adelaide, Australia p 1-49, 1978.
12. Elenkov IJ and Chrousos GP. Stress hormones, Th1/Th2 patterns, pro/anti-inflammatory cytokines and susceptibility to disease. 10: 359-368, 1991.
13. Fishman D, Faulds G, Jeffery R, Mohamed-Ali V, Yudkin JS, Humphries S and Woo P. The effect of novel polymorphisms in the Interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. J Clin Invest 102: 1369-1376, 1998.
14. Gaffen SL and Liu KD. Overview of interleukin-2 function, production and clinical applications. Cytokine 28: 109-123, 2004.
15. Gentile DA, Doyle WJ, Zeevi A, Piltcher O and Skoner DP. Cytokine gene polymorphisms moderate responses to respiratory syncytial virus in adults. Hum Immunol 64: 93-98, 2003.
16. Hoffmann SC, Stanley EM, Cox ED, Craighead N, DiMercurio BS, Koziol DE, Harlan DM, Kirk AD and Blair PJ. Association of cytokine polymorphic inheritance and in vitro cytokine production in anti-CD3/CD28-stimulated peripheral blood lymphocytes. Transplantation 72: 1444-1450, 2001.
17. Hull J, Ackerman H and Isles K. Association of respiratory syncytial virus bronchiolitis with the IL-8 gene region in UK families. Thorax 55: 1023-1027, 2000.

18. Konig D, Grathwohl D, Weinstock C, Northoff H and Berg A. Upper respiratory tract infection in athletes: Influence of lifestyle, type of sport, training effort and immunostimulant intake. Exerc Immunol Rev 6: 102-120, 2000.
19. Lowe PR, Galley HF, Abdel-Fattah A and Webster NR. Influence of interleukin-10 polymorphisms on interleukin-10 expression and survival in critically ill patients. Crit Care Med 31: 34-38, 2003.
20. Mackinnon LT. Immunity in Athletes. Int J Sports Med 18: S62-S68, 1997.
21. Noguchi E, Shibasaki M, Arinami T, Takeda K, Yokouchi Y, Kawashima T, Yanagi H, Matsui A and Hamaguchi H. Association of asthma and the interleukin-4 promoter gene in Japanese. Clin Exp Allergy 28: 449-453, 1998.
22. Peters-Futre EM. Vitamin C, Neutrophil function and upper respiratory tract infection risk in distance runners: The Missing Link. Exerc Immunol Rev 3: 32-52, 1996.
23. Pravica V, Perrey C, Stevens A, Lee J and Hutchinson IV. A single nucleotide polymorphism in the first intron of the human IFN-γ gene: absolute correlation with a polymorphic CA microsatellite marker of high IFN-γ production. Hum Immunol 61: 863-866, 2000.
24. Reid VL, Gleeson M, Williams N and Clancy RL. Clinical investigation of athletes with persistent fatigue and/or recurrent infection. Br J Sports Med 38: 42-45, 2004.
25. Rosenwasser LJ and Borish L. Genetics of atopy and asthma: The rationale behind promoter-based candidate gene studies (IL-4 and IL-10). Am J Respir Crit Care Med 156: S152-S155, 1997.
26. Sallakci N, Coskun M, Berber Z, Gurkan F, Kocamaz H, Uysal G, Bhuju S, Yavuer U, Singh M and Yegin O. Interferon-γ gene +874T-A polymorphism is associated with tuberculosis and gamma interferon response. Tuberculosis 87: 225-230, 2007.
27. Santtila S, Savinainen K and Hurme M. Presence of the IL-1RA allele 2 (IL1RN*2) is associated with enhanced IL-1B production in vitro. Scand J Immunol 47: 195-198, 1998.
28. Schaaf BM, Boehmke F, Esnaashari J, Seitzer U, Kothe H, Maass M, Zabel P and Dalhoff K. Pnemococcal septic shock is associated with the interleukin-10 -1082 gene promoter polymorphism. Am J Respir Crit Care Med 168: 476-480, 2003.
29. Schluter B, Raufhake C, Erren M, Schotte H, Kipp F, Rust S, Aken Hv, Assmann G and Berendes E. Effect of interluekin-6 promoter polymorphism (-174 G/C) on the incidence and outcome of sepsis. Crit Care Med 30: 32-37, 2002.
30. Schwellnus M, Kiessig M, Derman W and Noakes T. Fusafungine reduces symptoms of upper respiratory tract infections (URTI) in runners after a 56 km race. Med Sci Sports Exerc: S296, 1997.
31. Spence L, Brown WJ, Pyne DB, Nissen MD, Sloots TP, McCormack JG, Locke AS and Fricker PA. Incidence, etiology, and symptomatology of upper respiratory illness in elite athletes. Med Sci Sports Exerc 39: 577-586, 2007.
32. Stanilova SA, Miteva LD, Karakolev ZT and Stefanov CS. Interleukin-10-1082 promoter polymorphism in association with cytokine production and sepsis susceptibility. Intensive Care Med 32: 260-266, 2006.
33. Suzuki K, Nakaji S, Kurakake S, Totsuka M, Sato K, Kuriyama T, Fujimoto H, Shibusawa K, Machida K and Sugawara K. Exhaustive exercise and type-1/type-2 cytokine balance with special focus on Interleukin-12 p40/p70. Exerc Immunol Rev 9: 48-58, 2003.
34. Syvanen A. Accessing genetic variation: Genotyping single nucleotide polymorphisms. Nature Rev 2: 930-942, 2001.

35. Vollmer-Conna U, Piraino BF, Cameron B, Davenport T, Hickie I, Wakefield D and Lloyd AR. Cytokine polymorphisms have a synergistic effect on severity of the acute sickness response to infection. Clin Infect Dis 47: 1818-1425, 2008.