RESEARCH ARTICLE

Interleukin 10 rs1800872 T>G Polymorphism was Associated with an Increased Risk of Esophageal Cancer in a Chinese Population

Jia-Ming Sun[1&], Qiong Li[1&], Hai-Yong Gu[2], Yi-Jang Chen[3], Ji-Shu Wei[4], Quan Zhu[3]*, Liang Chen[3]*

Abstract

Aim: Esophageal cancer is the eighth most common cancer and sixth leading cause of cancer associated death worldwide. The 5 year survival rate for esophageal cancer patients is very poor and accounts for only 12.3%. Besides environmental risk factors, genetic factors might play an important role in the esophageal cancer carcinogenesis. Methods: We conducted a hospital based case–control study to evaluate the genetic effects of functional single nucleotide polymorphisms (SNPs): *interleukin* 9 (IL9) rs31563 C>T, IL9 rs31564 G>T, *IL10* rs1800872 T>G, IL12A rs2243115 T>G, IL12B rs3212227 T>G and *IL13* rs1800925 C>T on the development of esophageal cancer. A total of 380 esophageal squamous cell carcinoma (ESCC) cases and 380 controls were recruited for this study. The genotypes were determined using a custom-by-design 48-Plex SNPscan™ Kit. Results: The *IL10* rs1800872 T>G polymorphism was associated with an increased risk of ESCC. However, there were no significant links with the other five SNPs. Stratified analyses indicated no significant risk of ESCC associated with the *IL10* rs1800872 T>G polymorphism evident among any subgroups. Conclusion: These findings indicated that functional polymorphism *IL10* rs1800872 T>G might contribute to ESCC susceptibility. However, our results were obtained with a limited sample size, so that the power of our analysis was low. Future larger studies with more rigorous study designs of other ethnic populations are required to confirm the current findings.

Keywords: *IL10* - polymorphisms - esophageal cancer - molecular epidemiology - Chinese

Asian Pacific J Cancer Prev, **14 (6)**, 3443-3447

Introduction

Esophageal cancer is the eighth most common cancer and sixth leading cause of cancer associated death worldwide (Shinomiya et al., 1999; Parkin et al., 2005; Jemal et al., 2008). The 5 years survival rate for esophageal cancer patients is very poor and accounts only 12.3% (Berrino et al., 2007). The most frequent subtype of esophageal cancer, esophageal squamous cell carcinoma (ESCC), accounts for >90% of cases (Macfarlane et al., 1994). ESCC has multi-factorial etiology, besides environmental risk factors, accumulated evidence has shown genetic factors might play an important role in the ESCC carcinogenesis, including single nucleotide polymorphisms (SNPs) (Wu et al., 2011).

Chronic inflammation in esophageal tissues may play a role in ESCC development and multiple genes that play critical roles in inflammatory pathways may be associated with ESCC risk.

Interleukin 9 (*IL9*) was originally described as a growth factor for a subset of murine T cell clones (Uyttenhove et al., 1988). *IL9* is a pleiotropic cytokine in the T helper cell type 1 (Th1):Th2 pathway. Now, it was well known that a specialized subset termed Th9 cells selectively produce *IL9* (Goswami et al., 2011). CDKN2A is a major susceptibility gene for cutaneous malignant melanoma (CMM). *IL9* single nucleotide polymorphisms (SNPs) might have stronger risks of CMM in CDKN2A-positive families (Yang et al., 2009). *IL10* has anti-inflammatory and immunosuppressive effects by decreasing the production of pro-inflammatory mediators, *IL10* has also been shown to regulate the differentiation and proliferation of several immune cells (Couper et al., 2008). The *IL10* gene is located on chromosome 1q31-32. Studies have shown that *IL10* may be involved in the pathogenesis of many types of cancers (Howell et al., 2007). The maturation of Th1 cells from the naive CD4+ T cell pool is regulated by *IL12* (Trinchieri et al., 2003), *IL12* exhibits an immunoregulatory impact on T and NK cells by inducing IFN-γ biosynthesis from both cell types, augmenting

*[1]Department of Plastic Surgery, Wuhan Union Hospital, Tongji Medical College of Huazhong University of Science and Technology, Wuhan, [2]Department of Cardiothoracic Surgery, Affiliated People's Hospital of Jiangsu University, Zhenjiang, [3]Department of Thoracic & Cardiac Surgery, [4]Department of General Surgery, The First Affiliated Hospital of Nanjing Medical University, Nanjing, China [&]Equal contributors *For correspondence: zhuquan2003@yahoo.com.cn, clbright0909@hotmail.com*

their proliferation and cytotoxicity (Trinchieri et al., 2003). *IL12* displays anti-angiogenic activity, which may antagonize the pro-angiogenic signals whose presence has been demonstrated during the progression of malignancies (Imagawa et al., 2004). *IL13* play a central role in allergy by stimulating IgE synthesis (Akdis et al., 2006), show strong antitumor activity in mice, and inhibit proliferation of astrocytoma and low-grade glioma in human cell lines (Liu et al., 2000).

On the basis of the biological and pathologic significance of *IL9*, *IL10*, *IL12A*, *IL12B* and *IL13*, it is possible that functional genetic variations in these genes may contribute to the development of ESCC. The objective of this investigation was to evaluate the association between *IL9* rs31563 C>T, *IL9* rs31564 G>T, *IL10* rs1800872 T>G, *IL12A* rs2243115 T>G, *IL12*B rs3212227 T>G and *IL13* rs1800925 C>T genotypes and ESCC susceptibility in a hospital-based case-control study. We performed genotyping analyses for the six SNPs with 380 ESCC cases and 380 controls in a Chinese population.

Materials and Methods

Ethical approval of the study protocol

This hospital-based case-control study was approved by the Review Board of Jiangsu University (Zhenjiang, China). All subjects provided written informed consent to be included in the study.

Study subjects

Three-hundred and eighty subjects with esophageal cancer were consecutively recruited from the Affiliated People's Hospital of Jiangsu University and Affiliated Hospital of Jiangsu University (Zhenjiang, China) between October 2008 and November 2009. All cases of esophageal cancer were diagnosed as ESCC by pathologic means. The exclusion criteria were patients who previously had: cancer; any metastasized cancer; radiotherapy or chemotherapy. The controls were patients without cancer frequency-matched to the cases with regard to age (±5 years) and sex recruited from the two hospitals mentioned above during the same time period. Most of the controls were admitted to the hospitals for the treatment of trauma.

Each subject was personally questioned by trained interviewers using a pre-tested questionnaire to obtain information on demographic data (e.g., age, sex) and related risk factors (including tobacco smoking and alcohol consumption). After the interview, 2-mL samples of venous blood were collected from each subject. Individuals who smoked one cigarette per day for >1 year were defined as "smokers". Subjects who consumed ≥3 alcoholic drinks a week for >6 months were considered to be "alcohol drinkers".

Isolation of DNA and genotyping by a custom-by-design 48-Plex SNPscan™ Kit

Blood samples were collected from patients using Vacutainers and transferred to tubes lined with ethylenediamine tetra-acetic acid (EDTA). Genomic DNA was isolated from whole blood with the QIAamp DNA Blood Mini Kit (Qiagen, Berlin, Germany) (Gu et al., 2012). Sample DNA (10 ng) were amplified by PCR according to the manufacturer's recommendations. The SNP genotyping work was performed using a custom-by-design 48-Plex SNPscan™ Kit (Genesky Biotechnologies Inc., Shanghai, China) as previously described (Chen et al., 2012). This kit was developed according to patented SNP genotyping technology by Genesky Biotechnologies Inc., which was based on double ligation and multiplex fluorescence PCR. For quality control, repeated analyses were done for 4% of randomly selected samples with high DNA quality.

Statistical Analyses

Differences in the distributions of demographic characteristics, selected variables, and genotypes of the *IL9* rs31563 C>T, *IL9* rs31564 G>T, *IL10* rs1800872 T>G, *IL12*A rs2243115 T>G, *IL12*B rs3212227 T>G and *IL13* rs1800925 C>T variants between the cases and controls were evaluated using the χ^2 test. The associations between *IL9* rs31563 C>T, *IL9* rs31564 G>T, *IL10* rs1800872 T>G, *IL12*A rs2243115 T>G, *IL12*B rs3212227 T>G and *IL13* rs1800925 C>T genotypes and risk of ESCC were estimated by computing the ORs and their 95% CIs using logistic regression analyses for crude ORs and adjusted ORs when adjusting for age, sex, smoking and drinking status. The Hardy-Weinberg equilibrium (HWE) was tested by a goodness-of-fit χ^2 test to compare the observed genotype frequencies to the expected ones among the control subjects. All statistical analyses were performed with SAS 9.1.3 (SAS Institute, Cary, NC, USA).

Results

Characteristics of the study population

Characteristics of cases and controls included in the study are summarized in Table 1. The cases and controls appeared to be adequately matched on age and sex as suggested by the χ^2 tests (P = 0.056 and P = 0.346, respectively). As shown in Table 1, no significant difference was detected on drinking status between the cases and the controls (P = 0.183), but smoking rate was

Table 1. Distribution of Selected Demographic Variables and Risk Factors in ESCC Cases and Controls

Variable	Cases (n=380)		Controls (n=380)		*P**
	n	%	n	%	
Age (years)					0.056
< 60	142	37.4	117	30.8	
≥ 60	238	62.6	263	69.2	
Sex					0.346
Male	269	70.8	257	67.6	
Female	111	29.2	123	32.4	
Tobacco use					0.014
Never	220	57.9	253	66.6	
Ever	160	42.1	127	33.4	
Alcohol use					0.183
Never	253	66.6	270	71.1	
Ever	127	33.4	110	28.9	

* Two-sided χ^2 test

Table 2. Primary Information for Six Genotyped SNPs

Genotyped SNPs	Chr	Regulome DB Score[a]	TFBS[b]	Location	MAF[c] for Chinese in database	MAF in our controls (n = 380)	P value for HWE[d] test in our controls	% Genotyping value
IL9: rs31563 C>T	5	5	Y	5'-Flanking	0.116	0.13	0.933	95.39
IL9: rs31564 G>T	5	No Data	—	Intron3	0.419	0.432	0.864	97.11
IL10: rs1800872 T>G	1	5	Y	5'-Flanking	0.267	0.284	0.347	94.87
IL12A: rs2243115 T>G	3	4	Y	5'-Flanking	0.085	0.089	0.499	97.11
IL12B: rs3212227 T>G	5	No Data	—	3'-UTR	0.43	0.455	0.635	97.11
IL13: rs1800925 C>T	5	2b	Y	5'-Flanking	0.156	0.16	0.826	96.58

[a]http://www.regulomedb.org/; [b]TFBS: Transcription Factor Binding Site; [c]MAF: minor allele frequency; [d]HWE: Hardy-Weinberg equilibrium

Table 3. Logistic Regression Analyses of Associations Between Six Polymorphisms and Risk of ESCC

Genotype	Cases (n=380)		Controls (n=380)		Crude OR	*P*	Adjusted OR [a]	*P*
	n	%	n	%	(95%CI)		(95%CI)	
IL9: rs31563 C>T								
CC	271	75.3	276	75.6	1		1	
CT	85	23.6	83	22.7	1.04 (0.74-1.47)	0.811	1.00 (0.70-1.42)	0.991
TT	4	1.1	6	1.6	0.68 (0.19-2.43)	0.552	0.60 (0.16-2.20)	0.438
CT+TT	89	24.7	89	24.4	1.02 (0.73-1.43)	0.916	0.97 (0.69-1.37)	0.866
CC+CT	356	98.9	359	98.4	1		1	
TT	4	1.1	6	1.6	0.67 (0.19-2.40)	0.541	0.60 (0.16-2.19)	0.438
IL9: rs31564 G>T								
GG	102	27.7	120	32.4	1		1	
GT	200	54.3	180	48.6	1.31 (0.94-1.82)	0.114	1.30 (0.93-1.82)	0.126
TT	66	17.9	70	18.9	1.11 (0.72-1.70)	0.635	1.10 (0.72-1.70)	0.66
GT+TT	266	72.3	250	67.6	1.25 (0.91-1.72)	0.163	1.24 (0.91-1.71)	0.178
GG+GT	302	82.1	300	81.1	1		1	
TT	66	17.9	70	18.9	0.94 (0.65-1.36)	0.73	0.94 (0.64-1.36)	0.726
IL10: rs1800872 T>G								
TT	162	45.5	191	52.3	1		1	
TG	163	45.8	141	38.6	**1.36 (1.00-1.85)**	**0.049**	1.36 (1.00-1.85)	0.053
GG	31	8.7	33	9	1.11 (0.65-1.89)	0.707	1.08 (0.63-1.85)	0.782
TG+GG	194	54.5	174	47.7	1.32 (0.98-1.76)	0.067	1.31 (0.97-1.75)	0.078
TT+TG	325	91.3	332	91	1		1	
GG	31	8.7	33	9	0.96 (0.57-1.60)	0.875	0.94 (0.56-1.57)	0.805
IL12A: rs2243115 T>G								
TT	311	84.5	308	83.2	1		1	
TG	56	15.2	58	15.7	0.96 (0.64-1.43)	0.826	0.90 (0.60-1.34)	0.593
GG	1	0.3	4	1.1	0.25 (0.03-2.23)	0.213	0.25 (0.03-2.23)	0.212
TG+GG	57	15.5	62	16.8	0.91 (0.62-1.35)	0.64	0.85 (0.57-1.27)	0.434
TT+TG	367	99.7	366	98.9	1		1	
GG	1	0.3	4	1.1	0.25 (0.03-2.24)	0.215	0.25 (0.03-2.26)	0.217
IL12B: rs3212227 T>G								
TT	116	31.5	112	30.3	1		1	
TG	176	47.8	179	48.4	0.95 (0.68-1.32)	0.759	0.97 (0.70-1.36)	0.876
GG	76	20.7	79	21.4	0.93 (0.62-1.40)	0.723	0.92 (0.61-1.39)	0.69
TG+GG	252	68.5	258	69.7	0.94 (0.69-1.29)	0.713	0.96 (0.70-1.31)	0.784
TT+TG	292	79.3	291	78.6	1		1	
GG	76	20.7	79	21.4	0.96 (0.67-1.37)	0.816	0.93 (0.65-1.34)	0.711
IL13: rs1800925 C>T								
CC	254	69.6	261	70.7	1		1	
CT	98	26.8	98	26.6	1.03 (0.74-1.43)	0.871	1.01 (0.72-1.40)	0.971
TT	13	3.6	10	2.7	1.34 (0.58-3.10)	0.501	1.35 (0.58-3.16)	0.49
CT+TT	111	30.4	108	29.3	1.06 (0.77-1.45)	0.735	1.04 (0.75-1.43)	0.821
CC+CT	352	96.4	359	97.3	1		1	
TT	13	3.6	10	2.7	1.33 (0.57-3.06)	0.509	1.35 (0.58-3.14)	0.49

[a]Adjusted for age, sex, smoking and drinking status; Bold values are statistically significant ($P<0.05$)

higher in ESCC patients than in control subjects (P = 0.014). The primary information for six genotyped SNPs was in Table 2. For the six SNPs, the genotyping was successful ranging from 94.87% to 97.11% in all 760 samples. The concordance rates of repeated analyses were 100%. Minor allele frequency (MAF) in our controls was similar to MAF for Chinese in database for all six SNPs (Table 2). The observed genotype frequencies for these six polymorphisms in the controls were all in HWE (Table 2).

Associations between six polymorphisms and risk of ESCC

The genotype distributions of *IL9* rs31563 C>T, *IL9* rs31564 G>T, *IL10* rs1800872 T>G, *IL12*A rs2243115 T>G, *IL12*B rs3212227 T>G and *IL13* rs1800925 C>T in the cases and the controls are shown in Table 3. In the

Table 4. Stratified Analyses Between IL10: rs1800872 T>G Polymorphism and ESCC Risk by Sex, Age, Smoking Status and Alcohol Consumption

Variable	IL10: rs1800872 T>G (case/control)[a]				Adjusted OR[b] (95% CI); P				
	TT	TG	GG	TG+GG	TT	TG	GG	TG+GG	GG vs. (TG+TT)
Sex									
Male	116/131	115/98	20/23	135/121	1	1.32 (0.91-1.92); P: 0.144	0.98 (0.51-1.90); P: 0.957	1.26 (0.88-1.79); P: 0.208	0.86 (0.46-1.63); P: 0.653
Female	46/60	48/43	11/10	59/53	1	1.43 (0.82-2.52); P: 0.212	1.39 (0.54-3.59); P: 0.502	1.42 (0.83-2.44); P: 0.197	1.17 (0.47-2.91); P: 0.738
Age									
<60	59/57	61/45	13/9	74/54	1	1.33 (0.76-2.34); P: 0.320	1.42 (0.54-3.74); P: 0.483	1.35 (0.79-2.31); P: 0.280	1.23 (0.49-3.13); P: 0.659
≥60	103/134	102/96	18/24	120/120	1	1.41 (0.96-2.06); P: 0.078	1.01 (0.52-1.96); P: 0.983	1.33 (0.93-1.91); P: 0.124	0.86 (0.45-1.64); P: 0.648
Smoking status									
Never	92/128	93/96	19/18	112/114	1	1.31 (0.88-1.94); P: 0.184	1.35 (0.66-2.76); P: 0.408	1.32 (0.90-1.92); P: 0.157	1.19 (0.60-2.38); P: 0.615
Ever	70/63	70/45	12/15	82/60	1	1.32 (0.78-2.24); P: 0.307	0.73 (0.31-1.75); P: 0.483	1.17 (0.71-1.93); P: 0.529	0.65 (0.28-1.50); P: 0.309
Alcohol consumption									
Never	105/136	109/101	19/21	128/122	1	1.37 (0.94-2.00); P: 0.099	1.13 (0.57-2.24); P: 0.718	1.33 (0.93-1.91); P: 0.120	0.98 (0.51-1.89); P: 0.944
Ever	57/55	54/40	12/12	66/52	1	1.29 (0.72-2.34); P: 0.394	0.96 (0.37-2.52); P: 0.936	1.22 (0.70-2.13); P: 0.487	0.86 (0.34-2.15); P: 0.739

[a]The genotyping was successful in 356 (93.7%) ESCC cases, and 365 (96.1%) controls for IL10: rs1800872 T>G; [b]Adjusted for age, sex, smoking status and alcohol consumption (besides stratified factors accordingly) in a logistic regression model

single locus analyses, the genotype frequencies of *IL10* rs1800872 T>G were 45.5% (TT), 45.8% (TG), and 8.7% (GG) in the case patients and 52.3% (TT), 38.6% (TG), and 9.0% (GG) in the control subjects, and the difference was not statistically significant (P = 0.140). When the *IL10* rs1800872 TT homozygote genotype was used as the reference group, the TG genotype was associated with a significantly increased risk for ESCC (TG vs. TT: OR 1.36, 95% CI 1.00–1.85, P = 0.049). When the *IL10* rs1800872 TT homozygote genotype was used as the reference group, the GG genotype was not associated with the risk for ESCC (GG vs. TT: OR 1.11, 95% CI 0.65–1.89, P = 0.707). In the recessive model, when the *IL10* rs1800872 TT/TG genotypes were used as the reference group, the GG homozygote genotype was not associated with the risk for ESCC (OR 0.96, 95% CI 0.57–1.60, P = 0.875). In the dominant model, the *IL10* rs1800872 TG/GG variants were associated with a borderline statistically increased risk of ESCC, compared with the *IL10* rs1800872 TT genotype (TG/GG vs. TT: OR 1.32, 95% CI 0.98-1.76, P = 0.067) (Table 3). After adjusting for age, sex, smoking and drinking, a borderline statistically increased risk of ESCC was observed both in the heterozygote comparing model (TG vs. TT: adjusted OR 1.36, 95% CI 1.00–1.85, P = 0.053) and the dominant model (TG/GG vs. TT: adjusted OR 1.31, 95% CI 0.97-1.75, P = 0.078) (Table 3).

None of the *IL9* rs31563 C>T, *IL9* rs31564 G>T, *IL12*A rs2243115 T>G, *IL12*B rs3212227 T>G and *IL13* rs1800925 C>T polymorphisms achieved a significant difference in the genotype distributions between cases and controls (Table 3). Logistic regression analyses revealed that the five polymorphisms were not associated with the risk of ESCC (Table 3).

Stratification analyses of IL10 rs1800872 T>G polymorphisms and risk of ESCC

To evaluate the effects of *IL10* rs1800872 T>G genotypes on ESCC risk according to different age, sex, smoking and alcohol drinking status; we performed the stratification analyses. No significantly risk of ESCC associated with the *IL10* rs1800872 T>G polymorphism was evident among any subgroups (Table 4).

Discussion

In this hospital-based case-control study of ESCC, we investigated the associations of *IL9* rs31563 C>T, *IL9* rs31564 G>T, *IL10* rs1800872 T>G, *IL12*A rs2243115 T>G, *IL12*B rs3212227 T>G and *IL13* rs1800925 C>T SNPs with risk of ESCC in a high risk Chinese population. Our multivariable logistic analysis revealed that *IL10* rs1800872 TG genotype had an increased risk of ESCC.

Previous studies suggested that inflammatory cytokine gene SNPs such as *IL10* rs1800872 polymorphism is associated with smoking-related cancers (Oh et al., 2010). *IL10* gene is located on chromosome 1 (1q31-1q32), comprising five exons (Eskdale et al., 1997). Within the *IL10* gene promoter region, rs1800872 (-592 T>G), were reported to be associated with different *IL10* expres¬sion.

IL10 has been shown to regulate the differentiation and proliferation of several immune cells (Couper et al., 2008). *IL10* plays a key role in tumor development and metastasis. *IL10* has both tumor-promoting and tumor-inhibiting properties. Higher serum and peri-tumoral *IL10* levels had been reported in many malignancies (Jebreel et al., 2007). To date, lines of research have investigated the contributions of *IL10* gene polymor¬phisms to the predisposition to different cancer types, such as oral, stomach, liver, breast, ovarian, cervical, prostate, and so on (Howell et al., 2007). However, no positive association was found between *IL10* rs1800872 T>G SNP and ESCC till now. We found *IL10* rs1800872 TG variant heterozygote rather than *IL10* rs1800872 GG homozygote was associated with ESCC risk. *IL10* rs1800872 T>G

polymorphism was not associated with risk of ESCC among smoking or non-smoking subgroups. This might because our sample size was relatively small; the numbers of GG genotypes and GA genotypes in subgroups were not large enough.

There was no significantly association between the other five SNPs and ESCC risk in our population. These findings were consistent with some previous researches. The frequencies of genetic polymorphisms often vary between ethnic groups. In the present Chinese study, the allele frequency of *IL10* rs1800872 G was 0.284 among 380 control subjects, which is consistent with that of Chinese Han population (0.267) in SNP DataBase, but significantly lower than that of Sub-Saharan African (0.525) population and European (0.792) population (http://www.ncbi.nlm.nih.gov/SNP).

Considering *IL10* rs1800872 T>G mutant alleles in the control group, OR, ESCC samples and control samples, the power of our analysis ($\alpha = 0.05$) was 0.482 in 356 ESCC cases and 365 controls with adjusted OR 1.36 for *IL10* rs1800872 T>G.

Several limitations need to be addressed. The patients and controls were enrolled from hospitals and may not represent the general population, inherited biases may occur. The polymorphisms investigated in our study were chosen based on their functional considerations, and may not give a comprehensive view about genetic variability in *IL10*. Further fine-mapping studies in the susceptible region of the variants are needed. The moderate sample size limited the statistical power of our study. Further studies are warranted to confirm our findings, particularly the gene-environment interaction are warranted to clarify esophageal carcinogenesis genetic mechanism. Detailed information on cancer metastasis and survival information were not available till now, which restricted us from further analyses on the role of *IL10* polymorphisms in ESCC progression and prognosis.

In conclusion, our study provides evidence that functional *IL10* rs1800872 T>G polymorphism may contribute to the risk of ESCC. However, our results were obtained with a limited sample size, the power of our analysis was low, and therefore allowed us to draw just preliminary conclusions. Future larger studies with more rigorous study designs of other ethnic populations and tissue-specific biological characterization are required to confirm current findings.

Acknowledgements

This study was supported in part by National Natural Science Foundation of China (81101889) and Social Development Foundation of Zhenjiang (SH2010017). We wish to thank Dr. Da Ding and Dr. Yan Liu (Genesky Biotechnologies Inc., Shanghai, China) for technical support.

References

Akdis M (2006). Healthy immune response to allergens: T regulatory cells and more. *Curr Opin Immunol*, **18**, 738-44.

Berrino F, De Angelis R, Sant M, et al (2007). Survival for eight major cancers and all cancers combined for European adults diagnosed in 1995-99: results of the EUROCARE-4 study. *Lancet Oncol*, **8**, 773-83.

Chen X, Li S, Yang Y, et al (2012). Genome-wide association study validation identifies novel loci for atherosclerotic cardiovascular disease. *J Thromb Haemost*, **10**, 1508-14.

Couper KN, Blount DG, Riley EM (2008). IL-10: the master regulator of immunity to infection. *J Immunol*, **180**, 5771-7.

Eskdale J, Kube D, Tesch H, Gallagher G (1997) Mapping of the human *IL10* gene and further characterization of the 5' flanking sequence. *Immunogenetics*, **46**, 120-8.

Goswami R, Kaplan MH (2011). A brief history of IL-9. *J Immunol*, **186**, 3283-8.

Gu H, Ding G, Zhang W, et al (2012). Replication study of PLCE1 and C20orf54 polymorphism and risk of esophageal cancer in a Chinese population. *Mol Biol Rep*, **39**, 9105-11.

Howell WM, Rose-Zerilli MJ (2007). Cytokine gene polymorphisms, cancer susceptibility, and prognosis. *J Nutr*, **137**, S194-9.

Imagawa Y, Satake K, Kato Y, Tahara H, Tsukuda M (2004). Antitumor and antiangiogenic effects of interleukin 12 gene therapy in murine head and neck carcinoma model. *Auris Nasus Larynx*, **31**, 239-45.

Jebreel A, Mistry D, Loke D, et al (2007). Investigation of interleukin 10, 12 and 18 levels in patients with head and neck cancer. *J Laryngol Otol*, **121**, 246-52.

Jemal A, Siegel R, Ward E, et al (2008). Cancer statistics, 2008. *CA Cancer J Clin*, **58**, 71-96.

Liu H, Jacobs BS, Liu J, et al (2000). Interleukin-13 sensitivity and receptor phenotypes of human glial cell lines: non-neoplastic glia and low-grade astrocytoma differ from malignant glioma. *Cancer Immunol Immunother*, **49**, 319-24.

Macfarlane GJ, Boyle P (1994) The epidemiology of oesophageal cancer in the UK and other European countries. *J R Soc Med*, **87**, 334-7.

Oh SS, Chang SC, Cai L, et al (2010). Single nucleotide polymorphisms of 8 inflammation-related genes and their associations with smoking-related cancers. *Int J Cancer*, **127**, 2169-82.

Parkin DM, Bray F, Ferlay J, Pisani P (2005). Global cancer statistics, 2002. *CA Cancer J Clin*, **55**, 74-108.

Shinomiya T, Mori T, Ariyama Y, et al (1999). Comparative genomic hybridization of squamous cell carcinoma of the esophagus: the possible involvement of the DPI gene in the 13q34 amplicon. *Genes Chromosomes Cancer*, **24**, 337-44.

Trinchieri G (2003). Interleukin-12 and the regulation of innate resistance and adaptive immunity. *Nat Rev Immunol*, **3**, 133-46.

Uyttenhove C, Simpson RJ, Van Snick J (1988). Functional and structural characterization of P40, a mouse glycoprotein with T-cell growth factor activity. *Proc Natl Acad Sci U S A*, **85**, 6934-8.

Wu C, Hu Z, He Z, Jia W, et al (2011). Genome-wide association study identifies three new susceptibility loci for esophageal squamous-cell carcinoma in Chinese populations. *Nat Genet*, **43**, 679-84.

Yang XR, Pfeiffer RM, Wheeler W, et al (2009). Identification of modifier genes for cutaneous malignant melanoma in melanoma-prone families with and without CDKN2A mutations. *Int J Cancer*, **125**, 2912-7.