

Genetic polymorphisms in the *PTPN13* gene and risk of squamous cell carcinoma of head and neck

Jiangong Niu¹, Yu-Jing Huang¹, Li-E Wang¹, Erich M. Sturgis^{1,2} and Qingyi Wei^{1,*}

¹Department of Epidemiology and ²Department of Head and Neck Surgery, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA

*To whom correspondence should be addressed. Tel: +1 713 745 2481; Fax: +1 713 563 0999; Email: qwei@mdanderson.org

Fas-associated phosphatase-1 is encoded by the protein tyrosine phosphatase, non-receptor type 13 (*PTPN13*) gene and attributes to the resistance to Fas-mediated apoptosis in several tumors, including squamous cell carcinoma of the head and neck (SCCHN). However, no epidemiological studies have investigated the roles of *PTPN13* polymorphisms in SCCHN risk. In this hospital-based case-control study of 1069 SCCHN patients and 1102 non-Hispanic white cancer-free controls, we evaluated the associations between three single-nucleotide polymorphisms c.4068 T>G F1356L (rs10033029), c.4566 A>G I1522M (rs2230600) and c.6241 T>G Y2081D (rs989902) located in the coding region of *PTPN13* and SCCHN risk. We found that a significantly increased SCCHN risk was associated with the c.4566 I1522M GG genotype [odds ratio (OR), 1.89; 95% confidence interval (CI), 1.27–2.79] and c.6241 Y2081D GT genotype (OR, 1.26; 95% CI, 1.03–1.53) compared with the c.4566 I1522M AA and c.6241 Y2081D TT genotypes, respectively. Further stratified analyses showed that risk associated with the c.4566 I1522M GG genotype was more profound in the subgroups of young (≤ 57 years), males, never smokers, current drinkers and patients with pharyngeal cancer; that risk associated with c.6241 Y2081D GT genotype persisted in subgroups of old (> 57 years), males, current drinkers and patients with pharyngeal and laryngeal cancers and that risk associated with c.6241 Y2081D GG genotype was borderline in patients with laryngeal cancer. In conclusion, polymorphisms in the *PTPN13* coding region may be biomarkers for susceptibility to SCCHN in USA populations.

Introduction

Squamous cell carcinoma of the head and neck (SCCHN) is one of the common malignancies in the USA, accounting for ~3.2% of newly diagnosed cancer cases in 2009, with the estimated 48 010 new cases and 11 260 deaths (1). While the history of tobacco and alcohol use remains to be the main risk factors, many studies suggested that genetic predisposition may play an important role in the etiology of SCCHN (2,3). Indeed, our previous studies have suggested that polymorphisms in genes involved in cell cycle regulation, DNA repair and apoptosis were associated with the risk of SCCHN (4–7).

Apoptosis is a programmed cell death that ensures normal cell homeostasis, and resistance to apoptosis is one of the main characters of many tumors. The apoptosis processes can be divided into two categories: extrinsic and intrinsic (or mitochondrial) apoptosis pathways. Typically, the extrinsic apoptosis pathway is induced by binding of external cell death signals to the tumor necrosis factor receptor superfamily of death receptors. Among all death receptors, Fas/CD95

is the most frequently studied one. Binding of Fas ligand (FasL) to Fas triggers the recruitment of Fas-associated death domain, pro-caspase 8 and pro-caspase 10 to form the death-inducing signaling complex, which then leads to the activation of caspase 8 and caspase 10, the initiators of apoptosis. The activated caspase 8 and caspase 10 cleave effector caspases (e.g. caspase 3, caspase 6 and caspase 7) and then trigger apoptosis. Fas apoptosis signaling has also been suggested to be involved in activation of the mitochondrial death-signaling pathway through induction of cytochrome *c* release (8). Although Fas and FasL are normally expressed in many tumors, such as pancreatic adenocarcinoma, hepatocellular carcinoma and colon adenocarcinoma, these tumors are resistant to Fas-mediated apoptosis, suggesting an inhibitory mechanism of Fas signaling existed in tumors (9–11). Further studies showed that high expression of Fas-associated phosphatase-1 (FAP-1) might attribute to inhibition of the Fas-mediated apoptosis in these tumors (10–12).

FAP-1 is a 270 kDa non-transmembrane protein encoded by the *PTPN13* gene (protein tyrosine phosphatase, non-receptor type 13 gene, also known as PTPL1—protein tyrosine phosphatase 1 or FAP-1 gene) (8). FAP-1 interacts with the C-terminus of Fas through its PDZ domain and reduces the membrane localization of Fas, therefore inhibiting the Fas-mediated apoptosis, possibly through the dephosphorylation of Fas by FAP-1 (13–15). FAP-1 is highly expressed in various apoptosis-resistant tumor cells, including SCCHN (9–11,16,17). For example, studies showed that human SCCHN cells expressed Fas and FasL were resistant to the Fas-mediated apoptosis, that FAP-1 overexpression correlated with the downregulation of cell surface Fas in SCCHN cells and that FAP-1 induced the resistance of these cells to the Fas-mediated apoptosis through the activation of nuclear factor-kappaB (17,18). Moreover, increased FAP-1 expression was reported to be associated with the tumor progression toward malignancy during oral carcinogenesis (19), and inhibition of FAP-1 by Etodolac, a selective cyclooxygenase-2 inhibitor, has been shown to enhance carboplatin-induced apoptosis of human tongue carcinoma cells (20). These findings suggested that FAP-1 overexpression is correlated with the progressive tumor growth possibly due to the inhibition of Fas-mediated apoptosis in SCCHN cells, implying an important role of FAP-1 in the development of SCCHN.

The *PTPN13* gene is located on chromosome 4q21.3 with 48 exons. This gene reportedly has 865 variants (as of October, 2009; <http://www.ncbi.nlm.nih.gov/projects/SNP/>), but most of which are either extremely rare or non-detectable in the general population. There are only four common (minor allele frequency ≥ 0.05) and potentially functional single-nucleotide polymorphisms (SNPs) (rs10033029, rs2230600, rs12500797 and rs989902) because they are all in the exons and cause amino acid changes. Some studies suggested that the *PTPN13* polymorphisms may be associated with predisposition to cancer risk. Although the function of FAP-1 has been well studied, only few published association studies have evaluated the roles of genetic polymorphisms of the *PTPN13* gene on cancer risk, in which the *PTPN13* cSNP6304 (rs989902) GG genotype was found to be associated with significantly increased risk of hepatocellular carcinomas (21). To date, no published study has investigated the association between *PTPN13* polymorphisms and SCCHN risk. In the current study, we hypothesize that *PTPN13* polymorphisms are associated with risk of SCCHN. To test this hypothesis, we genotyped three functional polymorphisms in the *PTPN13* coding regions [c.4068 T>G F1356L (rs10033029), which is in linkage disequilibrium with c.4873 G>A E1625K (rs12500797), c.4566 A>G I1522M (rs2230600) and c.6241 T>G Y2081D (rs989902)] and evaluated their associations with SCCHN risk in a non-Hispanic white population.

Abbreviations: CI, confidence interval; FAP-1, Fas-associated phosphatase-1; FasL, Fas ligand; OR, odds ratio; PCR, polymerase chain reaction; *PTPN13*, protein tyrosine phosphatase, non-receptor type 13; RFLP, restriction fragment length polymorphism; SCCHN, squamous cell carcinoma of the head and neck; SNP, single-nucleotide polymorphism.

Materials and methods

Study subjects

The study population included 1069 patients with newly diagnosed SCCHN (i.e. cancers of the oral cavity, oropharynx, hypopharynx and larynx) at The University of Texas MD Anderson Cancer Center between October 1999 and October 2007 and 1102 cancer-free controls recruited during the same period. The patients with second primary tumors, primary tumors of nasopharynx or sinonasal tract or any histopathologic diagnoses other than SCCHN were excluded. The cancer-free controls were recruited from the visitors accompanying patients to clinics and were not genetically related to the patients. The cancer-free controls were frequency matched to cases by age (± 5 years) and sex. The response rate for SCCHN patients and cancer-free controls were ~ 93 and 85% , respectively. Having signed a written informed consent, the subjects were interviewed to obtain data on age, sex, ethnicity and the history of tobacco and alcohol use. Each subject donated 30 ml venous blood that was used for biomarker assays, including DNA extraction for genotyping. The research protocol was approved by the MD Anderson Institutional Review Board.

Genotyping of *PTPN13*

The SNPlex assays were used to genotype three *PTPN13* SNPs [c.4068 T>G F1356L (rs10033029), c.4566 A>G I1522M (rs2230600) and c.6241 T>G Y2081D (rs989902)] in the DNA Core Lab at MD Anderson Cancer Center, according to the protocol of manufacturer (Applied Biosystems, Foster City, CA). However, the SNP c.6241 T>G Y2081D (rs989902) failed in SNPlex assays and was then genotyped by the restriction fragment length polymorphism (RFLP)-polymerase chain reaction (PCR) method. The data generated by SNPlex assays were analyzed in GeneMapper software (Applied Biosystems) to determine the genotypes. The samples failed to be genotyped in SNPlex assays were also re-evaluated with RFLP. The primers we used in RFLP for three SNPs were c.4068 T>G F1356L (forward) 5'-AGGCTTAGGAGGTGATGGAG-3' and (reverse) 5'-TGACTCTAACAAAGAAATCTTTGTTT-3'; c.4566 A>G I1522M (forward) 5'-TTTTCTCGAGAAGATAATCTTGT-3' and (reverse) 5'-TCAAGATAACATCTCTACATCA-3' and c.6241 T>G Y2081D (forward) 5'-TCACGTACCTGGACCACAGGAGT-3' and (reverse) 5'-TCCCAA-GAAGCTGAAGTTATCCAGTC-3'. The PCRs were performed by 35 cycles with an annealing temperature of 53°C (c.4068 T>G F1356L), 48°C (c.4566 A>G I1522M) and 62°C (c.6241 T>G Y2081D), respectively. For c.4068 T>G F1356L, the 113 bp PCR products were digested with *BsmI* (New England Biolabs, Beverly, MA) at 55°C overnight, the G allele generated 91 and 22 bp bands, whereas T allele remained uncut; for c.4566 A>G I1522M, the 123 bp PCR products were digested with *RsaI* (New England Biolabs) at 37°C overnight, the G allele was uncut and the A allele was cut into 101 and 22 bp bands; for c.6241 T>G Y2081D, the 121 bp PCR products were digested with *BsmI* at 55°C overnight (New England Biolabs), the T allele produced 78 and 33 bp bands and G allele was cut into 51, 33 and 27 bp bands. For all three SNPs, $\sim 10\%$ of the samples were random selected and repeated with RFLP, and the error rate was $< 1\%$. Those samples with inconsistent genotype results were excluded from the final analysis.

Statistical analysis

All data were analyzed with SAS statistical software program (SAS/STAT version 9.1.3; SAS Institute Inc., Cary, NC). The chi-square tests were used to evaluate the differences in the frequency distributions of categorical variables, including demographic characteristics, tobacco smoking, alcohol use, and alleles and genotypes of *PTPN13* polymorphisms between the cases and controls. The associations between *PTPN13* polymorphisms and SCCHN risk were evaluated with unconditional univariate and multivariate logistic regression analyses through the estimation of odds ratios (ORs) and 95% confidence intervals (CIs) with or without adjustment for age, sex, smoking and drinking status. The associations of genotypes with SCCHN risk in subgroups were also assessed by stratification analysis by age, sex, smoking status, alcohol use and tumor sites. Homogeneity of ORs between different strata was tested as described previously (22) followed by analyses of gene-gene and gene-environment interactions, which were evaluated with the *P* value for the product of two variables of interest in multivariate logistic regression model with adjustment for age, sex, smoking and drinking status. Haplotypes for the *PTPN13* polymorphisms were constructed according to the PHASE program (23,24). In brief, each subject was assigned a pair of haplotypes with the highest estimated probabilities. The haplotype frequencies of $< 5\%$ were combined into one group representing all rare haplotypes, and the haplotype with the highest frequency in the controls was used as the reference group for comparisons. Pearson's chi-square test was used to test for the difference in haplotypes distributions between cases and controls. Diplotype analysis was conducted in a similar manner, with the assignment of a pair of haplotypes as a diplotype for each subject. All tests were two sided and *P* < 0.05 was considered significant.

Results

In the present study, the 1069 cases and 1102 controls of non-Hispanic whites were well matched by age and sex (*P* = 0.567 and 0.652, respectively) (Table I). The mean ages were 57.1 ± 11.1 (age range 18–90) for cases and 56.8 ± 11.1 (age range 20–87) for controls. There were more males in both cases and controls. The cases, compared with the controls, had more smokers (current smokers 37.9 versus 14.5% and former smokers 34.1 versus 36.8%) and more drinkers (current drinkers 50.8 versus 40.2% and former drinkers 21.7 versus 16.0%) (*P* < 0.001 for both smoking and drinking status). Smoking or drinking status appeared to be a risk factor for SCCHN in our study population. Therefore, these two variables were adjusted in the subsequent multivariate logistic regression analyses.

According to the single nucleotide polymorphism database from the National Center for Biotechnology Information, there were four common missense polymorphisms located in the coding regions of the *PTPN13* gene that had minor allele frequency $> 5\%$ in Caucasian populations, including c.4068 T>G F1356L (rs10033029), c.4566 A>G I1522M (rs2230600), c.4873 G>A E1625K (rs12500797) and c.6241 T>G Y2081D (rs989902). Among these four polymorphisms, c.4068 T>G F1356L was in linkage disequilibrium with c.4873 G>A E1625K ($r^2 = 0.93$) according to the HapMap; therefore, c.4873 G>A E1625K was excluded from genotyping in our study. The other three polymorphisms were genotyped by SNPlex assays, followed by RFLP. Figure 1A showed the locations of the SNPs in the gene and RFLP genotyping results of three SNPs. The strong linkage disequilibrium between rs10033029 and rs12500797 was displayed in Figure 1B, as shown by a high r^2 value ($r^2 = 0.93$). The distributions of different genotypes of these three SNPs in control subjects were in agreement with Hardy-Weinberg equilibrium (*P* = 0.099 for c.4068 T>G F1356L, 0.078 for c.4566 A>G I1522M and 0.629 for c.6241 T>G Y2081D).

When the c.4566 A>G I1522M AA genotype was used as the reference, the GG genotype, but not the AG genotype, was associated with a significantly increased risk to SCCHN (adjusted OR, 1.89; 95% CI, 1.27–2.79). When the c.6241 T>G Y2081D TT genotype was used as the reference, a significantly increased risk was associated with the GT genotype (adjusted OR, 1.26; 95% CI, 1.03–1.53) but not

Table I. Demographic characteristics of SCCHN cases and cancer-free controls

Variables	Cases n (%)	Controls n (%)	<i>P</i> value ^a
All subjects	1069 (100.0)	1102 (100.0)	
Age ^b (years)			
Range	18–90	20–87	0.567
Mean	57.1 \pm 11.1	56.8 \pm 11.1	
≤ 57	568 (53.1)	572 (51.9)	
> 57	501 (46.9)	530 (48.1)	
Sex			0.652
Female	264 (24.7)	263 (23.9)	
Males	805 (75.3)	839 (76.1)	
Smoking status			< 0.001
Never	300 (28.1)	536 (48.6)	
Former	364 (34.1)	406 (36.8)	
Current	405 (37.9)	160 (14.5)	
Drinking status			< 0.001
Never	294 (27.5)	483 (43.8)	
Former	232 (21.7)	176 (16.0)	
Current	543 (50.8)	443 (40.2)	
Tumor site			
Oral cavity	316 (29.6)		
Pharynx ^c	587 (54.9)		
Larynx	166 (15.5)		

^aTwo-sided chi-square test.

^bThe age group was divided by the median age of the controls.

^cIncluded both oropharyngeal and hypopharyngeal cancer cases.

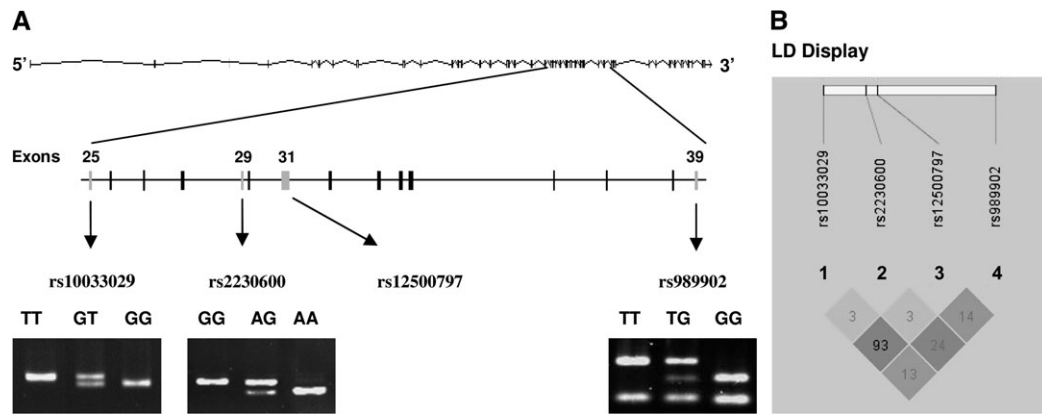


Fig. 1. *PTPN13* gene structure, genotypes and hypotypes with the display of four SNPs c.4068 T>G F1356L (rs10033029), c.4566 A>G I1522M (rs2230600), c.4873 G>A E1625K (rs12500797) and c.6241 T>G Y2081D (rs989902). (A) *PTPN13* gene structure and locations of rs10033029, rs2230600, rs12500797 and rs989902. RFLP genotyping results for rs10033029, rs2230600 and rs989902 were shown as displayed; (B) linkage disequilibrium (LD) display for the four SNPs of *PTPN13*. The colors were based on D' and logarithm (base 10) of odds (LOD) values. The dark gray area represented $D' = 1$ and $\text{LOD} \geq 2$, and the light gray area represented $D' < 1$ and $\text{LOD} \geq 2$. The numbers displayed in the diamonds are $r^2 \times 100$ for pairwise SNPs.

Table II. Genotyping distribution of *PTPN13* SNPs of SCCHN cases and cancer-free controls and their association with SCCHN risk

SNP position	Cases, n (%)	Controls, n (%)	P value	Crude OR (95% CI)	Adjusted OR (95% CI) ^a
All subjects	1069 (100.0)	1102 (100.0)			
c.4068 T>G F1356L (rs10033029)			0.326 ^b		
TT	815 (76.2)	857 (77.8)		1.00	1.00
GT	239 (22.4)	223 (20.2)		1.13 (0.92–1.39)	1.09 (0.88–1.35)
GG	15 (1.4)	22 (2.0)		0.72 (0.37–1.39)	0.61 (0.31–1.22)
G allele	0.126	0.121	0.640 ^c		
c.4566 A>G I1522M (rs2230600)			0.001 ^b		
AA	695 (65.0)	747 (67.8)		1.00	1.00
AG	290 (27.1)	310 (28.1)		1.01 (0.83–1.22)	1.00 (0.82–1.22)
GG	84 (7.9)	45 (4.1)		2.01 (1.38–2.92)	1.89 (1.27–2.79)
G allele	0.214	0.182	0.007 ^c		
c.6241 T>G Y2081D (rs989902)			0.014 ^b		
TT	332 (31.1)	392 (35.6)		1.00	1.00
GT	572 (53.5)	524 (47.6)		1.29 (1.07–1.56)	1.26 (1.03–1.53)
GG	165 (15.4)	186 (16.9)		1.05 (0.81–1.35)	1.00 (0.76–1.30)
G allele	0.422	0.407	0.304 ^c		

^aAdjusted for age, sex, smoking and drinking status in the logistic regression model.

^bDistribution of three genotypes.

^cTest for allele differences between cases and controls.

Table III. *PTPN13* haplotype and diplotype distributions of SCCHN cases and cancer-free controls and their association with SCCHN risk^a

Haplotype	Cases (n = 2138), chromosome no. (%)	Controls (n = 2204), chromosome no. (%)	Crude OR (95% CI)	P^b	Adjusted OR (95% CI) ^c	P^b
T-A-T	1123 (52.5)	1216 (55.2)	1.00		1.00	
T-A-G	311 (14.5)	332 (15.1)	1.03 (0.86–1.23)	0.736	1.02 (0.85–1.23)	0.828
T-G-G	357 (16.7)	327 (14.8)	1.17 (0.98–1.40)	0.081	1.14 (0.95–1.38)	0.157
G-A-G	228 (10.7)	233 (10.6)	1.05 (0.86–1.30)	0.620	1.00 (0.80–1.24)	0.973
Others ^d	119 (5.6)	96 (4.4)	1.27 (0.99–1.64)	0.063	1.23 (0.94–1.61)	0.132
Diplotype	Cases (n = 1069), n (%)	Controls (n = 1102), n (%)	Crude OR (95% CI)	P^b	Adjusted OR (95% CI) ^c	P^b
T-A-T/T-A-T	280 (26.2)	338 (30.7)	1.00		1.00	
T-A-T/T-A-G	199 (18.6)	188 (17.1)	1.26 (0.98–1.63)	0.072	1.25 (0.96–1.64)	0.101
T-A-T/T-G-G	192 (18.0)	191 (17.3)	1.22 (0.93–1.60)	0.162	1.18 (0.89–1.57)	0.254
T-A-T/G-A-G	137 (12.8)	118 (10.7)	1.40 (1.04–1.90)	0.029	1.34 (0.98–1.84)	0.070
Others ^e	261 (24.4)	267 (24.2)	1.16 (0.91–1.47)	0.232	1.10 (0.86–1.41)	0.464

^aThe P values for chi-square tests were 0.125 for haplotypes and 0.150 for diplotypes frequencies distributions. The haplotypes and diplotypes were based on the order of three SNPs c.4068 T>G F1356L, c.4566 A>G I1522M and c.6241 T>G Y2081D.

^bTwo-sided chi-square tests in logistic regression models.

^cAdjusted for age, sex, smoking and drinking status in the logistic regression model.

^dIncludes four minor haplotypes, each with a frequency <0.05.

^eIncludes 19 minor diplotypes, each with a frequency <0.05.

Table IV. Stratification analysis of the association between *PTPN13* SNPs and SCCHN risk

Variables	c.4566 A>G I1522M			c.6241 T>G Y2081D		
	AA	AG	GG	TT	GT	GG
Age						
≤57						
Cases/controls	358/388	162/158	48/26	181/195	299/276	88/101
Adjusted OR (95% CI) ^b	1.00	1.11 (0.84–1.45)	1.96 (1.17–3.28)	1.00	1.15 (0.88–1.51)	0.91 (0.63–1.30)
>57						
Cases/controls	337/359	128/152	36/19	151/197	273/248	77/85
Adjusted OR (95% CI) ^b	1.00	0.90 (0.67–1.21)	1.72 (0.94–3.16)	1.00	1.36 (1.01–1.81)	1.10 (0.74–1.64)
<i>P</i> for interaction ^a			0.126			0.327
Sex						
Female						
Cases/controls	170/191	75/60	19/12	78/87	145/134	41/42
Adjusted OR (95% CI) ^b	1.00	1.42 (0.93–2.17)	1.74 (0.79–3.84)	1.00	1.07 (0.71–1.61)	1.08 (0.61–1.89)
Male						
Cases/controls	525/556	215/250	65/33	254/305	427/390	124/144
Adjusted OR (95% CI) ^b	1.00	0.91 (0.72–1.14)	1.95 (1.24–3.07)	1.00	1.31 (1.05–1.65)	0.98 (0.72–1.32)
<i>P</i> for interaction ^a			0.310			0.974
Smoking status						
Never						
Cases/controls	197/374	81/146	22/16	103/193	152/257	45/86
Adjusted OR (95% CI) ^b	1.00	1.05 (0.76–1.45)	2.55 (1.31–4.98)	1.00	1.10 (0.81–1.51)	0.99 (0.64–1.52)
Former						
Cases/controls	230/262	108/125	26/19	109/143	202/195	53/68
Adjusted OR (95% CI) ^b	1.00	0.97 (0.71–1.32)	1.63 (0.8–3.04)	1.00	1.36 (0.99–1.87)	1.03 (0.66–1.60)
Current						
Cases/controls	268/111	101/39	36/10	120/56	218/72	67/32
Adjusted OR (95% CI) ^b	1.00	1.01 (0.64–1.58)	1.55 (0.73–3.31)	1.00	1.39 (0.90–2.14)	0.95 (0.55–1.65)
<i>P</i> for interaction ^a			0.539			0.794
Drinking status						
Never						
Cases/controls	201/330	68/131	25/22	97/165	149/240	48/78
Adjusted OR (95% CI) ^b	1.00	0.88 (0.62–1.24)	1.76 (0.96–3.23)	1.00	1.02 (0.74–1.42)	1.05 (0.67–1.63)
Former						
Cases/controls	148/116	64/50	20/10	69/69	125/76	38/31
Adjusted OR (95% CI) ^b	1.00	1.03 (0.65–1.63)	1.33 (0.58–3.02)	1.00	1.54 (0.97–2.42)	1.06 (0.58–1.94)
Current						
Cases/controls	346/301	158/129	39/13	166/158	298/208	79/77
Adjusted OR (95% CI) ^b	1.00	1.08 (0.80–1.45)	2.58 (1.30–5.08)	1.00	1.39 (1.03–1.87)	0.91 (0.60–1.36)
<i>P</i> for interaction ^a			0.371			0.980
Tumor site						
Oral cavity						
Cases/controls	220/747	76/310	20/45	108/392	170/524	38/186
Adjusted OR (95% CI) ^b	1.00	0.82 (0.60–1.12)	1.31 (0.73–2.35)	1.00	1.05 (0.78–1.41)	0.71 (0.46–1.09)
Pharynx						
Cases/controls	370/747	167/310	50/45	182/392	314/524	91/186
Adjusted OR (95% CI) ^b	1.00	1.08 (0.86–1.36)	2.18 (1.41–3.37)	1.00	1.31 (1.04–1.65)	1.03 (0.75–1.41)
Larynx						
Cases/controls	105/747	47/310	14/45	42/392	88/524	36/186
Adjusted OR (95% CI) ^b	1.00	1.18 (0.79–1.78)	1.78 (0.87–3.65)	1.00	1.63 (1.06–2.51)	1.71 (1.00–2.90)

^aThe interaction *P* value was obtained from the chi-square test for the product of two variables in multivariate logistic regression model.

^bAdjusted for age, sex, smoking and drinking status in the logistic regression model.

with the GG genotype (adjusted OR, 1.00; 95% CI, 0.76–1.30). No significantly altered risk of SCCHN was observed for c.4068 T>G F1356L GT and GG genotypes compared with the TT genotype (Table II). Distributions of the minor alleles were statistically different for the c.4566 A>G I1522M G allele (*P* = 0.007), but not for the 4068 T>G F1356L G allele or c.6241 T>G Y2081D G allele, between cases and controls (*P* = 0.640 and 0.304, respectively).

To determine the contribution of *PTPN13* SNPs to SCCHN risk, we also performed haplotypes and diplotypes analyses (Table III). Among all eight possible haplotypes we inferred, four had frequencies >5%. The overall distribution of *PTPN13* haplotypes was not statistically significant between cases and controls (global test: *P* = 0.125). The most frequent haplotype T-A-T (in the order of c.4068 T, c.4566 A and c.6241 T) (52.5% in cases and 55.2% in controls) was used as the reference, whereas all the haplotypes <5% were grouped as 'others', representing one group of rare haplotypes. We did not find

any statistically significantly altered risk associated with any of haplotypes compared with the reference. Similarly, among 23 possible diplotypes, four had a frequency >5%. The overall distribution of *PTPN13* diplotypes was not statistically significant (*P* = 0.150). Compared with the most frequent diplotype T-A-T/T-A-T (26.2% in cases and 30.7% in controls), the diplotype T-A-T/G-A-G had significantly increased risk only in univariate analysis (OR, 1.40; 95% CI, 1.04–1.90), suggesting the combination of G allele from c.4068 T>G F1356L and G allele from c.6241 T>G Y2081D may contribute to SCCHN risk. However, this risk was reduced to borderline significant after adjustment for age, sex, smoking and drinking status (OR, 1.34; 95% CI, 0.98–1.84), suggesting our study sample size was still not large enough to provide a sufficient study power.

We then evaluated the association between *PTPN13* variant genotypes and SCCHN risk stratified by age, sex, smoking and drinking status and tumor site. As shown in Table IV, the c.4566 A>G I1522M

GG genotype was associated with a significantly increased risk of SCCHN in the subgroups of ≤ 57 years (OR, 1.96; 95% CI, 1.17–3.28), males (OR = 1.95; 95% CI, 1.24–3.07), never smokers (OR, 2.55; 95% CI, 1.31–4.98), current drinkers (OR, 2.58; 95% CI, 1.30–5.08) and patients with cancer of pharynx (OR, 2.18; 95% CI, 1.41–3.37); the risk for the c.6241 Y2081D GT genotype was more pronounced in the subgroups of > 57 years (OR, 1.36; 95% CI, 1.01–1.81), males (OR, 1.31; 95% CI, 1.05–1.65), current drinkers (OR, 1.39 95% CI, 1.03–1.87) and patients with primary tumor sites of pharynx (OR, 1.31; 95% CI, 1.04–1.65) and larynx (OR, 1.63; 95% CI, 1.06–2.51) and the c.6241 Y2081D GG genotype was associated with a borderline risk of laryngeal cancer (OR, 1.71; 95% CI, 1.00–2.90; $P = 0.049$). These data suggested that risk factors may interact with genotypes of *PTPN13* SNPs in the etiology of SCCHN. However, there was no statistical evidence to support such interactions, as evaluated in our multivariate logistic regression model (Table IV).

Discussion

In our current hospital-based case-control study, we investigated the associations between three *PTPN13* functional polymorphisms and SCCHN risk. Individually, the c.4566 A>G I1522M GG genotype and the c.6241 T>G Y2081D GT genotype were associated with significantly increased risk of SCCHN. Further stratified analysis confined the risk associated with the c.4566 A>G I1522M GG genotype to subgroups of ≤ 57 years, males, never smokers, current drinkers and primary tumor site of the pharynx and the risk associated with the c.6241 Y2081D GT genotype to subgroups of > 57 years, males, current drinkers and patients with cancers of the pharynx and larynx. To our best knowledge, this is the largest case-control study on the susceptibility of *PTPN13* polymorphisms to cancer risk and the first on SCCHN.

PTPN13 encodes FAP-1, which is often overexpressed in multiple cancers and functions to resist Fas-related death signals. Tyrosine phosphorylation of Fas triggered by the treatment of FasL was thought to be the key step in the Fas-mediated apoptosis, and dephosphorylation of phosphor-tyrosine 275 in the carboxyl terminus of Fas by FAP-1 was suggested to be one of the mechanisms underlying the resistance to the Fas-mediated apoptosis by cancer cells (14). Besides, FAP-1-mediated activation of nuclear factor-kappaB was also suggested to be responsible for the resistance of SCCHN cells to the Fas-mediated apoptosis (17). Another study found that FAP-1 suppressed apoptosis pathway mediated by common neurotrophin receptor p75, which is a member of tumor necrosis factor receptor superfamily (25). Taken together, FAP-1 encoded by *PTPN13* plays an important role in the resistance of cancer cells to apoptosis. Therefore, it is biologically plausible that the functional polymorphisms in this gene may contribute to the altered functions of FAP-1. However, detailed mechanistic studies on the functions of these observed polymorphisms are needed to unravel the underlying molecular mechanisms.

In the literature, we only found one case-control study of the associations between *PTPN13* polymorphisms and cancer risk. In this study of 534 hepatocellular carcinoma cases and 295 control subjects, the *PTPN13* c.6241 T>G Y2081D (reported as cSNP6304) GG genotype was shown to be associated with significantly increased risk of multiplex familial hepatocellular carcinomas (OR, 2.67; 95% CI, 1.15–6.17) but not for the GT genotype (OR, 1.17; 95% CI, 0.58–2.40) nor for the c.4566 A>G I1522M (reported as cSNP4629) GG genotype (21). Interestingly, our much larger study showed the opposite results because we found that the c.4566 A>G I1522M GG genotype and the c.6241 T>G Y2081D GT, but not GG, genotype were associated with significantly altered risk of SCCHN. There are several explanations for these seemingly discrepant results. First, the risk associated with these genotypes may be tissue specific; second, the published study of hepatocellular carcinomas had a very limited sample size, with only 295 controls and 48 multiplex familial hepatocellular carcinoma cases, which may not have enough study power to detect the risks associated with these genotypes; third, the risk asso-

ciated with *PTPN13* variant genotypes differed by age, sex, smoking and drinking status and primary sites of tumors, such a stratification analysis needs a much larger sample size, as we demonstrated in the present study.

In our study population, ~50% of subjects had the c.6241 T>G Y2081D heterozygous genotype (GT) that was associated with a greater risk of SCCHN than was the homozygous GG genotype. As a result, some of the haplotypes we inferred was associated with a lower risk of SCCHN than individual polymorphisms we examined. One explanation to these results could be heterozygote advantage in the penetration of phenotype, i.e. alleles T and G with different functions could act together to exert stronger phenotype effects. Indeed, heterozygote advantage has been reported to explain the polymorphisms in major histocompatibility complex, as heterozygotes presented two sets of epitopes to T cells and could respond more efficiently to pathogens (26–28). However, further studies on the underlying molecular mechanisms are needed to determine whether heterozygote advantage does exist for the .6241 T>G Y2081D polymorphism.

In summary, our study indicated that subjects who carried the c.4566 A>G I1522M GG genotype and the c.6241 T>G Y2081D GT genotype had a significantly increased risk of SCCHN. These SNPs cause amino acids change that may lead to the altered function of FAP-1 but may not be reflected by the altered expression level. However, further functional assays addressing amino acid change for its functionality are not readily available. However, the results supported our hypothesis that functional polymorphisms of *PTPN13* are associated with SCCHN risk. Considering the large sample size of non-Hispanic whites, this study provides reliable risk estimates. Because of its nature of a hospital-based case-control study, an uncontrolled bias may exist in our selection of participants. Therefore, our results should be further validated by other larger, population-based, preferably prospective studies.

Funding

National Institute of Health (R01 CA131274, R01 ES011740) to Qingyi Wei (P50 CA097007), Scott Lippman; cancer prevention fellowship (R25T CA057730) to Robert Chamberlain and Shine Chang (P30 CA016672); The University of Texas MD Anderson Cancer Center.

Acknowledgements

We thank Margaret Lung and Kathryn L. Tipton for their assistance in recruiting the subjects and gathering the questionnaire information and Zhensheng Liu, Yawei Qiao, Jianzhong He, Kejing Xu and Min Zhao for laboratory assistance.

Conflict of Interest Statement: None declared.

References

- Jemal, A. *et al.* (2009) Cancer statistics, 2009. *CA Cancer J. Clin.*, **59**, 225–249.
- Hsu, T.C. *et al.* (1991) Mutagen sensitivity: a biological marker of cancer susceptibility. *Cancer Epidemiol. Biomarkers Prev.*, **1**, 83–89.
- Ho, T. *et al.* (2007) Epidemiology of carcinogen metabolism genes and risk of squamous cell carcinoma of the head and neck. *Head Neck*, **29**, 682–699.
- Chen, K. *et al.* (2007) Single-nucleotide polymorphisms at the TP53-binding or responsive promoter regions of BAX and BCL2 genes and risk of squamous cell carcinoma of the head and neck. *Carcinogenesis*, **28**, 2008–2012.
- Sturgis, E.M. *et al.* (2002) DNA repair gene ERCC1 and ERCC2/XPD polymorphisms and risk of squamous cell carcinoma of the head and neck. *Arch. Otolaryngol. Head Neck Surg.*, **128**, 1084–1088.
- Zhang, Z. *et al.* (2006) Polymorphisms of FAS and FAS ligand genes involved in the death pathway and risk and progression of squamous cell carcinoma of the head and neck. *Clin. Cancer Res.*, **12**, 5596–5602.

7. Chen, K. *et al.* (2008) CASP3 polymorphisms and risk of squamous cell carcinoma of the head and neck. *Clin. Cancer Res.*, **14**, 6343–6349.
8. Gloire, G. *et al.* (2008) Regulation of CD95/APO-1/Fas-induced apoptosis by protein phosphatases. *Biochem. Pharmacol.*, **76**, 1451–1458.
9. Ungefroren, H. *et al.* (1998) Human pancreatic adenocarcinomas express Fas and Fas ligand yet are resistant to Fas-mediated apoptosis. *Cancer Res.*, **58**, 1741–1749.
10. Lee, S.H. *et al.* (2001) Expression of Fas and Fas-related molecules in human hepatocellular carcinoma. *Hum. Pathol.*, **32**, 250–256.
11. Yao, H. *et al.* (2004) Expression of FAP-1 by human colon adenocarcinoma: implication for resistance against Fas-mediated apoptosis in cancer. *Br. J. Cancer*, **91**, 1718–1725.
12. Ungefroren, H. *et al.* (2001) FAP-1 in pancreatic cancer cells: functional and mechanistic studies on its inhibitory role in CD95-mediated apoptosis. *J. Cell Sci.*, **114**, 2735–2746.
13. Eischen, C.M. *et al.* (1994) Tyrosine kinase activation provides an early and requisite signal for Fas-induced apoptosis. *J. Immunol.*, **153**, 1947–1954.
14. Foehr, E.D. *et al.* (2005) FAS associated phosphatase (FAP-1) blocks apoptosis of astrocytomas through dephosphorylation of FAS. *J. Neurooncol.*, **74**, 241–248.
15. Yanagisawa, J. *et al.* (1997) The molecular interaction of Fas and FAP-1. A tripeptide blocker of human Fas interaction with FAP-1 promotes Fas-induced apoptosis. *J. Biol. Chem.*, **272**, 8539–8545.
16. Meinhold-Heerlein, I. *et al.* (2001) Expression and potential role of Fas-associated phosphatase-1 in ovarian cancer. *Am. J. Pathol.*, **158**, 1335–1344.
17. Wiecekowsk, E. *et al.* (2007) FAP-1-mediated activation of NF-kappaB induces resistance of head and neck cancer to Fas-induced apoptosis. *J. Cell Biochem.*, **100**, 16–28.
18. Gastman, B.R. *et al.* (1999) Fas ligand is expressed on human squamous cell carcinomas of the head and neck, and it promotes apoptosis of T lymphocytes. *Cancer Res.*, **59**, 5356–5364.
19. Itakura, M. *et al.* (2000) Both HPV and carcinogen contribute to the development of resistance to apoptosis during oral carcinogenesis. *Int. J. Oncol.*, **16**, 591–597.
20. Mishima, K. *et al.* (2005) Etodolac, a selective cyclo-oxygenase-2 inhibitor, enhances carboplatin-induced apoptosis of human tongue carcinoma cells by down-regulation of FAP-1 expression. *Oral Oncol.*, **41**, 77–81.
21. Yeh, S.H. *et al.* (2006) Genetic characterization of fas-associated phosphatase-1 as a putative tumor suppressor gene on chromosome 4q21.3 in hepatocellular carcinoma. *Clin. Cancer Res.*, **12**, 1097–1108.
22. Hong, Y. *et al.* (2005) The role of P53 and MDM2 polymorphisms in the risk of esophageal squamous cell carcinoma. *Cancer Res.*, **65**, 9582–9587.
23. Stephens, M. *et al.* (2005) Accounting for decay of linkage disequilibrium in haplotype inference and missing-data imputation. *Am. J. Hum. Genet.*, **76**, 449–462.
24. Stephens, M. *et al.* (2001) A new statistical method for haplotype reconstruction from population data. *Am. J. Hum. Genet.*, **68**, 978–989.
25. Irie, S. *et al.* (1999) Functional interaction of Fas-associated phosphatase-1 (FAP-1) with p75(NTR) and their effect on NF-kappaB activation. *FEBS Lett.*, **460**, 191–198.
26. Thursz, M.R. *et al.* (1997) Heterozygote advantage for HLA class-II type in hepatitis B virus infection. *Nat. Genet.*, **17**, 11–12.
27. Jeffery, K.J. *et al.* (2000) The influence of HLA class I alleles and heterozygosity on the outcome of human T cell lymphotropic virus type I infection. *J. Immunol.*, **165**, 7278–7284.
28. Carrington, M. *et al.* (1999) HLA and HIV-1: heterozygote advantage and B*35-Cw*04 disadvantage. *Science*, **283**, 1748–1752.

Received July 2, 2009; revised September 30, 2009; accepted October 17, 2009