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Distinct association of *VEGF-A* polymorphisms with laryngeal and nasopharyngeal cancer

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ABSTRACT

Background: We evaluated the association of common *VEGF-A* SNPs as potential risk factors for laryngeal cancer (LC) and nasopharyngeal carcinoma (NPC) in Tunisians.
Methods: Study subjects comprised 73 NPC and 48 LC patients, along with 125 cancer-free control subjects. *VEGF-A* genotyping was done by the allelic discrimination method.
Results: Minor allele frequency (MAF) of the 8 tested *VEGF-A* SNPs was comparable between LC patients and controls. Significantly higher MAF of rs2010963 and rs833070 were seen in NPC patients compared to controls. Increased nasopharyngeal cancer risk was seen with both rs2010963 and rs833070 as heterozygous, and more so as homozygous states, thus establishing a dose-dependent effect. In addition, increased NPC risk was associated with rs833068 only in heterozygous state. Increased frequency of CCGAACTC haplotype was seen in LC cases than controls. This was in sharp contrast to NPC, where highly significant positive association was seen with ATCGGCCC, ATGAGCCC, CCCAGTCC, and CCGAACCC haplotypes, while ATCAACCC, ATGGACCC, CCCAGCCC, CCCAGCCT, and ATGGATCC haplotypes are protective factors for NPC.
Conclusion: VEGF-A SNPs are associated with altered risk of NPC, but not with LC, among Tunisian subjects.

1. Introduction

Nasopharyngeal carcinoma (NPC) has distinctive geographic and ethnic distribution, with high incidence in Southeast Asia, intermediate incidence in North African countries (Tunisia, Algeria and Morocco), and low incidence in Europe, America, Australia and Japan (Hildesheim and Levin, 1993; Ferlay et al., 2001). NPC is epidemiologically, histopathologically, and clinically distinct from other head and neck cancers (Razak et al., 2010; Yoshizaki et al., 2012). On the other hand, laryngeal cancer (LC) is a common head and neck malignancy in males, but rare in females (Hildesheim and Levin, 1993; Choby et al., 2014). Like NPC, LC is a multifactorial malignancy, influenced by environmental, genetic and life style risk factors, and variable geographic and ethnic incidence (Atienza and Dasanu, 2012; Gong et al., 2013).

Angiogenesis plays a critical role in the development, growth, invasion, and metastasis of solid tumors (Carmeliet and Jain, 2000), and is tightly regulated by several factors, including vascular endothelial growth factor (*VEGF*) (Ferrara, 2004; Mittal et al., 2014). As a major angiogenic factor, VEGF acts by enhancing endothelial cell proliferation, migration and vascular permeability (Ferrara, 2004; Evensen et al., 2009). Heightened VEGF expression was associated with tumor progression, increased micro-vessel density, and with poor prognosis for various solid tumors, including laryngeal and pharyngeal cancer (Boonkitticharoen et al., 2008; Mittal et al., 2014), head and neck squamous cell carcinomas (Mineta et al., 2000; O-charoenrat et al., 2001), cancer of the piriformfossa (Homer et al., 2003), and cervical cancer (Fujiwaki et al., 2000; Lee et al., 2002).

The gene encoding VEGF, *VEGF-A*, is located on chromosome 6p21.3, and comprises a 14 Kb coding region with eight exons and seven introns (Tisher et al., 1991). Several *VEGF-A* polymorphisms have been identified, and were investigated for their association with cancer susceptibility and prognosis (Guan et al., 2009; Koukourakis et al., 2004; Lin et al., 2013; Zidi et al., 2014). These included: −2578C/A (rs699947), −634G/C (rs2010963), −460T/C (rs833061), −7C/T (rs25648), −583T/C (rs3025020), +936C/T (rs3025039), +398G/A (rs833068), and +497G/A (rs833070) variants, which were linked with varied effects on VEGF secretion (Al-Habboubi et al., 2011). Here we evaluate

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Table 1
Characteristics of study participants.

	Laryngeal			Nasopharyngeal		
	Cases n = 48 (%)	Controls n = 125 (%)	P	Cases (n = 73)	Controls n = 125 (%)	P
Characteristics						
Age (mean ± SD)	61.95 ± 10.2	45.14 ± 14.74		44.09 ± 16.31	45.14 ± 14.74	
Gender						
Male	43(89.6)	90(72)	0.000	55(75.35)	90(72)	0.452
Female	5(10.4)	35(28)		18(24.65)	35(28)	
Histology						
SCC	48(100)	N/A	N/A	–	N/A	N/A
UCNT	–	–		73(100)	N/A	N/A
Tumor size (T)	N/A	N/A			N/A	
T1				4 (5.48)		
T2				17 (23.29)		
T2b				6 (8.22)		
T3				25 (34.25)		
T4				21 (28.76)		
Lymph node (N)	N/A	N/A			N/A	N/A
N0				8 (10.96)		
N1				21 (28.77)		
N2				7 (9.59)		
N2a				1 (1.37)		
N2b				2 (2.74)		
N2c				3 (4.10)		
N3				30 (41.10)		
N2a				1 (1.37)		
Metastasis (M)	N/A	N/A			N/A	
M0				69 (94.52)		
M1				4 (5.48)		
TNM stage	N/A	N/A			N/A	
IIA				1 (1.37)		
IIB				10 (13.70)		
III				17 (23.29)		
IVA				13 (17.80)		
IVB				28 (38.36)		
IVC				4 (5.48)		

Pearson's Chi square/Fisher's exact test for categorical variables, t-test for continuous variables; number of subjects (percent total); N/A = not applicable; UCNT, undifferentiated carcinoma of nasopharyngeal type; SCC, squamous cell carcinoma.

the association of these eight *VEGF-A* SNPs as potential risk factors for LC compared to NPC in Tunisians. Although related by location and histology, LC and NPC head and neck carcinomas evolve in to variable cancer types, which differ in pathogenesis, biology, sub location, and treatment, and hence morbidity and mortality.

2. Subjects and methods

2.1. Study subjects

Between November 2012 and February 2014, 73 patients with NPC and 48 with LC were recruited from Salah Azeiz Oncology Institute (SAI, Tunisia). Patients were diagnosed by clinical examination and biopsy findings. Participants were interviewed using a structured questionnaire, and demographic and clinical data were collected from all participants, including treatment and history of substance abuse (alcohol drinking, and cigarette smoking). EDTA-anti-coagulated blood specimens were collected prior to radiotherapy or chemotherapy. The control group comprised 125 blood donors free of cancer, drug allergy, hypertension, diabetes, or cardiovascular disease, and no family history of cancer. All subjects were asked to sign a consent form agreeing to participate in the study; all institutional ethics requirements were met.

2.2. VEGF genotyping

Total DNA was extracted from peripheral blood samples using QIAamp® DNA blood Mini Kit, according to the instruction of the manufacturer (Qiagen GmbH, Hilden, Germany). We selected *VEGF-A* polymorphisms based on minor allele frequency (MAF) of >5% in Caucasians, using SNP browser software (version 4.0, Applied Biosystems, Foster City, CA, USA). Genotyping of *VEGF-A* SNPs was done by the allelic discrimination method, using VIC- and FAM-labeled primers, which were ordered through Applied Biosystems. The reaction was performed in 6 μl volume on StepOne/StepOne Plus real-time PCR systems, according to manufacturer's instructions (Applied Biosystems). Replicate blinded quality control samples were included to assess reproducibility of the genotyping procedure; concordance was >99%.

2.3. Statistical analysis

Statistical analysis was done on SPSS v. 21.0 (IBM, NY). Categorical data were expressed as percentages of total, while continuous variables were presented as mean ± SD. Student's *t*-test was used to determine differences in means, and Pearson χ^2 or Fisher's exact test was used to assess inter–group significance. Individual SNPs were tested for Hardy–Weinberg equilibrium (HWE) using Haploview version 4.2 (http://www.broad.mit.edu/mpg/haploview). All analyses were conducted under additive genetic, as it is the conservative model, using SNPStats software (bioinfo.iconcologia.net/snpstats/). Correction for multiple testing was done according to Bonferroni method, as per: Pc (corrected P) = $1 - [(1 - P)^n]$, where n = number of comparisons. Linkage disequilibrium analysis was performed using Haploview 4.2, and haplotype construction was done by the expectation maximization

Table 2
Distribution of *VEGF-A* SNPs alleles in laryngeal and nasopharyngeal cancer cases and control subjects[a].

SNP	Location[b]	Alleles	HWE	Controls[3]	Laryngeal[c]	*P*[d]	*Pc*[e]	Nasopharyngeal[c]	*P*[d]	*Pc*[e]
rs699947	43726139	C:**A**	0.257	0.34	0.35	0.75	1.000	0.34	1.00	1.000
rs833061	43737236	T:**C**	0.216	0.44	0.53	0.13	0.672	0.46	0.60	0.999
rs2010963	43738100	G:**C**	0.151	0.37	0.36	0.95	1.000	0.61	8.5×10^{-8}	6.8×10^{-7}
rs833068	43742277	G:**A**	0.364	0.37	0.31	0.38	0.978	0.43	0.18	0.796
rs833070	43742376	G:**A**	0.267	0.42	0.48	0.360	.972	0.60	6.6×10^{-5}	5.2×10^{-4}
rs3025020	43748860	T:**C**	0.816	0.21	0.19	0.64	1.000	0.20	0.67	1.000
rs3025039	43752286	C:**T**	0.140	0.09	0.13	0.28	0.928	0.11	0.55	0.998
rs25648	43738977	C:**T**	0.451	0.16	0.14	0.74	1.000	0.13	0.46	0.993

HWE, Hardy–Weinberg equilibrium; minor alleles are in bold
[a] Study subjects included 48 laryngeal and 73 nasopharyngeal cancer patients and 125 control subjects.
[b] Location on chromosome based on dbSNP build 125.
[c] Minor allele frequency.
[d] Adjusted *P* value, adjusted for age and gender.
[e] *Pc* = corrected *P* value as per: $Pc = 1 - [(1 - P)^8]$.