Association between the \(p73\) G4C14-to-A4T14 polymorphism and risk of nasopharyngeal carcinoma: a case–control and family-based study

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1p36, a structural and functional homolog of \(p53\), plays an important role in modulating cell cycle control and apoptosis. We examined whether the \(p73\) G4C14-to-A4T14 polymorphism was related to the risk of nasopharyngeal carcinoma (NPC) among Chinese populations. The \(G4C14\)-to-\(A4T14\) polymorphism was genotyped in 593 NPC cases and 480 controls, and in 102 NPC trios. Logistic regression analysis and transmission/disequilibrium tests (TDT) were performed to evaluate whether there was an association between the polymorphism and NPC, respectively. Functional analyses were conducted to verify the biological relevance of the polymorphism. We observed that compared with the GC/GC genotype, the genotypes containing AT allele (GC/AT + AT/AT genotypes) were associated with significantly increased susceptibility to NPC [odds ratio (OR) = 1.51; 95% confidence interval (CI) = 1.16–1.95; \(P = 0.002\)]. Furthermore, compared with the GC/GC genotype, the GC/AT + AT/AT genotypes were significantly associated with the advanced lymph node metastasis (OR = 1.47; 95% CI = 1.02–2.11; \(P = 0.041\)). A significantly greater than expected transmission of the AT allele from heterozygous parents to offspring was also observed (\(P = 0.049\)) using the TDT. By using the TDT-mediated duPT-biotin nick end labeling assay, we observed lower apoptosis in NPC tissues from the AT allele carriers compared with that from non-carriers. Furthermore, the relative \(T\Delta p73\) RNA levels of the AT allele were lower than those of the GC allele in heterozygous cells. Our findings suggest that the \(p73\) G4C14-to-A4T14 polymorphism may play a role in mediating the susceptibility to NPC in Chinese populations.

Introduction

Nasopharyngeal carcinoma (NPC) is a rare tumor in most parts of the world, but it occurs at relatively high rates in some geographic regions and among certain ethnic populations. The highest incidence of NPC in the world has been reported from the provinces of Guangdong, Guangxi and Fujian in southern China (1). In southern China, the incidence rate is ~15–50 per 100 000 person–years, which is 100-fold higher than those in western world (2). Over the years, numerous studies have revealed that NPC is a complex disease caused by an interaction of Epstein–Barr virus chronic infection, environment (including occupational exposure to formaldehyde, cigarette smoking and various dietary factors) and host genetics factors in a multistep process of carcinogenesis. Recently, our group and others have reported several genes contributing to the risk of this malignancy (3–7). Because the genetic susceptibility to NPC is determined at different functional levels, such as tumor antigen presentation, cell cycle regulation and apoptosis, we hypothesize that an unknown number of other unidentified genes are likely to modify the susceptibility to NPC.

The \(p73\) gene is a family member of the tumor suppressor gene \(p53\) (8). Both at the nucleotide and protein level, the \(p73\) shares very significant homology with the \(p53\). Consistently, the \(p73\) shows many \(p53\)-like properties: it transactivates several \(p53\)-responsive genes, induces apoptosis and inhibits proliferation (8, 9). The \(p73\) gene produces two types of proteins, full-length proteins with the N-terminal transactivation domain (proapoptotic TAp73) and N-terminal truncated proteins (antiapoptotic \(\Delta\)Np73) (10). The TA isoform shares functional similarity with \(p53\) by promoting cell death, but the \(\Delta\)N isoform antagonizes its proapoptotic effect (10). There is increasing evidence that the balance between the TAp73, \(p53\) and antiapoptotic \(\Delta\)Np73 plays an important role in regulating cell fate determination (11). These data, together with the localization of \(p73\) on chromosome 1p36, a region with frequent aberration in NPC and other cancers, led to the hypothesis that the \(p73\) may be an excellent candidate susceptibility gene for NPC (12). It is expected that the single nucleotide polymorphisms within \(p73\) could result in genotype-dependent differences in susceptibility to NPC.

The \(p73\) gene is polymorphic and among its validated polymorphisms, a dinucleotide polymorphism, which was called \(G4C14\)-to-\(A4T14\), has been extensively studied (13–25). The \(G4C14\)-to-\(A4T14\) dinucleotide polymorphism consists of two linked polymorphisms (rs2273953 and rs1801173, respectively) located at position 4 (G to A) and 14 (C to T) in the \(5′\)-untranslated region of exon 2, just upstream of the initial start codon of the \(p73\) gene. Because the two polymorphisms are in complete linkage disequilibrium, only three genotypes, GC/GC, GC/AT and AT/AT, are available. It has been shown that the GC to AT change of \(G4C14\)-to-\(A4T14\) may result in formation of a stem-loop structure and therefore may affect the translation efficiency of \(p73\) (8). Indeed, in recent years, the correlation of the \(G4C14\)-to-\(A4T14\) polymorphism with cancer risk has been investigated in a variety of cancers, and several studies have shown that subjects with the AT allele may have an increased risk of certain types of cancers (13–22, 25). The role of the \(G4C14\)-to-\(A4T14\) in NPC, however, has never been specifically investigated. In the present study, we examined whether the \(p73\) G4C14-to-A4T14 polymorphism has any bearing on the risk or severity of NPC in Chinese populations.

Materials and methods

Study subjects

This case–control study consisted of 593 patients with NPC and 480 controls. All subjects were unrelated ethnic Chinese and were enrolled from Nanning city and its surrounding regions in Guangxi province between September 2003 and July 2005. The characteristics of the subjects in the case–control study were described in detail previously (4, 5). A total of 102 NPC trios were also recruited from the same regions between April 2006 and January 2008 (see Supplementary Table S1, available at Carcinogenesis Online). The diagnosis of cases, the inclusion and exclusion criteria for cases and controls and the definition of smokers and drinkers were described previously (4, 5). For allelic-specific gene expression assay, blood samples were obtained from 18 healthy volunteers. All these volunteers had no history of malignant diseases.

From the 593 patients with incident NPC, 41 patients who had undergone resection before receiving any further treatment at Guangxi Cancer Hospital were selected, and primary NPC biopsies were collected from them (see Supplementary Table S2, available at Carcinogenesis Online). All the 41 tumor tissues were poorly differentiated squamous cell carcinoma (SCC).

Abbreviations: AI, apoptotic index; CI, confidence interval; NPC, nasopharyngeal carcinoma; OR, odds ratio; SCC, squamous cell carcinoma; TDT, transmission/disequilibrium test; TUNEL, TdT-mediated dUPT-biotin nick end labeling.

1These authors contributed equally to this work.
Histological non-cancerous nasopharyngeal epithelium tissues were collected from 13 of the 480 controls (subjects (see Supplementary Table S2, available at Carcinogenesis Online). All the tissues were fixed in paraffindehyde, embedded in paraffin wax and prepared for TdT-mediated dUTP-biotin nick end labeling (TUNEL) assay.

At recruitment informed consent was obtained from each subject, and personal information on demographic factors, medical history, tobacco and alcohol use and family history of NPC were collected via a structured questionnaire. This study was performed with the approval of the Ethical Committee of Beijing Institute of Radiation Medicine and conducted according to the principles expressed in the Declaration of Helsinki.

Genotype analysis

We extracted genomic DNA from peripheral blood leukocytes from 5 ml of whole blood using standard phenol/chloroform protocols. DNA samples were diluted to 10 ng/µl and were distributed to 96-well plates; each 96-well plate contained 94 samples and 2 no-DNA control with water. We analyzed samples for the G4C14-to-A4T14 polymorphism using PCR-based restriction fragment length polymorphism analysis. The primers 5′-CAGGAG AGCAGCAGGT-3′ and 5′-TGATGAG GTGTCGTACTGCTA-3′ were used for amplifying the target region containing the G4C14-to-A4T14 polymorphism (25). The PCR assay was performed in GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA). The PCR conditions were performed as described in the manufacturer’s protocol. The PCR reaction yielded a 433 bp amplification product. The PCR product was then digested with 3 unit of Styl (Takara BioTech, Dalian, China) and separated on a 2.5% agarose gel. The presence of the AT allele creates a Styl restriction site; digested amplicons from AT/AT homozygotes appear as a 364 bp and a 69 bp band, homozygotes for the GC allele appear as a 433 bp band and heterozygotes have all three of these bands. To ensure quality control, genotyping was performed without knowledge of case/ control status of the subjects, and the accuracy of genotyping data was validated by direct sequencing of a 15% masked, random sample of cases and controls.

Allele-specific gene expression

We used Q5™ Real-Time PCR System (Bio-Rad) for genotyping and allele-specific gene expression assay (Realtime PCR Master Mix; Takara). We followed the manufacturer’s protocol for the preparation of the PCR reactions. The primers (26) and TaqMan probes used for the G4C14-to-A4T14 polymorphism were as follows: 5′-GGCTGCG ACGGTCGACAG-3′ (forward primer), 5′-GCTCAAGA GATGGAAGTGGGC GTG-3′ (reverse primer), 5′-HEX AGCTGCC TTGGAGGCC GGCGT-BHQ-3′ (AT allele-specific probe), 5′-FAM-AGCTGCC CTGGAGG CGCGCGT-BHQ-3′ (GC allele-specific probe), and the underlined base indicates the polymorphic site (referred to as GenBank accession No. NM_005427). Total RNA and genomic DNA were extracted from peripheral blood mononuclear cells (PBMCs) of 18 healthy volunteers by use of Trizol (Invitrogen, Carlsbad, CA). Complementary DNA was prepared with oligo dT primer (Promega, Madison, WI). We first performed genotyping for the 18 subjects and identified 5 heterozygous samples. Then Taq73 nested PCR fragments were obtained by PCR amplification of complementary DNA from two homozygous individuals, one with the GC/GC genotype and the other with the AT/AT genotype, with the outer primers of the probes mentioned above. The amplified fragments were cloned into the pMD19-T vector and sequenced; they were named pMD19-T-GC and pMD19-T-AT. We mixed pMD19-T-GC and pMD19-T-AT in different ratios: 8:1, 4:1, 2:1, 1:1, 1:2, 1:4 and 1:8 (FAM allele/HEX allele). TaqMan assays were then performed. For each mixing ratio, we calculated the log of the fluorescent intensity ratio (FAM intensity/HEX intensity) at the last PCR cycle (cycle 40th). We generated a standard curve (linear regression line), y = a × bx, where y is the log_{10} of (FAM intensity/HEX intensity) at a given mixing ratio, x is the log of the mixing ratio, a is the intercept and b is the slope. Finally, we measured allele-specific expression of Taq73 from the five PBMCs identified as heterozygous samples using real-time quantitative PCR. We extrapolated the allele ratio on gene expression by interpolating log of (FAM intensity/HEX intensity) on the standard curve. Each sample was performed in triplicate in three independent experiments.

In situ detection of apoptosis in NPC tissues and non-tumor nasopharyngeal tissues

NPC tissues (n = 41) and non-tumor nasopharyngeal tissues (n = 13), which had been fixed with paraffindehyde and embedded in paraffin wax, were analyzed for TUNEL assay. Two slides from each biopsy were stained with hematoxylin and eosin for routine histological evaluation. Detection of apoptotic cells was performed with the TUNEL method. The TUNEL staining was performed using the In Situ Death Detection Kit, POD (Roche Diagnostics, Basel, Switzerland) following the manufacturer’s instructions. Briefly, the sections were counterstained with hematoxylin. Sections incubated with the TUNEL reaction mixture without TdT were used as negative controls, and sections treated with 0.7 mg/ml of Dase N1 (Roche) for 10 min at 25°C before the TUNEL reaction were used as positive controls. Apoptotic cells were determined with careful observation of TUNEL-stained and serial hematoxylin and eosin-stained sections. Apoptotic cells were analyzed independently by two pathologists (J.H.Wu and S.J.Liu) who did not have knowledge of G4C14-to-A4T14 genotype results and patient outcome. In each section, a total of 10 000 cells, i.e. 1000 cells each in 10 different fields chosen randomly, were evaluated at high magnification (×400). The apoptotic index (AI) was calculated as the number of TUNEL-positive cells per 1000 tumor cells.

Statistical analysis

Genotype and allele frequencies for the G4C14-to-A4T14 polymorphism were determined by gene counting and departures from the Hardy–Weinberg equilibrium were tested using the χ² test. Multivariate logistic regression analyses were performed to evaluate whether there was an association between the G4C14-to-A4T14 polymorphism and the risk and severity of NPC. The P values, odds ratios (ORs) and 95% confidence intervals (CIs) were calculated and adjusted for age, sex, smoking and drinking status, smoking level and nationality. All the tissues were fixed in paraformaldehyde, embedded in paraffin wax and prepared for TdT-mediated dUTP-biotin nick end labeling (TUNEL) assay.

When cases were limited to those with poorly differentiated SCC, the observed genotype frequencies for G4C14-to-A4T14 in both cases (P = 0.001) and controls (P = 0.04) differed from those among controls (P = 0.002). When cases were limited to those with poorly differentiated SCC, the observed genotype frequencies for G4C14-to-A4T14 in both cases (P = 0.001) and controls (P = 0.04) differed from those among controls (P = 0.002). When cases were limited to those with poorly differentiated SCC (n = 575), the general pattern of results was similar (data not shown).

The associations between the G4C14-A4T14 polymorphism and susceptibility to NPC were further examined with stratification by age, sex, smoking and drinking status, smoking level and nationality (see Supplementary Table S3, available at Carcinogenesis Online). Although the susceptibility to NPC associated with the GC/AT + AT/AT genotypes appeared to be more pronounced in subjects who were males, younger (<47 years), non-smokers, heavier smokers (>19 pack-years), drinkers and non-Han Chinese, these differences
The G4C14-to-A4T14 polymorphism is associated with the severity of NPC

We next assessed the effect of G4C14-to-A4T14 genotypes on the severity of NPC (as measured by tumor-node-metastasis staging system). The distributions of the G4C14-to-A4T14 genotypes were not statistically significantly different in the subgroups with different T or M classifications of the cancer (data not shown). However, with regard to the N classification, we did note a trend in the patients with more frequent involvement of the lymph node (P = 0.046, test for trend). After adjustment for age, sex, smoking and drinking status, smoking level and nationality, multivariate regression analyses revealed that patients with the GC/AT + AT/AT genotype, compared those with the GC/GC genotype, had an OR of 1.47 (95% CI = 1.02–2.115; P = 0.040) for being more frequent involvement of the lymph node (N2 + N3 versus N0 + N1; Table IV). In the stratification analyses, sex, age, and smoking and drinking had no modification effect on the risk of more frequent involvement of lymph node related to the GT + GG genotype (all P > 0.20, test for homogeneity within each strata).

Allele-specific expression difference of TAp73

The G4C14-to-A4T14 polymorphism lies in the 5'-untranslated region of the TAp73 gene. This allowed us to use this marker to determine the allele-specific transcription difference by real-time quantitative PCR of TAp73 messenger RNA in heterozygous individuals. Using the TaqMan assay, we observed preferential expression of the GC allele, with significantly higher than that of the AT allele carriers having lower AI than the GC/GC genotype carriers (t-test; P < 0.05, Figure 1). We conclude, therefore, that the biological effects of the genetic associations between the risk of NPC and G4C14-to-A4T14 polymorphism might be at least in part attributable to the difference in allele-specific expression levels of TAp73.

Effects of the G4C14-to-A4T14 polymorphism on apoptosis in NPC tissues and non-cancerous nasopharyngeal tissues

We assessed the apoptosis in NPC tissues and non-cancerous nasopharyngeal tissues by TUNEL assay (Figure 2). Significant lower AI was observed in the NPC tissues, compared with that in the non-cancer nasopharyngeal tissues (1.54 ± 0.68 versus 3.22 ± 0.73; P < 0.001). In NPC tissues, the AI of patients with the GC/AT + AT/AT genotype was significantly lower than those with the GC/GC or AT/AT genotypes (1.30 ± 0.31 versus 1.92 ± 0.23; P < 0.001). However, there was no significant association between the G4C14-to-A4T14 genotypes and AI in the non-cancerous nasopharyngeal tissues (2.99 ± 0.72 versus 3.40 ± 0.70; P = 0.07).

Table I. Selected characteristics of patients with nasopharyngeal carcinoma and controls in the Guangxi population

<table>
<thead>
<tr>
<th>Category</th>
<th>Cases (n = 593)</th>
<th>Controls (n = 480)</th>
<th>P valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years Mean (SD)</td>
<td>46.6 (11.3)</td>
<td>48.6 (10.0)</td>
<td>0.003</td>
</tr>
<tr>
<td>≤47, N (%)</td>
<td>311 (52.4)</td>
<td>229 (47.7)</td>
<td>0.12</td>
</tr>
<tr>
<td>Men, n (%)</td>
<td>428 (72.2)</td>
<td>384 (80.0)</td>
<td>0.003</td>
</tr>
<tr>
<td>Smoker, n (%)</td>
<td>191 (32.2)</td>
<td>152 (31.7)</td>
<td>0.85</td>
</tr>
<tr>
<td>Smoking level, pack–years Mean (SD)</td>
<td>22.7 (13.4)</td>
<td>19.1 (15.9)</td>
<td>0.03</td>
</tr>
<tr>
<td>≤19, N (%)</td>
<td>62 (32.5)</td>
<td>88 (57.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Drinker, n (%)</td>
<td>236 (39.8)</td>
<td>121 (21.2)</td>
<td>0.001</td>
</tr>
<tr>
<td>Nationality, n (%)</td>
<td>Han</td>
<td>409 (69.0)</td>
<td>0.04</td>
</tr>
<tr>
<td>Non-Hanb</td>
<td>184 (31.0)</td>
<td>121 (21.2)</td>
<td></td>
</tr>
<tr>
<td>Histological type, n (%)</td>
<td>Poorly differentiated squamous cell carcinoma</td>
<td>575 (97.0)</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>18 (3.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinical stage, n (%)</td>
<td>I</td>
<td>32 (5.4)</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>252 (42.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>203 (34.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>106 (17.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Local tumor invasion (T classification), n (%)</td>
<td>T1</td>
<td>104 (17.5)</td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>311 (52.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>120 (20.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T4</td>
<td>58 (9.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymph node involvement (N classification), n (%)</td>
<td>N0</td>
<td>129 (21.8)</td>
<td></td>
</tr>
<tr>
<td>N1</td>
<td>291 (49.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N2</td>
<td>129 (21.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N3</td>
<td>44 (7.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distance metastasis (M classification), n (%)</td>
<td>M0</td>
<td>578 (97.5)</td>
<td></td>
</tr>
<tr>
<td>M1</td>
<td>15 (2.5)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(\chi^2\) test for categorical variables and the t-test for continuous variables.

\(\chi^2\) test for P trend of genotypes (df = 2).

Due to genotyping failure, the actual sample size was 569 and 479 for the cases and controls, respectively.

Table II. The genotype and allele frequencies of the p73 G4C14-to-A4T14 polymorphism in patients with nasopharyngeal carcinoma and controls

<table>
<thead>
<tr>
<th>Genotypes and alleles</th>
<th>Cases, n (%, n = 569)</th>
<th>Controls, n (%, n = 479)</th>
<th>OR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GC/GC</td>
<td>323 (56.8)</td>
<td>315 (65.8)</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>GC/AT</td>
<td>220 (38.7)</td>
<td>147 (30.7)</td>
<td>1.51 (1.16–1.97)</td>
<td>0.003a</td>
</tr>
<tr>
<td>AT/AT</td>
<td>26 (4.5)</td>
<td>17 (3.5)</td>
<td>1.47 (0.76–2.82)</td>
<td>0.25</td>
</tr>
<tr>
<td>GC/AT + AT/AT</td>
<td>246 (43.2)</td>
<td>164 (34.2)</td>
<td>1.51 (1.16–1.95)</td>
<td>0.002a</td>
</tr>
<tr>
<td>P trend</td>
<td></td>
<td></td>
<td></td>
<td>0.005b</td>
</tr>
<tr>
<td>Alleles</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GC</td>
<td>866 (76.1)</td>
<td>777 (81.1)</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>AT</td>
<td>272 (23.9)</td>
<td>181 (18.9)</td>
<td>1.35 (1.09–1.68)</td>
<td>0.006c</td>
</tr>
</tbody>
</table>

Due to genotyping failure, the actual sample size was 569 and 479 for the cases and controls, respectively.

\(\chi^2\) test for P trend of genotypes (df = 2).

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In this study, we investigated the genetic associations of the $p73$ G4C14-to-A4T14 polymorphism with the risk of occurrence and progression of NPC in populations in southern China. By case–control study, we found that the $p73$ G4C14-to-A4T14 GC/AT + AT/AT genotypes were associated with a significantly increased risk of occurrence of NPC. A significantly greater than expected transmission of the AT allele from heterozygous parents to offsprings with NPC was also observed using the TDT. The consistency between the family-based TDT investigation and the case–control study is strength of the genetic association. We also observed that the GC/AT + AT/AT genotypes were significantly associated with the advanced neck lymph node metastasis of NPC. Our data, together with the recent evidence for an association of the G4C14-to-A4T14 polymorphism with the risk of other cancers, including hepatocellular carcinoma, esophageal and lung cancers (19, 20, 25), suggest that the G4C14-to-A4T14 GC/AT + AT/AT genotypes are potent genetic risk factors for both onset and advanced neck lymph node metastasis of NPC.

Our observed genetic associations are plausible from a biological perspective. It is well known that the growth of tumor is determined by cell proliferation and apoptosis. The $p73$ is a member of the p53 family of transcription factors, which has essential roles in apoptosis and tumor suppression (8, 28). The $p73$ gene encodes two types of isoforms: TAp73 and ΔNp73. The full-length TAp73 isoform contains a p53-like transactivation domain and can activate a number of p53 target genes and shares a number of common p53 activators and inhibitors (10). Knockout mice for the TAp73 isoform have confirmed that the TAp73 acts as a tumor suppressor gene (29). Accumulating evidence suggests the importance of the $p73$ G4C14-to-A4T14 polymorphism in the risk of other cancers.
data indicate that the balance between the various proapoptotic TA and antiapoptotic ΔN isoforms determines whether specific signaling pathways lead to apoptosis or survival in tumor cells in response to specific stimuli (30). In this study, we found that the apoptosis was lower in the NPC tissues as compared with the non-cancer nasopharyngeal tissues. Our results are consistent with those of previous studies showing that the balance between the p73 isoforms and the tightly regulated pro- and antiapoptotic signals was disturbed in NPC (31,32).

The p73 G4C14-to-A4T14 polymorphism occurs in a region of the transcript that could theoretically form a stem-loop structure, possibly an indication of regulatory function (10). In our study population, the carriers of the AT allele were overrepresented in cases compared with controls, suggesting that the AT allele is risk allele for NPC. This hypothesis was then confirmed by our functional results. By allele-specific gene expression assay, we found that the TAp73 messenger RNA levels transcribed by the AT allele were lower than those by the GC allele in heterozygous cells. Furthermore, the AT allele carriers were found to have lower apoptosis than GC/GC genotype carriers in NPC tissues. Given the role of TAp73 in the development of cancers, it can reasonably expected, therefore, that individuals who carry the AT allele, in whom TAp73 is downregulated with a consequent decrease in the TAp73:ΔTAp73 ratio, would have a higher susceptibility to NPC, and a higher risk of advanced lymph node metastasis after the establishment of this malignancy.

Recently, many case–control studies have been reported with regard to the associations between the p73 G4C14-to-A4T14 polymorphism and risk of many cancers, and thus provide comparisons with our findings. Some studies have reported an association of the AT allele with the risk of prostate, breast and digestive tract cancer, SCC of the head and neck and hepatocellular carcinoma (13–15,18–20,25). These findings are in line with our results showing that the AT allele is a risk allele for NPC. However, several reports showed no associations between the G4C14-to-A4T14 and risk of prostate, breast and digestive tract cancer (23,24,33,34); two reports revealed reverse associations of the AT allele with the risk of lung and esophageal cancer (35,36). These conflicting results may be attributable to the different molecular mechanisms of carcinogenesis among cancers and/or different ethnicities of study populations. Additionally, other factors in the studies, such as small sample size or inadequate adjustment for confounding factors, could also cause the inconsistent results. Consequently, additional well-designed case–control studies in a wide spectrum of cancers with ethnically diverse populations are warranted to understand the roles of the G4C14-to-A4T14 polymorphism in the etiology of cancers.

Our study is subject to several limitations. One limitation is the selection bias or unknown confounding factors. As a hospital-based study, our cases were enrolled from the hospitals and the controls were selected from the community population. Consequently, inherent selection bias might have occurred. To overcome this limitation, we matched cases and controls by age and residential area. Moreover, any inadequacy in matching has been controlled in data analyses with further adjustment and stratification. Second, in this study, we selected the G4C14-to-A4T14 polymorphism in the p73 gene because it was most extensively studied polymorphisms in this gene and was potentially functional. However, because we have not performed a systematic screen for variants in the p73 gene, we cannot exclude that this polymorphism is in linkage disequilibrium with a nearby causative variant. Deep resequencing of this gene may provide further help to uncover additional associated variants and facilitate selection of potential causal variants for further functional studies. Third, the exact functional relevance of the G4C14-to-A4T14 polymorphism remains largely unknown. In this study, we found that the AT allele was associated with lower TAp73 messenger RNA levels and apoptosis when compared with the GC allele. Our results provide new insights into functional relevance of this polymorphism. However, further studies are warranted to elucidate the molecular mechanism of this important polymorphism.

In conclusion, our results reveal, for the first time, an association between the p73 G4C14-to-A4T14 polymorphism and the risk of occurrence and advanced lymph node metastasis of NPC in Chinese populations. These associations are supported by our functional data showing allele-specific downregulation of the TAp73 gene. These findings may provide support for the importance of p73 in the pathogenesis of NPC. If confirmed by other studies, knowledge of genetic factors contributing to the pathogenesis of the NPC as presented here may have implications for the screening and treatment of this disorder.

Fig. 2. Apoptosis assessed by the TUNEL assay. TUNEL, TdT-mediated dUTP-biotin nick end labeling. (a and b) Tumor tissues; (c and d) non-tumor nasopharyngeal tissues. The scale bar represents 50 μm in a and c, and 10 μm in b and d.
Supplementary material

Supplementary Tables S1–S3 can be found at http://carcin.oxfordjournals.org/

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References


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