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p53 Arg72Pro, MDM2 T309G and *CCND1 G870A* polymorphisms are not associated with susceptibility to esophageal adenocarcinoma

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Abstract

p53 Arg72Pro, MDM2 T309G, and *CCND1 G870A* are functional single nucleotide polymorphisms (SNPs) in key genes that regulate apoptosis and cell cycle. Variant genotypes of these SNPs have been associated with increased risk and earlier age of onset in some cancers. We investigated the association of these SNPs with susceptibility to esophageal adenocarcinoma in a large, North American case-control study. 312 cases and 454 cancer-free controls recruited in Boston, USA were genotyped for each of the three SNPs, and demographic and clinical data were collected. Genotype frequencies for each of the three SNPs did not deviate from Hardy-Weinberg equilibrium, and did not differ between cases and controls. Odds ratios (OR), adjusted for clinical risk factors, for the homozygous variant genotypes were 0.99 (95% CI 0.57 – 1.72) for *p53 Pro/Pro*, 0.81 (95% CI 0.52 – 1.28) for *MDM2 G/G*, and 0.97 (95% CI 0.64 – 1.49) for *CCND1 A/A*. The analysis was adequately powered (80%) to detect ORs of 1.37. 1.35 and 1.34 for each SNP respectively. In contrast to the results of smaller published studies, no association between *p53 Arg72Pro, MDM2 T309G*, and *CCND1 G870A* SNPs and susceptibility to esophageal adenocarcinoma, age of onset, or stage of disease at diagnosis was detected.

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CONFLICT OF INTEREST STATEMENT
The authors have no conflicts to declare.

Keywords

Esophageal adenocarcinoma; molecular epidemiology; single nucleotide polymorphism (SNP); risk factors; p53

INTRODUCTION

Esophageal adenocarcinoma (EA) is the predominant form of esophageal cancer in Western populations, and its incidence is rising dramatically.1 Despite its high morbidity and mortality, there have been few studies examining the molecular epidemiology of this disease and, of those published, most are small and their results inconsistent.2 A better understanding of the genetic risk factors that underlie EA could lead to improved strategies for screening and prevention.

p53, its negative regulator *MDM2*, and cyclin D1 (*CCND1*) are important regulators of cell cycle and apoptosis; and each contains functional single nucleotide polymorphisms (SNPs) (*p53 Arg72Pro*, *MDM2 T309G*, and *CCND1 G870A*) that have been implicated in the susceptibility and outcome of various human malignancies.3–5 The variants alleles of both *p53 Arg72Pro* and *MDM2 T309G* putatively impair apoptosis, which could contribute to esophageal carcinogenesis: *p53 Pro72* codes for a protein with reduced apoptotic potential, while *MDM2 309G* is a promoter SNP that results in the upregulation of *MDM2* and the consequent downregulation of the *p53* pathway. *CCND1 G870A* alters a transcriptional splice site, with the resulting transcript leading to constitutive nuclear cyclin D1 localization and an increased in vitro transforming capacity through mechanisms not fully elucidated.4 Two studies, with conflicting findings, have evaluated the association between *CCND1 G870A* and EA risk.6,7 *p53 Arg72Pro* and *MDM2 T309G* have not been studied in this disease despite the importance of the p53 pathway in esophageal cancer, and associations with risk in other aerodigestive cancers.8–12

We sought to evaluate the association of these SNPs with EA risk in a large North American case-control study. Subgroups of females and smokers, in whom the variant alleles might exert a stronger biologic effect,13 were analyzed. We also explored whether a relationship exists between these SNPs and age of onset and stage of disease at diagnosis, both of which have been demonstrated previously.13,14

MATERIALS AND METHODS

Case and control population

Since 1999, patients with histologically-confirmed esophageal adenocarcinoma were recruited from Massachusetts General Hospital (Boston, MA).15 Patients were also enrolled from the Dana Farber Cancer Institute (Boston, MA) beginning in 2003. The current study includes the cohort enrolled up to September, 2005. Healthy unrelated age-, sex-, and gender-matched visitor controls with no history of cancer or GERD were recruited from the same institutions. A more detailed description of the recruitment of this cohort has been recently published.16 For both cases and controls, the rate of recruitment exceeded 85% of individuals who were approached for participation. Informed consent was provided by all participants, and the study protocol was approved by the institutional review boards of the participating hospitals and universities.

Variables

Demographic information, detailed medical and family histories, adult body mass index (BMI, defined using healthy weight between ages 20 and 30), smoking, and alcohol consumption habits were collected by trained interviewers. Smoking habits were defined as never, former, current smokers according to standard definitions. Alcohol use was dichotomized into never-drinker (lifetime average ≤1 standard drink/year) and drinkers.

Genotyping

DNA was extracted from peripheral blood using the Puregene DNA Isolation Kit (Gentra Systems, Minneapolis, USA). Genotyping for *p53 Arg72Pro* (rs1042522), *MDM2 T309G* (rs2279744) and *CCND1 G870A* (rs603965) were performed as previously described using Taqman assays.17 Probe and primer sequences are available upon request.

Statistical analysis

Sex and age distribution matching were confirmed between cases and controls. Demographic and clinical variables were compared across cases and controls, and across genotypes in the case cohort using Fisher`s exact tests (categorical variables) and non-parametric Wilcoxon rank sum tests (continuous variables) where appropriate. Unconditional logistic regression models were used to analyze associations between genotypes and risk of EA, as previously described.15 Recessive, additive and dominant models were considered. Analyses were adjusted for smoking status and adult BMI. Subgroup analyses were performed by gender and smoking status. Stage of disease and age at diagnosis were compared across genotypes using Fisher exact and Wilcoxon rank tests. P values of 0.05 were considered significant. All statistical testing was performed using SAS 9.1 (SAS Institute, Cary, NC, USA).

RESULTS

312 cases and 454 controls were included in the analysis. Demographic and clinical variables, as well as genotype frequencies are shown in Table 1. Smoking, BMI and alcohol use, all putative risk factors for esophageal cancer, were more common in cases than controls. Genotyping for each of the three SNPs was complete in 98–99.7% of individuals. Genotype frequencies, as well as crude and adjusted ORs for EA risk for all SNPs are shown in Table 2. There were no differences in genotype distribution between cases and control for any of the three SNPs. The observed frequencies were similar to previous reports, and both cases and controls did not deviate from Hardy Weinberg equilibrium ($p>0.05$).

Subgroup analyses were performed to examine the association of SNPs and EA risk by gender and smoking status. Analyses adjusted for alcohol consumption were also carried out in the subgroup of patients with available alcohol use data. No significant associations were shown in any subgroup explored ($p>0.10$ for each comparison).

Neither stage of disease at diagnosis, nor age at diagnosis was associated with any of the three SNPs ($p>0.10$ for each comparison).

DISCUSSION

In a large esophageal adenocarcinoma genetic case-control study, we found no association between *p53 Arg72Pro*, *MDM2 T309G* or *CCND1 G870A* and EA susceptibility. Our study was adequately powered (80%) to detect ORs of 1.37, 1.35, and 1.34 for the *p53*, *MDM2* and *CCND1* SNPs respectively, and included several times more patients than any previous study that has evaluated these associations. We considered subgroups of patients in whom

the SNPs might be more likely to modulate disease risk, and no positive associations were found. In addition, we found no association between any of the three SNPs and age or stage of disease at diagnosis.

The lack of association between *p53 Arg72Pro, MDM2 T309G* and risk or age of onset in EA is important and adds to the growing literature about *p53* pathway SNPs and cancer susceptibility. The conflicting reports among tumor types suggests that any impact on cancer susceptibility is likely disease-site specific, and may be limited to subgroups of patients within tumor types. In our study we had limited ability to examine the interaction between SNP genotypes and other patient factors and EA susceptibility; and as our population was almost entirely Caucasian, our null results may not apply to other ethnic groups. Furthermore, because these SNPs affect a common pathway, a combined analysis of the *MDM2* and *p53* SNPs is clearly warranted; however, because of the low prevalence of each variant allele, we had limited power to analyze SNP combinations.

Our null result for *CCND1 G870A* and EA risk contrasts with one small case-control study (cases, n=56; OR 5.99, 95% CI 1.89–18.96, for A/A vs. G/G), but consistent with another (n=56) that did not find a significant association.6,7 In addition, we did not confirm the results of a case series (n=124) that found a positive association between *CCND1 A/-* genotypes and both age of onset and frequency of distant metastases at diagnosis.14,18 While the affected population in that series had similar ethnic and sex distributions as our own, a greater proportion of our patients had stage IV disease. These results highlight the importance of validating SNP-susceptibility associations.

In conclusion, our findings do not support an association between *p53 Arg72Pro, MDM2 T309G*, and *CCND1 G870A* SNPs and esophageal adenocarcinoma susceptibility in a North American Caucasian-predominant population. As demonstrated in the current and other recently published studies,15,16,19–21 the large and well-characterized cohort examined here provides a powerful resource for characterizing the molecular epidemiology of esophageal adenocarcinoma.

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REFERENCES

1. National Cancer Institute. Bethesda, MD: 2007. SEER Cancer Statistics Review, 1975–2004. http://seer.cancer.gov/csr/1975_2004/, based on November 2006 SEER data submission, posted to the SEER web site
2. Hiyama T, Yoshihara M, Tanaka S, Chayama K. Genetic polymorphisms and esophageal cancer risk. Int J Cancer. 2007; 121:1643–1658. [PubMed: 17674367]
3. Bond GL, Hu W, Levine A. A single nucleotide polymorphism in the MDM2 gene: from a molecular and cellular explanation to clinical effect. Cancer Res. 2005; 65:5481–5484. [PubMed: 15994915]
4. Knudsen KE, Diehl JA, Haiman CA, Knudsen ES. Cyclin D1: polymorphism, aberrant splicing and cancer risk. Oncogene. 2006; 25:1620–1628. [PubMed: 16550162]
5. Pietsch EC, Humbey O, Murphy ME. Polymorphisms in the p53 pathway. Oncogene. 2006; 25:1602–1611. [PubMed: 16550160]
6. Casson AG, Zheng Z, Evans SC, et al. Cyclin D1 polymorphism (G870A) and risk for esophageal adenocarcinoma. Cancer. 2005; 104:730–739. [PubMed: 15971196]

7. Geddert H, Kiel S, Zotz RB, et al. Polymorphism of p16 INK4A and cyclin D1 in adenocarcinomas of the upper gastrointestinal tract. J Cancer Res Clin Oncol. 2005; 131:803–808. [PubMed: 16163549]
8. Yang M, Guo Y, Zhang X, et al. Interaction of P53 Arg72Pro and MDM2 T309G polymorphisms and their associations with risk of gastric cardia cancer. Carcinogenesis. 2007; 28:1996–2001. [PubMed: 17638920]
9. Wilkening S, Bermejo JL, Hemminki K. MDM2 SNP309 and cancer risk: a combined analysis. Carcinogenesis. 2007; 28:2262–2267. [PubMed: 17827408]
10. Ohmiya N, Taguchi A, Mabuchi N, et al. MDM2 promoter polymorphism is associated with both an increased susceptibility to gastric carcinoma and poor prognosis. J Clin Oncol. 2006; 24:4434–4440. [PubMed: 16983111]
11. Hu Z, Jin G, Wang L, Chen F, Wang X, Shen H. MDM2 promoter polymorphism SNP309 contributes to tumor susceptibility: evidence from 21 case-control studies. Cancer Epidemiol Biomarkers Prev. 2007; 16:2717–2723. [PubMed: 18086778]
12. Hong Y, Miao X, Zhang X, et al. The role of P53 and MDM2 polymorphisms in the risk of esophageal squamous cell carcinoma. Cancer Res. 2005; 65:9582–9587. [PubMed: 16230424]
13. Bond GL, Levine AJ. A single nucleotide polymorphism in the p53 pathway interacts with gender, environmental stresses and tumor genetics to influence cancer in humans. Oncogene. 2007; 26:1317–1323. [PubMed: 17322917]
14. Izzo JG, Wu TT, Wu X, et al. Cyclin D1 guanine/adenine 870 polymorphism with altered protein expression is associated with genomic instability and aggressive clinical biology of esophageal adenocarcinoma. J Clin Oncol. 2007; 25:698–707. [PubMed: 17308274]
15. Liu G, Zhou W, Yeap BY, et al. XRCC1 and XPD polymorphisms and esophageal adenocarcinoma risk. Carcinogenesis. 2007; 28:1254–1258. [PubMed: 17264068]
16. Tse D, Zhai R, Zhou W, et al. Polymorphisms of the NER pathway genes, ERCC1 and XPD are associated with esophageal adenocarcinoma risk. Cancer Causes Control. 2008 Dec; 19(10):1077–1083. [PubMed: 18478337]
17. Heist RS, Zhou W, Chirieac LR, et al. MDM2 polymorphism, survival, and histology in early-stage non-small-cell lung cancer. J Clin Oncol. 2007; 25:2243–2247. [PubMed: 17538168]
18. Izzo JG, Malhotra U, Wu TT, et al. Impact of cyclin D1 A870G polymorphism in esophageal adenocarcinoma tumorigenesis. Semin Oncol. 2005; 32:S11–S15. [PubMed: 16399423]
19. Zhai R, Liu G, Asomaning K, et al. Genetic polymorphisms of VEGF, interactions with cigarette smoking exposure and esophageal adenocarcinoma risk. Carcinogenesis. 2008; 29:2330–2334. [PubMed: 18780893]
20. Lanuti M, Liu G, Goodwin JM, et al. A functional epidermal growth factor (EGF) polymorphism, EGF serum levels, and esophageal adenocarcinoma risk and outcome. Clin Cancer Res. 2008; 14:3216–3222. [PubMed: 18483390]
21. Fruh M, Zhou W, Zhai R, et al. Polymorphisms of inflammatory and metalloproteinase genes, Helicobacter pylori infection and the risk of oesophageal adenocarcinoma. Br J Cancer. 2008; 98:689–692. [PubMed: 18253117]

Table 1

Demographic characteristics of cases and controls

	Case (n=312)	Control (n=454)	P-value
Gender			
Male	279 (89%)	397 (87%)	
Female	33 (11%)	57 (13%)	0.40
Median Age (range), years	64 (21–91)	64 (19–96)	0.64
Race			
Caucasian	302 (98%)	446 (98%)	
Other	7 (2%)	8 (2%)	0.62
Median Adult BMI (range)	23 (15–39)	22 (14–36)	0.002
<=25	216 (69%)	374 (82%)	
>25–30	76 (24%)	68 (15%)	
>30	20 (6%)	12 (3%)	<0.0001
Smoking Status			
Non-smokers	62 (20%)	144 (32%)	
Ex-smokers	171 (55%)	233 (51%)	
Current smokers	77 (25%)	77 (17%)	0.0004
Alcohol Use†			
Never	28 (11%)	53 (18%)	
Ever	233 (89%)	237 (82%)	0.01
Stage			
Stage 1	22 (7%)		
Stage 2a	70 (22%)		
Stage 2b	56 (18%)	N/A	
Stage 3	79 (25%)		
Stage 4a	28 (9%)		
Stage 4b	57 (18%)		

† Alcohol use data was available for only 261 cases, and 290 controls

Percentages may not add up to 100 due to rounding

BMI = body mass index

Table 2

Genotype frequencies for *p53 Arg72Pro*, *MDM2* T*309G* and *CCND1* G*870A* polymorphisms and crude and adjusted ORs for their risk of esophageal adenocarcinoma.

SNP Genotype	Cases (%)	Control (%)	Crude OR (95% CI)	Adjusted† OR (95% CI)
p53 Arg72Pro				
Arg/Arg	160 (53)	256 (57)	—	—
Pro/Arg	117 (39)	159 (35)	1.18 (0.64 – 1.61)	1.16 (0.85 – 1.60)
Pro/Pro	25 (8)	38 (8)	1.05 (0.61 – 1.81)	0.99 (0.57 – 1.72)
Pro/-	142 (47)	197 (43)	0.87 (0.65 – 1.16)	0.89 (0.66 – 1.20)
MDM2 T309G				
T/T	116 (37)	175 (39)	—	—
T/G	154 (50)	199 (44)	1.17 (0.85 – 1.60)	1.12 (0.81 – 1.55)
G/G	41 (13)	80 (18)	0.77 (0.50 – 1.21)	0.81 (0.52 – 1.28)
G/-	195 (63)	279 (61)	0.95 (0.70 – 1.28)	0.97 (0.71 – 1.31)
CCND1 G870A				
G/G	79 (26)	128 (28)	—	—
G/A	154 (52)	215 (48)	1.16 (0.82 – 1.64)	1.21 (0.84 – 1.73)
A/A	66 (22)	107 (24)	1.00 (0.66 – 1.51)	0.97 (0.64 – 1.49)
A/-	220 (74)	322 (72)	0.90 (0.64 – 1.26)	0.89 (0.63 – 1.24)

†Adjusted for smoking status and adult body mass index. When adjustment for alcohol use was included (which decreased the sample size as shown in Table 1), results were similar.