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ORIGINAL ARTICLE

Functional variants of *IL4* and *IL6* genes and risk of tobacco-related oral carcinoma in high-risk Asian Indians

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BACKGROUND: Tobacco-related oral squamous cell carcinoma (OSCC) is one of the most common cancers involving Indian males. We assessed the association of *IL4* promoter –589 T>C, –33 T>C, and *IL6* –174 G>C functional genetic polymorphisms with tobacco-related OSCC in Asian Indians.

PATIENTS AND METHODS: The *IL4* and *IL6* promoter polymorphisms were assessed in 140 patients with OSCC and 120 normal subjects by PCR–RFLP technique, and significance of the data was determined using chi-square test.

RESULTS: The frequency of TC, CC genotype, and C allele at *IL4* promoter sites –589 and –33 were higher in patients when compared with controls. Consequently, TC/CC genotypes and C allele at both sites appeared as susceptible. However, *IL6* –174 G>C single-nucleotide polymorphisms (SNP) appeared to be protective in patients with OSCC. Of eight haplotypes, five were associated with two- to seven-fold increased risk of tobacco-related OSCC. These SNPs further showed heterogeneity among different ethnic population, but their distribution in Asian Indians stand closer to other Asian populations.

CONCLUSIONS: In this study, *IL4* –589 CC, –33 CC genotype, and *C allele at both sites appeared to be susceptible, while *IL6* –174 CC genotype and *C allele appeared to be protective in patients with OSCC; hence, these SNPs may be a potential prognostic markers for tobacco-related OSCC in Asian Indians.

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Keywords: IL4; IL6; single-nucleotide polymorphisms; haplotype; oral cancer; Asian Indians

Introduction

Oral cancer is the largest category of head and neck cancer. Worldwide an estimated 400 000 new cases of oral cancer are diagnosed every year with two-thirds of the cases occurring in developing countries like Sri Lanka, India, Pakistan, and Bangladesh (GLOBOCAN, 2005). Although external carcinogens and poor lifestyle habits such as tobacco, alcohol consumption and low-grade diet play a pivotal role in oral cancer development, strong genetic predisposition may further contribute to the susceptibility to the disease. Cytokines have been considered to play an important role in carcinogenesis. They may either be involved in the anti-tumor effector immune mechanisms or may enhance malignant transformation and tumor growth. They are produced by both host stromal and immune cells as well as by the cancer cells in the same microenvironment. But their relative concentration, receptor expression patterns, etc. decide the direction of their action. Broadly, Th1 type cytokines like IFN-γ, IL2, and IL12 are required for anti-tumor immunity, whereas Th2 type and several inflammatory cytokines like CSF-1, IL1 family, TNF, and TGF-β favor tumor development (Smyth *et al*, 2004). We have earlier reported deregulated expression of Th2 cytokines in tobacco-related oral squamous cell carcinoma (Agarwal *et al*, 2003).

IL4, a 20-kDa glycoprotein, is a member of four α-helical cytokine family. It is produced by activated $CD4^+$ T cells, mast cells, and basophils. It is an autocrine growth factor for differentiation and expansion of Th2 subset, responsible for B cell switching to IgE production, antagonizes IFN-γ function, inhibits macrophage activation and reportedly shows anti-tumor activity on different cancer cells such as colon, breast (Toi *et al*, 1992), and renal carcinoma (Golumbek *et al*, 1991; Yu *et al*, 2004). However, we have earlier shown an up-regulated expression of IL4 in patients with tobacco-related oral squamous cell carcinoma (OSCC) (Agarwal *et al*, 2003; Manchanda *et al*, 2006).

Interleukin 6 (IL6), another cytokine of Th2 type, is a 25-kDa-long glycoprotein having multifunctional effect on various physiological and pathophysiological processes like inflammation, bone metabolism, synthesis of CRP, and carcinogenesis (Diehl and Rincon, 2002). It is synthesized by phagocytes, vascular endothelial cells, and fibroblasts. It has been demonstrated that IL6 acts as a potent stimulator of cancer metastasis by

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up-regulating the expression of intercellular and leukocyte adhesion molecule-1 on endothelial cells (Hutchins and Steel, 1994). In addition, it stimulates the production of hepatocyte (De Jong *et al*, 2001) and vascular endothelial growth factors (Cohen *et al*, 1996) that aids in tumor-cell proliferation.

Polymorphisms in the promoter or other regulatory regions of the cytokine gene may affect its expression (Terry *et al*, 2000), and thus, the genetic variants have been suggested as risk-modifying factor for variety of cancers (Jin *et al*, 2004). In this study, we therefore evaluated the association of select functional single-nucleotide polymorphisms (SNPs) in *IL4* [*IL4* −589 T > C (rs2243250), *IL4* −33 T > C (rs2070874)] and *IL6* [*IL6* −174 G > C (rs1800795)] genes with the development of tobacco-related OSCC in Asian Indians. *IL4* gene is located on chromosome 5q31.1 and *IL6* is on 7p21.24, and above mentioned SNPs are associated with 5′ UTR region containing promoter and thus affect their transcription rate and hence serum levels (Terry *et al*, 2000; Nakashima *et al*, 2002). *IL4 −589 T* and *−33 T* alleles are associated with three-folds higher transcriptional activity *in vitro* and *in vivo* (Nakashima *et al*, 2002). Similarly, a transition from G to C at −174 position in *IL6* gene creates a site for *NF-1* transcription factor that acts as a repressor. Thus, *IL6* −174 C allele is associated with lower serum levels of IL6 (Terry *et al*, 2000). A recent study on Swedish patients with colorectal cancer established association of *IL4* −589T allele with longer survival and thus suggested it to be protective (Wilkening *et al*, 2008). However, results of such association studies for *IL6 −174* G > C polymorphism are contradictory. While in breast cancer, increased serum levels of IL6 was associated with advancing stage, more metastatic sites, poor clinical outcome, and resistance to chemotherapy and hormone therapy (DeMichele *et al*, 2003), some other studies have reported *IL6* −174 C allele (low producer of IL6) as a risk factor for developing colorectal (Landi *et al*, 2003) and breast cancer (Hefler *et al*, 2005). These controversial results led us to investigate for the first time the association between these SNPs and tobacco-related OSCC in Asian Indians.

Materials and methods

Patients and controls

This study included 140 patients with oral cancer attending BRA-Institute Rotary Cancer Hospital of the All India Institute of Medical Sciences (New Delhi, India) and 120 age-, sex- and ethnicity-matched healthy individuals. All the patients had history of tobacco usage either in smoke or smokeless forms from 6 months to 30 years. The normal subjects had no history of tobacco usage, and none of them had any oral lesion. All the patients had biopsy-proven OSCC of various sites. Clinical staging of the tumor was performed as per UICC criteria (Sobin and Wittekind, 2002). The study protocol was approved by ''Ethics Committee'' of the All India Institute of Medical Sciences, New Delhi, (approbation No. IESC/T-04/28.08.09), and a written informed consent was obtained from each study subjects.

Methods

Ten milliliters venous blood was drawn aseptically by venipuncture. The genomic DNA was isolated from whole blood using sodium perchlorate method as described earlier (Baniasadi *et al*, 2006) and stored at −20°C until further use.

Genotyping by (PCR)-RFLP for IL4 *−589 T > C, −33 T > C, and* IL6 *−174 G > C*

The genotyping for *IL4* −589 T > C, −33 T > C, and *IL6* −174 G > C SNPs was performed by PCR–RFLP using endonucleases *Bsm*FI, *Bsm*AI, and *Nla*III (NEB, Hitchin, Hertfordshire, UK), respectively. Briefly, the isolated DNA sample was subjected to PCR amplification with specific primers for detecting *IL4* −589 T > C SNP and −33 T > C SNP (F, 5′-actaggcctcacctgatacg-3′; R, 5′-aggtgtcgatttgcagtgac-3′) and *IL6* −174 G > C SNP (F, 5′-atgccaagtgctgagtcacta- 3′; R, 5′-tcgagggcagaatgagcctc-3′). The restriction digestion of 646-bp amplicon of *IL4* −589 locus with *Bsm*FI yielded fragments of 601 and 45 bp, and the digestion of 646-bp amplicon of −33 locus with *BsmAI* gave rise to 622- and 24-bp fragments. Similarly, 305-bp amplicon of *IL6* −174 locus was cleaved to 230- and 75-bp fragments in case with G allele or 121-, 109- and 75-bp fragments in case with C allele by the endonuclease *Nla*III. The restricted fragments were resolved on 3% agarose gel (*IL4* −589 T > C and *IL6* −174 G > C) and 8% native polyacrylamide gel (*IL4* −33 T > C) respectively.

Statistical analysis

The differences in the frequency of various alleles and genotypes between patients and healthy controls were evaluated by χ^2 test. The calculated *P*-values were corrected (P_c) for number of alleles and P_c value < 0.05 at the 95% CI was considered significant. The odd ratio (OR) and confidence interval (CI) was determined using Hutchon.net software (http://www.hutchon.net/ConfidOR.htm). Unconditional logistic regression was used to estimate the risk of polymorphic genotype or allele with respect to wild type. All the SNPs were tested for Hardy–Weinberg equilibrium, both in the patients and control groups by χ^2 –goodness-of-fit test. Haplotype analysis was performed by manual counting, and linkage disequilibrium was evaluated by calculating Lewinton's coefficient |D'|.

Results

Clinicopathological features of patients with tobacco-related OSCC

The clinicopathological features of the patients with OSCC have been shown in Table 1. Most of the patients registered for the cohort study were men (85%, $n = 119$) and only 15% ($n = 21$) were women. The mean age of the patients' group was 51.4 ± 13.6 years, with a range of 20–90 years. Tongue was the most common site of lesion seen in 61.4% cases. Majority of

Table 1 Clinical and histopathological parameters of patients with oral cancer

Characteristics	*Values*
Age (year)	
Range	20–90
Mean ± s.d.	51.4 ± 13.6
Gender	No. (%)
Male	119 (85)
Female	21 (15)
Site	
Tongue	86 (61.4)
Buccal mucosa	21 (15)
Floor of mouth	8 (5.7)
Alveolus	7 (5)
Lips	5 (3.6)
Retro molar trigone	5 (3.6)
Gingivo buccal sulcus	5 (3.6)
Palate	3 (2.1)
Tumor size	
$T_1 + T_2$	31 (22.1)
$T_3 + T_4$	109 (77.9)
Lymph node involvement	
N_0	39 (27.9)
N_+	101 (72.1)
Clinical stage	
Early stage (I + II)	32 (22.9)
Late stage (III + IV)	108 (77.1)

the patients (77.1%) presented with advanced stage (III and IV) tumors with cervical lymph node involvement in 101/140 (72.1%) cases.

The genotype and allele frequencies of IL4 and IL6

The frequency of various alleles and genotypes of *IL4* −589 T > C, −33 T > C, and *IL6* −174 G > C SNPs are shown in Table 2 and Figure 1 (a, b, and c respectively). In the single locus analysis, −589 TT homozygous was found to be more and CC homozygous less prevalent both in patients and normal subjects. When compared between the two groups, TT genotype was less frequent in patients as compared to controls, whereas the prevalence of TC and CC genotypes was greater in patients than in normal subjects; however, the difference between two groups was statistically insignificant. In unconditional logistic regression analysis of genotypes, *IL4* −589 TC (OR = 1.78, 95% CI, 1.04–3.05) and −589 CC (OR = 2.27, 95% CI, 0.96–5.39) were found to be at higher risk as compared to wild-type −589 TT genotype. Moreover, at this site, T allele showed significantly lower (67.5% *vs* 77.9%) and C allele significantly (P_c = 0.032) higher prevalence (32.5% *vs* 22.1%) in patients with OSCC as compared to the normal subjects. In addition, occurrence of *C allele at this site appears to increase the OSCC risk by 1.7-fold (OR = 1.70, 95% CI, 1.14–2.52) as compared to T allele.

Similar pattern was observed in the genotype distribution at *IL4* −33 sites, besides TT genotype being significantly (P_c = 0.036) in low prevalence in case with patients (42.1%) as compared to normal subjects (59.2%). In unconditional logistic regression analysis of genotypes, −33 TC and −33 CC genotypes with OR = 1.93, 95% CI, 1.13–3.29 and OR = 2.19, 95% CI, 0.97–4.93, respectively, appeared to be susceptible genotypes for developing tobacco-related OSCC. The allelic distribution showed remarkably significant difference between the two groups T allele being less frequent in patients when compared with normal subjects (63.9% *vs* 75.0%, respectively, P_c = 0.024), while *C allele showed significantly higher prevalence in patients with OSCC as compared to normal subjects (36.1% *vs*

Table 2 Risk association of *IL4 and IL6* single-nucleotide polymorphisms with tobacco-related OSCC in Asian Indians

	Patient (%) (n = *140*)	*Normal (%)* (n = *120*)	P-*value*	P_c	*OR (95% CI)*	*OR (95% C.I.)*[a]
IL4 −589(T > C)						
TT	67 (47.9)	76 (63.3)	0.0124	NS	0.53 (0.32–0.87	1 (Reference)
TC	55 (39.3)	35 (29.2)	0.087	NS	1.57 (0.93–2.64)	1.78 (1.04–3.05)
CC	18 (12.8)	9 (7.5)	0.158	NS	1.82 (0.79–4.22)	2.27 (0.96–5.39)
Alleles						
T	189 (67.5)	187 (77.9)	0.008	0.032	0.59 (0.40–0.87)	1 (Reference)
C	91 (32.5)	53 (22.1)	0.008	0.032	1.70 (1.14–2.52)	1.70 (1.14–2.52)
IL4 −33(T > C)						
TT	59 (42.1)	71 (59.2)	0.006	0.036	0.50 (0.31–0.82)	1 (Reference)
TC	61 (43.6)	38 (31.7)	0.048	NS	1.67 (1.0–2.8)	1.93 (1.13–3.29)
CC	20 (14.3)	11 (9.1)	0.20	NS	1.65 (0.76–3.60)	2.19 (0.97–4.93)
Alleles						
T	179 (63.9)	180 (75.0)	0.006	0.024	0.59 (0.40–0.86)	1 (Reference)
C	101 (36.1)	60 (25.0)	0.006	0.024	1.69 (1.16–2.48)	1.69 (1.16–2.48)
IL6 −174(G > C)						
GG	98 (70.0)	65 (54.1)	0.008	0.048	1.97 (1.19–3.29)	1 (Reference)
GC	35 (25.0)	41 (34.2)	0.105	NS	0.64 (0.38–1.09)	0.57 (0.33–0.98)
CC	7 (5.0)	14 (11.7)	0.0492	NS	0.40 (0.16–1.02)	0.33 (0.13–0.87)
Alleles						
G	231 (82.5)	171 (71.2)	0.002	0.008	1.9 (1.25–2.88)	1 (Reference)
C	49 (17.5)	69 (28.8)	0.002	0.008	0.53 (0.35–0.80)	0.53 (0.35–0.80)

NS, not significant; OSCC, oral squamous cell carcinoma.
[a]Logistic regression analysis.

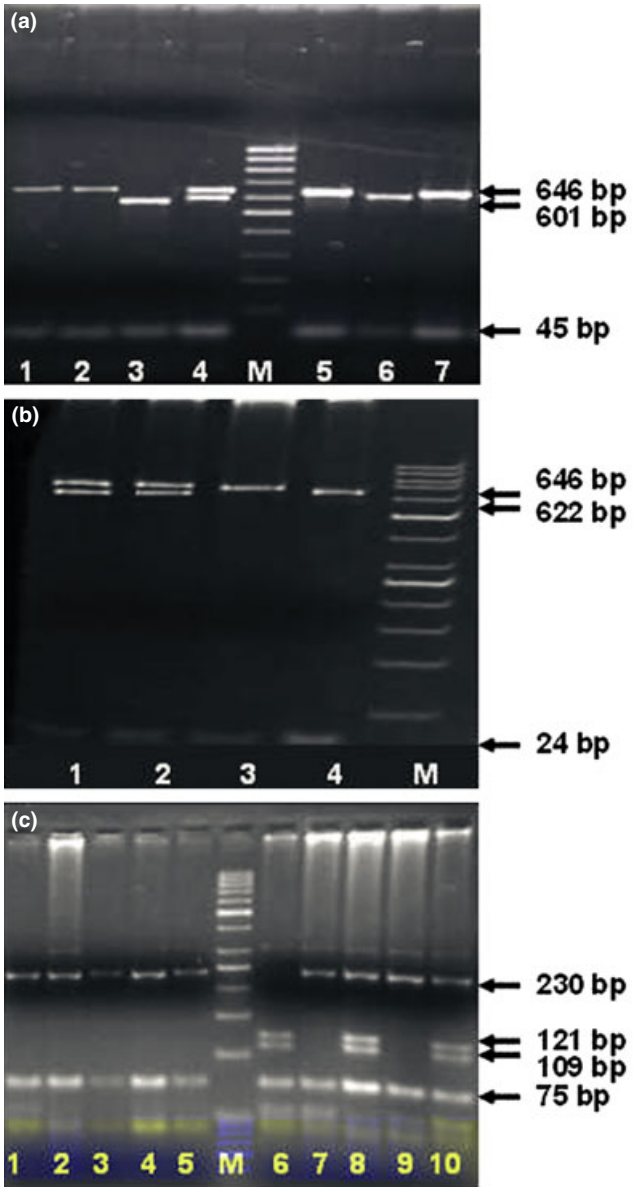

Figure 1 PCR–RFLP analysis of *IL4* and *IL6* single-nucleotide polymorphisms (SNPs): (**a**) 3% agarose gel showing *IL4* −589 T > C SNP (lanes 1, 2, 5, 7: TT homozygous; lanes 3, 6: CC homozygous; lane M: 50 bp marker; lane 4: TC heterozygous) (**b**) 8% PAGE showing *IL4* −33 T > C SNP (lanes 1, 2: TC heterozygous; lane 3: CC homozygous; lane 4: TT homozygous; lane M: 50 bp marker) (**c**) 3% agarose gel showing *IL6* −174 G > C SNP (lanes 1–5, 7, 9: GG homozygous; lane M: 50 bp marker; lane 6: CC homozygous; lanes 8, 10: GC heterozygous)

25.0%, respectively, P_c = 0.024). In addition, *IL4* −33 *C allele appeared to be associated with 1.7-fold higher risk as compared to the wild-type T allele (OR = 1.69, 95% CI, 1.16–2.48).

In case with *IL6* −174 G > C SNP, the frequency of GG genotype was significantly higher (P_c = 0.048) in patients as compared to normal subjects (70% *vs* 54.1%; P_c = 0.048), while GC and CC genotypes tended to be relatively more frequent in normal controls. Consequently, G allele had significantly higher prevalence in patients with OSCC than in normals (82.5% *vs* 71.2%; P_c = 0.008). In unconditional logistic regression analysis, *IL6* −174 GC (OR = 0.57, 95% CI, 0.33–0.98) and −174 CC (OR = 0.33, 95% CI, 0.13–0.87) were found to be protective genotypes. Moreover, C allele at *IL6* −174 locus appears to be protective than the G allele at the same position (OR = 0.53, 95% CI, 0.35–0.80). Thus, *IL4* −589 CC genotype, −33 CC genotype, and *C allele at both promoter sites appear to be susceptible, while *IL6* −174 CC genotype and −174 *C allele appear to be protective in Asian Indians.

Table 3 Haplotype distribution in the patients with OSCC and in normals

Haplotypes	*Patients (2n = 280) no. (%)*	*Normals (2n = 240) no. (%)*	*OR (95% CI)*[a]	P-*value*
$T_{-589}T_{-33}G_{-174}$	126 (45.0)	142 (59.2)	1 (Reference)	–
$*C_{-589}*C_{-33}*C_{-174}$	18 (6.4)	31 (12.9)	0.65 (0.35–1.23)	0.18
$T_{-589}T_{-33}*C_{-174}$	7 (2.5)	20 (8.3)	0.39 (0.16–0.96)	0.035
$*C_{-589}T_{-33}G_{-174}$	33 (11.9)	6 (2.5)	6.20 (2.51–15.28)	< 0.0001
$*C_{-589}T_{-33}*C_{-174}$	13 (4.6)	12 (5.0)	1.22 (0.54–2.77)	0.63
$T_{-589}*C_{-33}G_{-174}$	45 (16.1)	19 (7.9)	2.67 (1.48–4.80)	0.0008
$T_{-589}*C_{-33}*C_{-174}$	11 (3.9)	6 (2.5)	2.07 (0.74–5.75)	0.16
$*C_{-589}*C_{-33}G_{-174}$	27 (9.6)	4 (1.7)	7.61 (2.59–22.34)	< 0.0001

OSCC, oral squamous cell carcinoma.
[a]Logistic regression analysis.

Haplotype frequencies of IL4 *and* IL6

Table 3 shows frequencies of all possible haplotypes and their distribution among patients and normals. The risk associated with different haplotypes was assessed using unconditional logistic regression analysis. Wild-type haplotype $T_{-589}T_{-33}G_{-174}$ was most common haplotype both in patients with OSCC and normal population (45% and 59.2%, respectively), while with a prevalence rate of 2.5%, $T_{-589}T_{-33}*C_{-174}$ was found to be a rare haplotype in patients and $*C_{-589}*C_{-33}G_{-174}$ was rare (1.7%) in normal subjects. In logistic regression analysis, $*C_{-589}T_{-33}G_{-174}$ (OR = 6.20, 95% CI, 2.51–15.28, $P < 0.0001$) and $*C_{-589}*C_{-33}G_{-174}$ (OR = 7.61, 95% CI, 2.59–22.34, $P < 0.0001$) haplotypes appeared to pose six- to seven-fold increased risk of developing OSCC as compared to wild-type $T_{-589}T_{-33}G_{-174}$. Additionally, $C_{-589}T_{-33}C_{-174}$, $T_{-589}C_{-33}G_{-174}$, and $T_{-589}C_{-33}C_{-174}$ haplotypes were also found to be at higher risk with OR = 1.22, 95% CI, 0.54–2.77; OR = 2.67, 95% CI, 1.48–4.80 and OR = 2.07, 95% CI, 0.74–5.75, respectively. In contrast, $C_{-589}C_{-33}C_{-174}$ (OR = 0.65, 95% CI, 0.35–1.23) and $T_{-589}T_{-33}C_{-174}$ (OR = 0.39, 95% CI, 0.16–0.96) appeared to be protective. All three SNPs appeared to be in strong linkage disequilibrium in patients as well as in normals ($|D'| \sim 1.0$ for all three combinations).

IL4 *−589 T > C, −33 T > C, and* IL6 *−174 G > C allele frequencies in different ethnic groups*

The comparison of allelic frequencies at *IL4* −589, −33, and *IL6* −174 in Asian Indians with other ethnic groups has been shown in Table 4. Similar to other Asiatic

Table 4 Allele frequencies at −589 and −33 sites of *IL4* and −174 of *IL6* genes in various ethnic groups

		IL4 −589		*IL4 −33*		*IL6 −174*		
S. no.	*Ethnic Population*	*Allele T*	*Allele C*	*Allele T*	*Allele C*	*Allele G*	*Allele C*	*References*
1	Swedish	0.25	0.75	–	–	0.53	0.47	Wilkening *et al* (2008)
2	Caucasian	0.12	0.88	0.11	0.89	0.63	0.37	Hefler *et al* (2005); Kleinrath *et al* (2007)
3	Spanish	–	–	–	–	0.68	0.32	Landi *et al* (2003)
4	Italian	–	–	–	–	0.63	0.37	Boiardi *et al* (2006)
5	Greek	0.11	0.89	–	–	0.78	0.22	Tsezou *et al* (2008)
6	Korean	0.77	0.23	0.77	0.23	–	–	Choi *et al* (2002)
7	Iranian	0.46	0.54	0.28	0.72	–	–	Amirzargar *et al* (2009)
8	Japanese	0.68	0.32	0.67	0.33	–	–	Hegab *et al* (2004)
9	Egyptian	0.32	0.68	0.34	0.66	–	–	Hegab *et al* (2004)
10	Chinese	0.76	0.24	–	–	–	–	Zhu *et al* (2010)
11	Asian Indian	0.78	0.22	0.75	0.25	0.71	0.29	Present study

population such as Chinese, Japanese, and Koreans, Asian Indians showed lower frequency of *IL4* −589 *C and −33 *C alleles as compared to Caucasians, Europeans, and Iranians. Similarly, the *IL6* −174*C allele was found to be less prevalent in Asian Indians than in those of Caucasians and Europeans. Thus, the polymorphic alleles of *IL4* and *IL6* promoters (*IL4* −589*C and −33*C and *IL6* −174*C) in Asian Indians stand closer to other Asians but distant from Caucasians and Europeans.

Discussion

In the present study, we found that functional polymorphism at *IL4* −589 T > C, −33 T > C, and *IL6* −174 G > C have significant effects on the risk of tobacco-related OSCC development. Individuals carrying variant allele at both the loci of *IL4* gene were associated with fairly increased risk of developing the disease, while at *IL6* −174 G > C SNP, C allele appeared to be protective as compared to *IL6* −174 G allele.

IL4 is an anti-inflammatory cytokine and various *in vitro* studies have documented its anti-tumor activity on breast and colon cancers (Toi *et al*, 1992). In colon carcinoma, it inhibited matrix metalloproteinases (MMP-1, MMP-2, and MMP-9), hepatocyte growth factor induced invasion and migration of tumor cells (Uchiyama *et al*, 1996). It also directly modulates proliferation of various cancer cell types including gastric and renal cancers by increasing expression of $p21^{WAFI}$ and interferon regulating factor (IRF-1) and decreasing cyclin-dependent kinase (CDK)-2 activities besides facilitating the infiltration of inflammatory cells such as macrophages, eosinophils, and neutrophils (Yu *et al*, 2004).

Based on these findings, it is conceivable that in our study *IL4* −589 C and −33 C alleles are associated with increased risk of OSCC as these are suggested to decrease the transcription rate of *IL4* gene which may have anti-tumor effects in patients with tobacco-related OSCC. Similarly, in Swedish patients with colorectal carcinoma, *IL4* −589 T allele was associated with longer survival (Wilkening *et al*, 2008). Another statistical study has also reported the association of −589 T and −33 T with lower incidence of kidney cancer in Asians (Ries *et al*, 2005). Some recent studies have also reported a significantly reduced risk of colorectal and gastric cancer development with *IL4* −589T allele (Landi *et al*, 2007; Wu *et al*, 2009). However, other studies have reported −589 CC and −33 CC genotypes as protective in patients with renal cell carcinoma (Kleinrath *et al*, 2007; Zhu *et al*, 2010).

Interestingly, despite being from the same Th2 cytokine category, IL6 has shown opposite effects on cancer. It is a pro-inflammatory cytokine, and it is well established that cancer arises and progresses in chronically inflamed tissue (Coussens and Werb, 2002); particularly, colorectal cancer, hepatocellular carcinoma, and lung adenocarcinoma have strong inflammatory basis (Wilkening *et al*, 2008). Thus, one can predict its role in promoting carcinogenesis. Although exact mechanism of its role in carcinogenesis is yet unknown, *in vitro* studies on ovarian cancer cells have implicated it in favoring tumor growth by modulating host immune responses (Watson *et al*, 1990; Hefler *et al*, 2003). IL6 inhibits anti-tumor T-cell functions by shifting immune response toward Th2/Tc2 type by enhancing IL4 production and inhibits Th1-cell development via SOCS1 (suppressor of cytokine family) which prevents IFN-γ signaling. It also prevents APCs from activating anergic T cells that have potential implications for tumor vaccines (Spaner, 2004). Noticeably, in case with breast cancer, it has been shown to inhibit cell growth *in vitro* (Asgeirsson *et al*, 1998) and had anti-adhesive effects (Badache and Hynes, 2001).

Regarding *IL6* −174 G > C SNP, our results did not show significant risk association with tobacco-related OSCC, although −174 C allele appeared to be protective that is reportedly associated with low serum levels of IL6 (Terry *et al*, 2000). However, the influential role of this SNP on the cytokine expression and different cancer susceptibility is ambiguous and controversial. While a recent meta-analysis reported no association of this SNP with the breast cancer risk (Yu *et al*, 2010), another study has also shown the lack of association between *IL6* −174 G > C, its expression and susceptibility to sporadic colon cancer (Cacev *et al*, 2010). On the other hand, a study on ovarian cancer in Caucasian

subjects has shown the disease-free survival and overall survival is higher in *IL6* −174 CC or GC as compared to GG (Hefler *et al*, 2003). Similarly, this SNP also appeared to be associated with the risk of papillary thyroid carcinoma in Turkish population (Ozgen *et al*, 2009). These contradictions can be attributed to various reasons. First, *IL6* promoter harbors four functional SNPs (−597, −572, −373, and −174), and it is not one polymorphism that makes the difference but other SNPs in the vicinity that act synergistically (Terry *et al*, 2000). So, whole array of SNPs in a particular gene should be studied. Second, IL6 has both pro- as well as anti-inflammatory properties and it seems to be regulated differently depending on the type of cell it is expressed in. Lastly, the confusion in the nomenclature of *IL6* −174 alleles because the GG/CC allelic frequencies are close to 50% in Caucasians and both the alleles are the complementary nucleotides C and G. Thus, the allele annotation depends on which DNA strand is used as a reference sequence (Wilkening *et al*, 2008).

We also analyzed the distribution of *IL4* −589 T > C, −33 T > C, and *IL6* −174 G > C allele frequencies among different ethnic populations, and found that *IL4* allele frequencies were significantly different between Asian and Caucasian populations. In the present study, *IL4* −589 C (0.22) and −33 C (0.25) frequencies in normal population were close to that of Korean (0.23 and 0.23, respectively), Chinese (0.24) and Japanese (0.32 and 0.33, respectively), but was far apart from Caucasians (0.88 and 0.89, respectively) (Table 4). This racial heterogeneity further explains the discrepancies between risk associations of these SNPs with different cancers in different ethnic groups.

Although we have analyzed relatively smaller sample size, our results convincingly show significant risk of tobacco-related OSCC in patients with *IL4* −589 T > C and −33 T > C functional polymorphisms. We also found that the mutant CC genotype was associated with an increased risk of OSCC compared with their wild-type TT homozygote and lack of association between *IL6* −174 G > C SNP and the OSCC susceptibility. Thus, *IL4* −589 T > C and −33 T > C functional polymorphisms may represent a novel and independent prognostic marker for tobacco-related OSCC in Asian Indians.

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Author contributions

Dr. Das and Poonam were responsible for study design, planning the experiments, data analysis, manuscript preparation and revision; Poonam and Manasi were responsible for sample preparation, Poonam was responsible for experiments and Dr. Mohanti for recruitment and clinical evaluation of patients and review of the manuscript.

Conflict of interests

There is no conflict of interests.

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