Published in final edited form as:
Surgery. 2013 May ; 153(5): 711–717. doi:10.1016/j.surg.2012.11.009.

Significance of *MDM2* and *P14^{ARF}* polymorphisms in susceptibility to differentiated thyroid carcinoma

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Abstract

Background—Murine double minute 2 (MDM2) oncoprotein and p14^{ARF} tumor suppressor play pivotal roles in regulating p53 and function in the MAPK pathway, which is frequently mutated in differentiated thyroid carcinoma (DTC). We hypothesized that functional polymorphisms in the promoters of *MDM2* and *p14^{ARF}* contribute to the inter-individual difference in predisposition to DTC.

Methods—*MDM2*-rs2279744, *MDM2*-rs937283, *p14^{ARF}*-rs3731217, and *p14^{ARF}*-rs3088440 were genotyped in 303 patients with DTC and 511 cancer-free controls. Multivariate logistic regression analysis was performed to calculate odds ratios (ORs) and 95% confidence intervals (CIs).

Results—*MDM2*-rs2279744 and *p14^{ARF}*-rs3731217 were associated with a significantly increased risk of DTC (*MDM2*-rs2279744: TT vs. TG/GG, OR = 1.5, 95% CI, 1.1–2.0; *p14^{ARF}*-rs3731217: TG/GG vs. TT, OR = 1.7, 95% CI, 1.2–2.3). No association was found for *MDM2*-rs937283 or *p14^{ARF}*-rs3088440. Individuals carrying 3–4 risk genotypes of *MDM2* and *p14^{ARF}* had 2.2 times (95% CI, 1.4–3.5) the DTC risk of individuals carrying 0–1 risk genotypes (P_{trend} = 0.021). The combined effect of *MDM2* and *p14^{ARF}* on DTC risk was confined to young subjects (≤45 years), non-smokers, non-drinkers, and subjects with a first-degree family history of cancer. These associations were quite similar in strength when cases were restricted to those with papillary thyroid cancer.

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Conflict of interest: The authors declare no conflict of interest.

Conclusion—Our results suggest that polymorphisms of *MDM2* and *p14*^{ARF} contribute to the inter-individual difference in susceptibility to DTC, either alone or more likely jointly. The observed associations warrant further confirmation in independent studies.

Keywords

papillary thyroid carcinoma; p53 pathway; case-control study

Introduction

Differentiated thyroid carcinoma (DTC) accounts for more than 90% of all thyroid cancer cases and consists of three histological types: papillary, follicular, and Hürthle cell. The incidence of thyroid cancer in the United States has been increasing sharply since the mid-1990s, with 56,460 new thyroid cancer cases expected in 2012.[1] Remarkably, the increasing incidence has been observed for all subgroups of sex, age and races/ethnicities and for tumors of all stages.[2] Although the rise in incidence has been thought to be partly due to improved diagnosis, a complete explanation remains unknown.[2] Exposure to ionizing radiation related to medical treatment or fallout from nuclear accidents during childhood is a confirmed exogenous risk factor for DTC, though only a fraction of exposed individuals develop DTC, suggesting the significance of genetic factors in the predisposition to DTC.[3]

Activating mutations of *BRAF*, *RAS* or *RET/PTC* are observed in more than two-thirds of cases of human papillary thyroid carcinoma (PTC), supporting activation of the MAPK pathway as a major genetic event in PTC carcinogenesis.[4] The activated MAP kinases phosphorylate a number of substrates, including p53. Specifically, p53 is a principal mediator of response to ionizing radiation, in which normal p53 is activated and induces cell cycle arrest, apoptosis or senescence as appropriate.[5] Somatic mutations that inactivate the *p53* gene are detected in approximately 50% of human cancers,[6] but in thyroid cancer, the mutation rate is very low, except in the rare anaplastic histotype.[7] Alternatively, p53 can be inactivated through regulatory mechanisms, such as the p14^{ARF}-MDM2-p53 pathway. Indeed, abnormal expression of p14^{ARF} and MDM2 (murine double minute 2) has been observed in PTC tumor tissues compared to paired normal tissues, supporting involvement of the p14^{ARF}-MDM2-p53 pathway in thyroid tumorigenesis.[8–10]

In the p14^{ARF}-MDM2-p53 pathway, MDM2 negatively regulates p53 via several different mechanisms, including inhibition of p53-mediated transcriptional activity through binding with the p53 trans-activation domain and degradation of p53 through directly functioning as an E3 ubiquitin ligase or shuttling p53 from nucleus to cytoplasm to expose it to proteasome.[11] On the other hand, p14^{ARF} acts as an activator of p53 by interfering directly with MDM2 and neutralizing its inhibitory effects on p53.[12] Coordinately, MDM2 and p14^{ARF} regulate stabilization and activation of p53 in a delicately controlled manner through an autoregulatory feedback mechanism, which is critical to the p53-mediated stress response. [13] Besides, both MDM2 and p14^{ARF} interact with the MAPK signaling pathway directly in a p53-independent manner.[14–16] Interestingly, oncogenic Ras induces expression of both MDM2 and p14^{ARF}, though these interactions vary by cell type and result in different downstream effects on cell cycle control and/or apoptosis.[14, 17] Moreover, MDM2 and p14^{ARF} display p53-independent oncogenic and tumor suppressor activities, respectively, through their interactions with a number of other proteins that are important in cell cycle control, such as E2F/DP1, ATM and RB.[12, 18]

Given the pivotal roles of MDM2 and p14^{ARF} in regulating p53 activity and function in the MAPK pathway, it is biologically plausible that genetic variations of *MDM2* and *p14*^{ARF} may affect the p53-mediated response to environmental stressors, including ionizing

radiation, leading to inter-individual differences in predisposition to DTC. Indeed, numerous studies have suggested that *MDM2* and $p14^{ARF}$ polymorphisms are potential susceptibility biomarkers for cancer risk;[19–23] however, no study of the impact of such polymorphisms on thyroid cancer risk has been reported. To explore the impact of *MDM2* and $p14^{ARF}$ polymorphisms on DTC risk, we chose 4 common single-nucleotide polymorphisms (SNPs) in *MDM2* (rs2279744, rs937283) and $p14^{ARF}$ (rs3731217, rs3088440). We chose these SNPs because they all 1) reside in the promoter regions of genes with potential functional significance, 2) have a minor allele frequency > 10% in Caucasian populations (resource: dbSNP and SNP500Cancer project, maintained by the National Cancer Institute), and 3) have been associated with cancer risk.[19, 20, 24, 25]

Materials and Methods

Study subjects

The case-control study within which this analysis was performed was conducted at MD Anderson Cancer Center, as described previously.[26, 27] In brief, 303 patients with DTC were recruited from November 1999 through October 2008 with a final diagnosis confirmed by histopathology, and 511 cancer-free controls were visitors to the same institution recruited from November 1996 to March 2005 for a molecular epidemiological study of squamous cell carcinoma of the head and neck. Exclusion criteria for cases and controls included age younger than 18 years, prior malignancy (except for nonmelanoma skin cancer), blood transfusion in the past 6 months, or current receipt of immunosuppressant medications. The study was approved by the Institutional Review Board, and each participant gave written informed consent prior to recruitment.

All recruited subjects completed a self-administered questionnaire and donated 20 ml of blood for laboratory analysis. Race/ethnicity was self-reported and categorized as non-Hispanic white or other. Subjects who had smoked more than 100 cigarettes during their lifetimes were defined as smokers, and those who had quit smoking for at least 1 year before enrollment were defined as former smokers. Subjects who consumed alcohol at least once a week for more than 1 year were defined as drinkers, and those who had quit such alcohol use for at least 1 year before enrollment were defined as former drinkers. Radiation exposure was defined as previous whole-body or head-and-neck-specific medical irradiation.

MDM2 and $p14^{ARF}$ genotyping

Genomic DNA was extracted from blood samples using the QIAamp DNA blood mini kit (QIAGEN Inc, Valencia, CA) according to the manufacturer's instructions. We genotyped the selected SNPs of *MDM2* and $p14^{ARF}$ genes by polymerase chain reaction–restriction fragment length polymorphism assay, as described in detail previously.[20, 22, 23] Genotyping was performed by laboratory personnel blinded to case-control status. Greater than 99% concordance was observed in the repeated analysis in a randomly selected subset of 10% of the samples.

Statistical analysis

The chi-square test was used to compare selected demographic characteristics and *MDM2* and $p14^{ARF}$ genotype frequencies between cases and controls. The chi-square test for Hardy-Weinberg equilibrium was performed for each SNP in controls. Odds ratios and 95% CIs were calculated for risk of DTC in association with *MDM2* and $p14^{ARF}$ genotypes, individually and in combination, by using a multivariate logistic regression model with adjustment for potential confounders. The analyses were further stratified by age, sex, race/ethnicity, smoking, alcohol drinking and first-degree family history of cancer. We also estimated the association between PTC risk and *MDM2* and $p14^{ARF}$ genotypes using the

same analyses and covariates as above. All statistical tests were 2-sided, and $P < 0.05$ was accepted as statistically significant. All analyses were performed using SAS software, version 9.2 (SAS Institute Inc., Cary, NC).

Results

The demographic characteristics of the patients with DTC and controls are presented in Table 1. The majority of the cases were diagnosed with PTC (273, 90.1%). Significant differences in sex, age, race/ethnicity and smoking status were observed between DTC (and PTC) cases and controls. A predominance of subjects had no history of radiation exposure (> 97%), and there was no difference between cases and controls in the proportion exposed to radiation.

Among the controls, the genotype distributions of the 4 SNPs were in Hardy-Weinberg equilibrium ($P > 0.05$). The genotype frequencies of *MDM2* and $p14^{ARF}$ and genotype-specific risk estimates for DTC and PTC are shown in Table 2. Two SNPs, *MDM2*-rs2279744 and $p14^{ARF}$-rs3731217, were significantly associated with DTC risk. The adjusted odds ratios (ORs) were 1.5 ($P = 0.011$) and 1.7 ($P = 0.002$) for rs2279744 TT genotype and rs3731217 TG/GG genotypes, respectively. No association was found between *MDM2*-rs937283 or $p14^{ARF}$-rs3088440 and DTC risk. Similar to the findings for DTC risk, *MDM2*-rs2279744 and $p14^{ARF}$-rs3731217 were associated with a moderately increased risk of PTC.

On the basis of the risk estimates in Table 2, we grouped subjects according to the number of risk genotypes (Table 3). The risk genotypes were *MDM2*-rs2279744 TT genotype, *MDM2*-rs937283 AG/GG genotypes, $p14^{ARF}$-rs3731217 TG/GG genotypes, and $p14^{ARF}$-rs3088440 GA/AA genotypes. As shown in Table 3, when subjects were divided into 3 groups—those carrying 0–1, 2 or 3–4 risk genotypes—those carrying 3–4 risk genotypes had 2.2 times the DTC risk of those with 0–1 risk genotypes and also a higher risk than those with only 2 risk genotypes (OR = 1.2) ($P_{trend} = 0.021$). A similar trend was found for PTC risk ($P_{trend} = 0.014$). When subjects were dichotomized into those with 0–1 or 2–4 risk genotypes, subjects with at least 2 risk genotypes had a significantly increased risk of developing DTC (OR = 1.4, 95% CI, 1.1–1.9) or PTC (OR = 1.5, 95% CI, 1.1–2.0). In addition, the combined risk genotypes of four variants were significantly associated with patient's stage. Compared with the DTC patients with 0–2 risk genotypes of the four variants, those carrying 3–4 risk genotypes were approximately 2.2 times more like to have an early disease stage (I–II) (OR, 2.2; 95% CI, 1.4 – 3.4) and 2.5 times more likely to have a late disease stage (III–IV) (OR, 2.5; 95% CI, 1.3 – 4.4), and the association was in a significant dose-effect relationship (Trend test, $P<0.001$).

Stratification analysis with dichotomized risk genotypes is shown in Table 4. A significant association between DTC risk and 2 or more risk genotypes appeared restricted to specific subgroups, though no significant interaction was found between the risk genotypes and the stratified factors ($P > 0.05$). The risk of DTC was significantly higher for individuals in the high-risk genotype group (2–4 risk genotypes) for young subjects (≤ 45 years) (OR = 1.6, 95% CI, 1.0–2.5, $P = 0.048$), non-smokers (OR = 1.5, 95% CI, 1.1–2.2, $P = 0.022$), non-drinkers (OR = 1.6, 95% CI, 1.0–2.3, $P = 0.032$), and subjects with a first-degree family history of cancer (OR = 1.5, 95% CI, 1.0–2.3, $P = 0.049$).

Discussion

In the present study, we found that *MDM2*-rs2279744 and $p14^{ARF}$-rs3731217 were significantly associated with a moderately increased risk of developing DTC, particularly

PTC. Moreover, subjects who carried at least 3 risk genotypes of *MDM2* and $p14^{ARF}$ genes had an approximately 2.2-fold increased risk of DTC compared to those who carried 0–1 risk genotypes. MDM2 and $p14^{ARF}$ lie within the same pathway in regulation of p53's tumor suppressor function. Therefore, it is plausible that polymorphisms of *MDM2* and $p14^{ARF}$ could jointly affect individual susceptibility to cancer. Indeed, our results showed an increased risk of DTC in association with increased number of risk genotypes of *MDM2* and $p14^{ARF}$ in a dose-response manner.

In the present study, the combined effect of *MDM2* and $p14^{ARF}$ risk genotypes on DTC risk was more pronounced in non-smokers and non-drinkers. This seems to be paradoxical because smoking and alcohol use, both of which are known environmental stresses, could trigger p53-mediated stress response and individuals with inherited differences in p53 activity would respond differently to such stresses. Indeed, one previous study found that individuals who were smokers and carried the risk allele of *MDM2*-rs2279744 had a significantly greater risk for lung cancer than those who were either smokers or carried the risk allele but not both.[28] Smoking is a risk factor for lung cancer, but in terms of thyroid cancer, a number of epidemiological studies observed reduced risk of thyroid cancer for smokers, in agreement with what was observed in our case-control population, suggesting a different mechanism of smoking in thyroid cancer development.[29] A recent study observed a significantly lower level of thyroid-stimulating hormone linked to smoking, suggesting an inhibitory effect of smoking on the thyroid,[30] which may override the risk effect of exposure to tobacco carcinogens in thyroid tumorigenesis. Therefore, our findings of greater risk in non-smokers may suggest an effect of p53-mediated stress response associated with other risk factors. We also observed a greater risk associated with *MDM2* and $p14^{ARF}$ risk genotypes in younger subjects (≤45 years) and subjects with a first-degree family history of cancer, suggesting an early age of onset of cancer, a characteristic of inherited susceptibility to DTC. It is noted, however, that no significant interaction was detected between risk genotypes and these factors (age, smoking, alcohol drinking and family history of cancer). On the other hand, we could not exclude the possibility that the non-significant results in smokers, drinkers and subjects with no family history of cancer are because of the small numbers of subjects in these subgroups. Consequently, larger studies are warranted to confirm our findings. In addition, although a significantly increased risk associated with the combined risk genotypes was found in subjects other than non-Hispanic whites, the mixed racial/ethnic background and relatively limited number of subjects made it impossible to attribute the significant association to any specific race/ethnicity group.

MDM2-rs2279744 (also referred to as SNP309, T/G) is located in the first intron of the *MDM2* promoter, which drives transcription of the *MDM2* gene.[14] Bond *et al.* initially described this polymorphism and found that the GG genotype enhanced the binding affinity of the transcriptional activator Sp1, which results in over-expression of MDM2 and attenuation of p53 stress response.[31] In our study, however, the variant TG/GG genotypes were associated with reduced risk of DTC, which seems to contradict the evidence for functional effect. But such an observation is not odd for *MDM2*-rs2279744. As reviewed in a recent meta-analysis, the association between rs2279744 and cancer risk varies by cancer type, with the TG/GG genotypes associated with an increased risk of lung cancer and colorectal cancer but no risk of breast cancer or ovarian cancer and even a reduced risk of prostate cancer.[19] The inconsistency between results of *in vitro* functional assays and epidemiology studies may reflect the complex effect of SNPs on tumorigenesis, which shows tissue-specific effects and is influenced by other genes and environmental exposures.[32] Thus, our findings need to be validated in future studies.

It has been long suspected that female-specific hormones including estrogen contribute to thyroid cancer development, given the higher incidence of thyroid cancer in females (the

female-to-male ratio of 3:1), which peaks at puberty.[1] Expression of estrogen receptor-α was previously observed in human PTC, whereas no expression was observed in normal thyroid cells.[33] Interestingly, the expression of estrogen receptor-α was shown to induce *MDM2* transcription, which is mediated, at least in part, by the promoter region containing rs2279744.[34] Bond *et al.* suggested that an active estrogen-signaling pathway was needed for rs2279744 to exhibit its effect on human tumor formation, and indeed they observed gender-specific differences in development of breast cancer and colorectal cancer in association with rs2279744.[35, 36] Consistent with those findings, in the present study, the significant association between rs2279744 and DTC risk was likely confined to women, though we did not find a significant interaction with sex. The lack of significant interaction could be due to the limited sample size.

We observed a significant association between $p14^{ARF}$-rs3731217 and DTC risk. This SNP has been used as a tag SNP for a 174-kb region of linkage disequilibrium at 9p21.3, which has been previously reported to be associated with risk of melanoma and leukemia.[25, 37] Specifically, rs3731217 was in a strong association with risk of childhood acute lymphoblastic leukemia,[25] for which radiation exposure is a well-known risk factor as well. Our finding of an association between $p14^{ARF}$-rs3731217 and DTC risk is also in line with a previous study reporting that the TG/GG genotypes of rs3731217 were in association with a moderately increased risk of developing a second primary malignancy in patients with squamous cell carcinoma of the head and neck.[20]

The results of the present study need to be interpreted with caution. Since the majority of the recruited subjects in this study were non-Hispanic whites, our results are not generalizable to other races/ethnicities. In addition, we used a hospital-based case-control study design. Therefore, the potential for selection bias needs to be considered. Furthermore, we cannot rule out the possibility that the significant SNPs may not be the causal loci but rather be in linkage disequilibrium with the causal loci. Finally, the sample size limited the statistical power to detect potential subtle effects of SNPs on cancer risk, especially in stratification analysis.

In summary, the data we present support our hypothesis that polymorphisms of *MDM2* and $p14^{ARF}$ contribute to the inter-individual difference in susceptibility to DTC and suggest that inherited genetic variations in the MDM2-$p14^{ARF}$-p53 pathway likely affect susceptibility to DTC either alone or more likely jointly. These findings argue that low-penetrance genes (polymorphisms) account for a significant proportion of the predisposition to sporadic DTC and that multiple genetic variations together contribute to DTC susceptibility.[38] To confirm the role of *MDM2* and $p14^{ARF}$ polymorphisms in thyroid cancer risk, further validation in larger population-based studies and assessment of functional significance of these variants are anticipated.

Acknowledgments

Funding: This work was supported in part by an American Thyroid Association Thyroid Cancer Grant (to E.M.S.), The University of Texas MD Anderson Cancer Center start-up funds (to E.M.S.), National Institutes of Health grants U01 DE019765-01 (to Adel K. El-Naggar), R01 ES-11740 (to Q.W.), CA016672 (to John Mendelsohn), CA135679 (to G.L.), and CA133099 (to G.L.), and a cancer prevention fellowship (to L.X.) supported by Halliburton Employees Fellow in Cancer Prevention funds (Shine Chang, Principal Investigator).

The authors thank Margaret Lung, Kathyrn Patterson, Liliana Mugartegui, and Jenny Vo for their help with subject recruitment, Chong Zhao and Yingdong Li for DNA extraction and genotyping analysis, and Stephanie P Deming for manuscript editing.

Abbreviations

DTC	differentiated thyroid carcinoma
MDM2	murine double minute 2
PTC	papillary thyroid carcinoma
OR	odds ratio
CI	confidence interval

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Table 1

Demographic and exposure characteristics of thyroid cancer case and control subjects

	Controls (No. = 511)		DTC cases (No. = 303)		P^*	PTC cases (No. = 273)		P^*
Variable	**No.**	**(%)**	**No.**	**(%)**		**No.**	**(%)**	
Age, years								
≤45	198	(38.7)	162	(53.5)	<0.001	153	(56.0)	<0.001
> 45	313	(61.3)	141	(46.5)		120	(44.0)	
Sex								
Male	245	(47.9)	103	(34.0)	<0.001	89	(32.6)	<0.001
Female	266	(52.1)	200	(66.0)		184	(67.4)	
Race/ethnicity								
Non-Hispanic white	401	(78.5)	214	(70.6)	0.012	191	(70.0)	0.008
Other	110	(21.5)	89	(29.4)		82	(30.0)	
First-degree family history of cancer								
Yes	257	(51.4)	151	(50.0)	0.701	133	(48.9)	0.506
No	243	(48.6)	151	(50.0)		139	(51.1)	
Smoking status								
Current	99	(19.6)	29	(9.6)	<0.001	28	(10.3)	0.002
Former	114	(22.5)	69	(22.8)		59	(21.7)	
Never	293	(57.9)	204	(67.6)		185	(68.0)	
Alcohol drinking status								
Current	171	(33.8)	95	(31.4)	0.112	89	(32.7)	0.107
Former	75	(14.8)	32	(10.6)		27	(10.0)	
Never	260	(51.4)	175	(58.0)		156	(57.3)	
Radiation exposure								
No	504	(98.6)	293	(97.0)	0.110	264	(97.1)	0.127
Yes	7	(1.4)	9	(3.0)		8	(2.9)	

*Chi-square test, compared to controls.

Abbreviations: DTC, differentiated thyroid carcinoma; PTC, papillary thyroid carcinoma.

Table 2

MDM2 and *p14*^{ARF} genotype frequencies and risk estimates for DTC and PTC

	Controls (No. = 511)		DTC cases (No. = 303)			PTC cases (No. = 273)			Adjusted OR† (95% CI)	
Genotypes	**No.**	**(%)**	**No.**	**(%)**	***P****	**No.**	**(%)**	***P****	**DTC**	**PTC**
MDM2 rs2279744										
TG/GG	341	(66.7)	173	(57.1)		118	(43.2)		1.0	1.0
TT	170	(33.3)	130	(42.9)	0.006	155	(56.8)	0.006	1.5 (1.1–2.0)	1.5 (1.1–2.1)
MDM2 rs937283										
AA	173	(33.9)	104	(34.3)		92	(33.7)		1.0	1.0
AG/GG	338	(66.1)	199	(65.7)	0.892	181	(66.3)	0.965	1.0 (0.7–1.3)	1.0 (0.7–1.4)
p14^{ARF} rs3731217										
TT	388	(75.9)	198	(65.4)		181	(66.3)		1.0	1.0
TG/GG	123	(24.1)	105	(34.6)	0.001	92	(33.7)	0.004	1.7 (1.2–2.3)	1.5 (1.1–2.2)
p14^{ARF} rs3088440										
GG	402	(78.7)	241	(79.5)		216	(79.1)		1.0	1.0
GA/AA	109	(21.3)	62	(20.5)	0.769	57	(20.9)	0.883	1.1 (0.7–1.5)	1.1 (0.7–1.6)

*Chi-square test, compared to controls.

†Adjusted for age, sex, race/ethnicity, first degree family history of cancer, smoking status, alcohol drinking status, and radiation exposure.

Abbreviations: *MDM2*, murine double minute 2; DTC, differentiated thyroid carcinoma; OR, odds ratio; PTC, papillary thyroid carcinoma.

Table 3

Combination effect of *MDM2* and $P14^{ARF}$ genotypes on risk of DTC and PTC

	Controls (No. = 511)		DTC cases (No. = 303)			PTC cases (No. = 273)			Adjusted OR† (95% CI)	
No. of risk genotypes	No.	(%)	No.	(%)	*P**	No.	(%)	*P**	DTC	PTC
0–1	267	(52.3)	132	(43.6)		116	(42.5)		1.0	1.0
2	188	(36.8)	110	(36.3)		103	(37.7)		1.2 (0.9–1.6)	1.3 (0.9–1.8)
3–4	56	(10.9)	61	(20.1)	0.009	54	(19.8)	0.001	2.2 (1.4–3.5)	2.2 (1.4–3.5)
									P_{trend} = 0.021	P_{trend} = 0.014
0–1	267	(52.3)	132	(43.6)		116	(42.5)		1.0	1.0
2–4	244	(47.7)	171	(56.4)	0.017	157	(57.5)	0.009	1.4 (1.1–1.9)	1.5 (1.1–2.0)

* Chi-square test, compared to controls.

† Adjusted for age, sex, race/ethnicity, first-degree family history of cancer, smoking status, alcohol drinking status and radiation exposure.

Abbreviations: *MDM2*, murine double minute 2; DTC, differentiated thyroid carcinoma; OR, odds ratio; PTC, papillary thyroid carcinoma.

Table 4

Subgroup analysis of combined risk genotypes in association with DTC risk

	Low-risk (ref.) genotype (0–1)			High-risk genotype (2–4)		
Stratification variable	**Controls No. (%)**	**DTC cases No. (%)**	**Adjusted OR* (95% CI)**	**Controls No. (%)**	**DTC cases No. (%)**	**Adjusted OR* (95% CI)**
Age, years						
≤45	103 (52.0)	66 (40.7)	1.0	95 (48.0)	96 (59.3)	1.6 (1.0–2.5)
> 45	164 (52.4)	66 (46.8)	1.0	149 (47.6)	75 (53.2)	1.2 (0.8–1.7)
Sex						
Female	139 (52.3)	84 (42.0)	1.0	127(47.7)	116 (58.0)	1.5 (1.0–2.2)
Male	128 (52.2)	48 (46.6)	1.0	117(47.8)	55 (53.4)	1.3 (0.8–2.1)
Race						
Non-Hispanic white	195 (48.6)	88 (41.1)	1.0	206 (51.3)	126 (58.88)	1.3 (0.9–1.8)
Other	72 (65.5)	44 (49.4)	1.0	38 (34.5)	45 (50.6)	2.0 (1.1–3.7)
Smoking status						
Ever	112 (52.6)	48 (49.0)	1.0	101 (47.4)	50 (51.0)	1.3 (0.8–2.1)
Never	153 (52.2)	83 (40.7)	1.0	140 (47.8)	121 (59.3)	1.5 (1.1–2.2)
Alcohol drinking status						
Ever	125 (50.8)	57 (44.9)	1.0	121 (49.2)	70 (57.7)	1.3 (0.8–2.0)
Never	140 (53.9)	74 (42.3)	1.0	120 (46.1)	101 (55.1)	1.6 (1.0–2.3)
First-degree family history of cancer						
Yes	128 (49.8)	59 (39.1)	1.0	129 (50.2)	92 (60.9)	1.5 (1.0–2.3)
No	134 (55.1)	72 (47.7)	1.0	109 (44.9)	79 (52.3)	1.3 (0.9–2.0)

*Adjusted for age, sex, race/ethnicity, first-degree family history of cancer, smoking status, alcohol drinking status and radiation exposure.

Abbreviations: DTC, differentiated thyroid carcinoma; OR, odds ratio.