Interactions between genetic polymorphisms in the apoptotic pathway and environmental factors on esophageal adenocarcinoma risk

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How genetic variations in apoptosis pathway interact with environmental factors to contribute to esophageal adenocarcinoma (EA) risk has not been comprehensively investigated. We conducted a case-only analysis in 335 Caucasian EA patients that were genotyped for 242 single nucleotide polymorphisms (SNPs) in 43 apoptotic genes. Gene–environment interactions were assessed using a two-step approach. First, random forest algorithm was used to screen for the potential interacting markers. Next, we used case-only logistic regression model to estimate the effects of gene–environment interactions on EA risk. Four SNPs (PERP rs648802, PIK3CA rs4855094, rs7644468 and TNFRSF1A rs4149579) had significant interaction with gastroesophageal reflux disease (GERD). The presence of variant alleles in PIK3CA and TNFRSF1A enhances the risk of smoking by 2.08–2.58 times [interaction odds ratio (ORI) = 2.08–2.58, adjusted P-value (Padj) = 0.02–0.04]. Compared with patients carrying ≤1 risk genotype, the risk of GERD on EA was increased in persons with two (ORI = 1.89, Padj = 0.016) or ≥3 (ORI = 4.30, Padj < 0.0001) risk genotypes. Compared with cases with ≤1 risk genotype, smoking-associated EA risk increased by 3.15 times when ≥2 risk genotypes were present (ORI = 3.15, Padj < 0.0001). In conclusion, interactions among apoptotic SNPs and GERD or smoking play an important role in EA development.

Introduction

Cumulative evidence has indicated that symptomatic gastroesophageal reflux disease (GERD), obesity, smoking and male gender are four major risk factors for developing esophageal adenocarcinoma (EA) (1,2). Although GERD is the strongest individual risk factor and subjects who have the most frequent reflux symptom carry 4-fold EA risk, most of them follow an indolent course for the entire life (3). On the other hand, nearly 50% of EA patients do not experience GERD-associated symptoms (2,4,5). Therefore, challenges arise when considering the benefit and cost of endoscopic surveillance for EA among subjects with these risk factors, especially GERD (6). One of the explanations for this phenomenon stems from the complex interactions between lifestyle/environmental exposure and genetic factors. Since most EA cases are sporadic, genetic influences are more probably to be polymorphisms in multiple genes instead of single gene mutation. Evidence from pathway-based studies suggests that genetic variance may modulate the susceptibility of developing EA (7–9). Incorporating one’s genetic background in the clinical setting could be a better strategy for risk assessment and cancer surveillance, and possibly provide a better understanding about how complex factors contribute to EA risk jointly. However, fewer studies have assessed interactions using a more comprehensive approach.

Apoptosis, or programmed cell death, is essential for normal tissues to regulate cell number and to eliminate unwanted or aging cells as an organism develops. Mutations and single nucleotide polymorphisms (SNPs) in apoptotic pathway genes that alter the ability of the cell to undergo apoptosis may induce cancers by allowing transformed cells to keep accumulating rather than dying (10). Our recent studies indicated that genetic polymorphisms in the apoptosis pathway, by themselves or through interaction with environmental factors, play an important role in the carcinogenesis of EA (7,11). In a case–control study covering 1330 functional/tagging SNPs categorized into14 cancer-related pathways, two apoptotic SNPs (Caspase 7 rs312707 and Caspase 9 rs4661636) were significantly associated with EA risk. Moreover, apoptosis pathway was found to be the most important one in pathway-based analysis (11). However, it is possible that other SNPs further down the significance list of individual effects exert their importance through gene–environment interaction. Here, we conducted a pathway-based case-only study to further explore the interactions between 242 apoptosis-related SNPs and those well-known EA risk factors. We applied a two-step approach to identify gene–environment interaction markers in EA cases. Our results showed that gene–environment interactions play important roles in the development of EA.

Materials and methods

Study population, interview and DNA preparation

Details of patient recruitment were described in our previous paper (11). In brief, they were Caucasian, >18 years old and histologically confirmed to have EA at Massachusetts General Hospital between 1999 and 2005 and at Dana Farber Cancer Institute between 2004 and 2005. All of them had a tumor center located at or above the gastroesophageal junction and had at least two-thirds of the bulk tumor located in the esophagus. Patients with secondary or recurrent cancers were excluded. The recruitment rate was 86%. Participants were interviewed using a modified questionnaire (12) immediately after enrolment to collect information of their demographic characteristics and smoking history. Smoking status was defined based on whether the patient smoked 1 year prior to diagnosis. Lifetime GERD-related symptoms were collected based on the questions described before (13). The presence of GERD was defined as having heartburn or regurgitation symptoms at least once per month for more than six continuous months in one’s lifetime (7).

Blood samples were collected at the time of recruitment and DNA was extracted using the Puregene DNA Isolation Kit (Gentra Systems/Qiagen, Valencia, CA). This study was approved by the Human Subjects Committees of Massachusetts General Hospital, Dana Farber Cancer Institute and the Harvard School of Public Health (Boston, MA). All subjects signed the informed consent prior to study participation.

SNP selection, pathway categorization and genotyping

The criteria for SNP selection in the GoldenGate assay was described previously (11). The candidate SNPs selected in the apoptosis pathway were common SNPs with minor allele frequency (MAF) ≥ 5% in the HapMap Caucasian population (CEU). They were either missense/exonic SNPs, SNPs within untranslated regions and 2 kb 5’ of the gene or tagSNPs for genes with
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Results

The mean age of the EA cases was 62.9 years and 295 (88%) were males. About half of them had history of GERD symptoms and 80.3% were ever smokers. Other demographic characteristics were shown in Table I. In random forest analysis, MDA scores were obtained by SNP–GERD, SNP-smoking and SNP–BMI models, respectively. To minimize random bias, 100,000 trees were constructed in each RF model, allowing each marker to have \(~100\%\) of probability to be tested for 500 times. Based on MDA plot curves, the most important SNPs that had interaction with GERD (22 SNPs), smoking (17 SNPs) and BMI (15 SNPs) were selected for subsequent case-only logistic regression analysis (Figure 1).

In case-only logistic regression analysis, we found that five SNPs (rs651662, rs648802, rs4855094, rs7644468 and rs4149579) in three genes (PERP, PIK3CA and TNFRSF1A) had significant interaction with GERD; these interactions remained significant even after adjusting for covariates and false discovery rate (Table II). Two SNPs (rs651662 and rs648802) were in the gene PERP and were in high linkage disequilibrium (\(r^2 = 0.99\)). Another two significant SNPs in PIK3CA (rs4855094 and rs7644468) were not tagged to each other. Subjects carrying the variant allele of rs651662, rs648802, rs4855094 or rs7644468 significantly enhanced the risk of GERD to develop EA compared with subjects carrying homozygous wild genotypes. On the contrary, the risk of GERD was significantly reduced when one carries the wide-type allele of rs1419579 (ORi \(= 0.42\), 95% CI 0.22–0.81, \(P_{\text{adj}} = 0.04\)) (Table II). Since rs651662 and rs648802 were in linkage disequilibrium (\(r^2 = 0.99\)) and the later was a non-synonymous SNP, whereas the former located in intron, only rs648802 was used to combine with the other three SNPs to calculate the joint effect. There was a significant cumulative risk when those four SNPs were considered jointly. The risk of GERD on EA was increased when one has two (ORi = 1.89, 95% CI 1.13–3.16) or three (ORi = 4.30, 95% CI 2.25–8.21) risk genotypes comparing with those with \(\leq 1\) risk genotype (Table II).

For the SNP-smoking interaction, 4 of the top 17 SNPs were identified to be significant in logistic regression models (Table III). The presence of variant alleles in TP53BP1 rs560191, rs2602141, CASP7 rs7907519 or BCL2 rs12454712 enhanced the risk of smoking by 2.08–2.58 times (ORi \(= 1.89\), 95% CI 1.13–3.16) or \(\geq 3\) (ORi \(= 4.30\), 95% CI 2.25–8.21) risk genotypes comparing with those with \(\leq 1\) risk genotype (Table II).

The allele frequencies of the SNPs with significant interaction with environmental factors were shown in Supplementary Table S2, available at Carcinogenesis Online.

Discussion

This is a comprehensive study covering 242 apoptotic SNPs to evaluate the gene–environment interaction in EA. Case-only study with random forest analysis has been proven to be a reliable method to investigate gene–environment interaction in complex diseases (20). The EA patients in this study were incident cases; therefore, the ORi estimated the interaction under the assumption that the genetic and

Table I. Characteristics of 335 EA cases

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>EA cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years, mean ± SD)</td>
<td>62.9 ± 1.18</td>
</tr>
<tr>
<td>Gender, male (%)</td>
<td>295 (88.0%)</td>
</tr>
<tr>
<td>GERD(^1), yes (%)</td>
<td>173 (50.6%)</td>
</tr>
<tr>
<td>Smoking, yes (%)</td>
<td>269 (80.3%)</td>
</tr>
<tr>
<td>Smoking (pack-years), mean ± SD, medium (minimum to maximum)</td>
<td>30.5 ± 31.0, 24.4 (0–212.0)</td>
</tr>
<tr>
<td>BMI at age 18, (kg/m(^2), mean ± SD)</td>
<td>23.6 ± 3.8</td>
</tr>
<tr>
<td>BMI ≥ 25 at age 18 (%)</td>
<td>104 (31.0%)</td>
</tr>
<tr>
<td>Stage</td>
<td></td>
</tr>
<tr>
<td>I–IIA</td>
<td>98 (29.3%)</td>
</tr>
<tr>
<td>IIB–IV</td>
<td>237 (70.7%)</td>
</tr>
</tbody>
</table>

\(^1\) 7% (2%) missing and imputed.
environment factors were independent. In addition to identifying potential modifiers, we also demonstrated increased gene–GERD and gene–smoking interactions when those significant SNPs were considered jointly. Our results provide more evidence to how apoptotic genes modify the risk of GERD and smoking on EA risk. Dominant model of each SNP was used to calculate the OR because it was more convenient and the sample size was limited in this study. We also used case–control model to test the interaction between smoking and the four significant SNPs found in this case-only analysis. The control subjects were described in our previous study (11). Three of them had very consistent results (rs560191, rs260214 and rs12454712; ORi 5 2.11–2.28, P-value 5 0.02–0.04). Although we did not find significant interaction between CASP7 rs7907519 and smoking in case–control model, the trend was consistent (ORi 5 1.20, 95% CI, 0.58–2.48, P-value 5 0.61). Because GERD information was missing in 50% of our control subjects (11), we cannot perform case–control analysis for genotype–reflux interaction.

Evading apoptosis, a characteristic of transformed cells, has been well documented in Barrett’s carcinogenesis (23,24). Gastric acid and bile acid are two principle components of refluxate that cause chronic esophageal inflammation and Barrett’s transformation. Direct exposure of an EA cell line to acid leads to suppression of apoptotic genes, which may occur via p53-dependent mechanism (25). TP53 tumor suppressor induces the intrinsic apoptotic pathway and activates many downstream genes, including PERP (TP53 apoptosis effector related to PMP-22) (26). Previous study also suggests that there is a reciprocal regulation between p53 and the PI3K–AKT pathway (27). The PIK3CA gene encodes the 110 kDa subunit of PI3K, which has been found in many human cancers (39,40). Treatment with a PI3K inhibitor decreases proliferation and increases apoptosis; therefore, it has become a target for cancer therapy (28). Genetic polymorphisms of PIK3CA have been studied in the susceptibility of ovarian cancer (31). In addition to PERP and PIK3CA, we also found a significant interaction between the TNFRSF1A polymorphism and GERD. TNFRSF1A is one of the cognate receptors that binds to tumor necrosis factor family members to activate the extrinsic apoptotic pathway (32). TNFRSF1A rs149570 has been linked to the survival of early-stage non-small cell lung cancer (33). However, no studies investigated the association between PERP, PIK3CA or TNFRSF1A polymorphism and esophageal cancer risk.

Our data revealed that polymorphisms in CASP7, TP53BP1 and BCL2 modified the risk of smoking to develop EA. Long-term exposure to carcinogens in cigarette smoke such as polycyclic aromatic hydrocarbons leads to DNA damage, which accumulates if a cell evades cell cycle regulation and apoptotic mechanism. Caspase activation plays a primary role in the apoptotic cascade. A previous report has shown an inactivation mutation of CASP7 in esophageal cancer cells (34). Polymorphisms in CASP7 have been examined in several human cancers (35,36) but not in esophageal cancer until our recent report (11). In this study, we identified a significant interaction between CASP7 rs7907519 and smoking in EA cases. Such information adds more evidence to the importance of genes in caspase cascade in the susceptibility of EA. TP53BP1 binds to TP53 and plays a role in responses to DNA damage. A study in Japan revealed that TP53BP1 Asp353Glu (rs560191) and TP53 Arg72Pro polymorphism had significant interaction in lung cancer risk. However, no gene–smoking interaction was found (37). BCL2 protein is one of the products of BCL2 family and has anti-apoptotic function. It has been found to aberrantly expressed in Barrett’s carcinogenesis (38). Polymorphisms in BCL2 were reported in many human cancers such as ovarian cancer, leukemia and esophageal cancer (39–41). However, no study examined the association of TP53BP1 or BCL2 polymorphism and EA risk among Caucasians.

Fig. 1. Random forest importance plots for interaction between apoptotic SNPs and GERD (A), smoking (B) or body mass index (C).
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<table>
<thead>
<tr>
<th>SNPs</th>
<th>GERD</th>
<th>ORI ($) (95% CI)</th>
<th>( P_{\text{adj}}^{a} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>PERP rs651662</td>
<td>GG</td>
<td>68 (41.97)</td>
<td>43 (24.86)</td>
</tr>
<tr>
<td></td>
<td>GA + AA</td>
<td>94 (58.03)</td>
<td>130 (75.14)</td>
</tr>
<tr>
<td>PERP rs648802</td>
<td>CC</td>
<td>67 (41.36)</td>
<td>43 (24.86)</td>
</tr>
<tr>
<td></td>
<td>CG + GG</td>
<td>95 (58.64)</td>
<td>130 (75.14)</td>
</tr>
<tr>
<td>PIK3CA rs4855094</td>
<td>GG</td>
<td>151 (93.21)</td>
<td>145 (83.82)</td>
</tr>
<tr>
<td></td>
<td>GA + AA</td>
<td>11 (6.79)</td>
<td>28 (16.18)</td>
</tr>
<tr>
<td>PIK3CA rs7644648</td>
<td>AA</td>
<td>123 (75.93)</td>
<td>109 (63.00)</td>
</tr>
<tr>
<td></td>
<td>GA + GG</td>
<td>39 (24.07)</td>
<td>64 (37.00)</td>
</tr>
<tr>
<td>TNFRSF1A rs4149579</td>
<td>GG</td>
<td>130 (80.25)</td>
<td>157 (90.75)</td>
</tr>
<tr>
<td></td>
<td>GA + AA</td>
<td>32 (19.76)</td>
<td>16 (9.25)</td>
</tr>
</tbody>
</table>

*Adjusted for age, sex, BMI at age 18 (<25 versus ≥25) and smoking (+/-) (also false discovery rate for \( P_{\text{adj}}^{a} \)).

Since rs651662 and rs648802 were in linkage disequilibrium, only the later was used to calculate the cumulative risks.

Obesity not only promotes reflux symptoms but also contributes to the progression of EA by inhibiting apoptosis through a reduced adiponectin (anti-inflammatory adipokine from adipose tissue) and ghrelin levels (42). It is possible that there is an interaction between increased BMI and apoptotic SNPs; however, our data does not support this hypothesis. This might suggest that the effect of obesity on EA involves in mechanisms other than polymorphisms in apoptotic genes. It is also possible that there is a high-dimensional interaction involving multiple SNPs and BMI, which is not easy to investigate. Because we only included 40 (12%) female cases, the interaction with obesity and BMI, which is not easy to investigate.

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References

42. Benjamini, Y. et al. (2005) Quantitative trait Loci analysis using the false discovery rate. Genetics, 171, 783–790.