Null Results in Brief

No Association between hOGG1, XRCC1, and XPD Polymorphisms and Risk of Reflux Esophagitis, Barrett’s Esophagus, or Esophageal Adenocarcinoma: Results from the Factors Influencing the Barrett’s Adenocarcinoma Relationship Case-Control Study

Heather R. Ferguson,1 Christopher P. Wild,5 Lesley A. Anderson,2 Seamus J. Murphy,6 Brian T. Johnston,2 Liam J. Murray,2 R.G. Peter Watson,3 Jim McGuigan,4 John V. Reynolds,7 and Laura J. Hardie5

1Division of Gastroenterology, Belfast City Hospital; Centre for Clinical and Population Sciences, Queen’s University; Divisions of Gastroenterology and Surgery, Royal Group of Hospitals, Belfast, United Kingdom; Centre for Epidemiology and Biostatistics, University of Leeds, Leeds, United Kingdom; Division of Gastroenterology, Daisy Hill Hospital, Newry, United Kingdom; and Division of Surgery, St. James’s Hospital, Dublin, Ireland

Abstract

Reflux of gastric contents can lead to development of reflux esophagitis and Barrett’s esophagus. Barrett’s esophagus is a risk factor for esophageal adenocarcinoma. Damage to DNA may lead to carcinogenesis but is repaired through activation of pathways involving polymorphic enzymes, including human 8-oxoguanine glycosylase 1 (hOGG1), X-ray repair cross-complementing 1 (XRCC1), and xeroderma pigmentosum group D (XPD). Of the single nucleotide polymorphisms identified in these genes, hOGG1 Ser326Cys, XRCC1 Arg399Gln, and XPD Lys751Gln are particularly common in Caucasians and have been associated with lower DNA repair capacity. Small studies have reported associations with XPD Lys751Gln and esophageal adenocarcinoma. XRCC1 Arg399Gln has been linked to Barrett’s esophagus and reflux esophagitis. In a population-based case-control study, we examined associations of the hOGG1 Ser326Cys, XRCC1 Arg399Gln, and XPD Lys751Gln polymorphisms with risk of esophageal adenocarcinoma, Barrett’s esophagus, and reflux esophagitis. Genomic DNA was extracted from blood samples collected from cases of esophageal adenocarcinoma (n = 210), Barrett’s esophagus (n = 212), reflux esophagitis (n = 230), and normal population controls frequency matched for age and sex (n = 248). Polymorphisms were genotyped using TaqMan allelic discrimination assays. Odds ratios and 95% confidence intervals were obtained from logistic regression models adjusted for potential confounding factors. There were no statistically significant associations between these polymorphisms and risk of esophageal adenocarcinoma, Barrett’s esophagus, or reflux esophagitis. (Cancer Epidemiol Biomarkers Prev 2008;17(3):736–9)

Introduction

Reflux of gastric contents can lead to development of reflux esophagitis and Barrett’s esophagus. Barrett’s esophagus is an important risk factor for esophageal adenocarcinoma, the incidence of which has increased in recent years (1, 2). Damage to DNA may lead to carcinogenesis (3) but is repaired through activation of pathways involving polymorphic enzymes (4). Human 8-oxoguanine glycosylase 1 (hOGG1) and X-ray repair cross-complementing 1 (XRCC1) act on the base excision repair pathway. Xeroderma pigmentosum group D (XPD) enzyme is involved in the nucleotide excision repair pathway (5). Various single nucleotide polymorphisms have been identified in these genes, but hOGG1 Ser326Cys, XRCC1 Arg399Gln, and XPD Lys751Gln polymorphisms with lower DNA repair capacity (6-8). Small studies have reported associations with XPD Lys751Gln and esophageal adenocarcinoma (9-11). XRCC1 Arg399Gln has been linked to Barrett’s esophagus and reflux esophagitis. In a large case-control study, we investigated associations between hOGG1 Ser326Cys, XRCC1 Arg399Gln, and XPD Lys751Gln and risk of esophageal adenocarcinoma, Barrett’s esophagus, and reflux esophagitis.
Materials and Methods

Design. The study methods have been described previously in detail (12). In summary, the Factors Influencing the Barrett’s Adenocarcinoma Relationship study was carried out in Ireland between March 2002 and July 2005. Data and samples were collected from Caucasians with (a) esophageal adenocarcinoma, (b) Barrett’s esophagus, (c) reflux esophagitis, and (d) normal controls.

Genotyping. The Puregene DNA purification kit (Gentra Systems) was used to extract DNA from blood samples. The hOGG1 Ser326Cys (rs1052133), XRCC1 Arg399Gln (rs25487), and XPD Lys751Gln (rs13181) polymorphisms were genotyped using TaqMan allelic discrimination assays (Applied Biosystems). As a quality-control measure, genotyping was repeated for 10% of the samples, and the replicates were 100% concordant.

Statistical Analysis. Departures from Hardy-Weinberg equilibrium were tested for using the goodness-of-fit test. Genotype frequencies among cases of esophageal adenocarcinoma, Barrett’s esophagus, and reflux esophagitis were compared with controls using the \( \chi^2 \) test for association while adjusting for potential confounding factors by logistic regression. Statistical significance was set at \( P < 0.05 \). Analyses were done using SPSS for Windows version 13.0 (SPSS).

Results

The Factors Influencing the Barrett’s Adenocarcinoma Relationship study recruited a total of 227 esophageal adenocarcinoma patients, 224 Barrett’s esophagus patients, 230 reflux esophagitis patients, and 260 population controls. The demographic characteristics of each group are presented in Table 1. The participation rates of Barrett’s esophagus and reflux esophagitis patients and control subjects were 82.4%, 68.7%, and 41.8%, respectively. The participation rate of eligible, alive esophageal adenocarcinoma patients was 74.2% and the overall esophageal adenocarcinoma response rate was 63.9%. DNA samples were available for analysis from 210 (92.5%) esophageal adenocarcinoma patients, 212 (94.6%) Barrett’s esophagus patients, 230 (100%) reflux esophagitis patients, and 248 (95.4%) controls.

The distribution and frequency of genotypes for cases and controls are shown in Table 2. There were no statistically significant departures from Hardy-Weinberg equilibrium for any of the polymorphisms in any of the subject groups. For controls, the frequency of polymorphic variants was 0.24 (hOGG1 Ser326Cys), 0.36 (XRCC1 Arg399Gln), and 0.38 (XPD Lys751Gln). These were similar to frequencies reported in Caucasians in the SNP500 Database (http://snp500cancer.nci.nih.gov).

There were no statistically significant associations between the hOGG1 Ser326Cys, XRCC1 Arg399Gln, and XPD Lys751Gln polymorphisms and the risk of esophageal adenocarcinoma, Barrett’s esophagus, or reflux esophagitis.

Discussion

To date, this is the largest case-control study to explore the association between DNA repair gene polymorphisms and risk of esophageal adenocarcinoma. In addition, it is the first population-based study to evaluate the role of these polymorphisms in Barrett’s esophagus and reflux esophagitis. All three polymorphisms were in Hardy-Weinberg equilibrium and had sufficient statistical power (>80%) to detect an odds ratio (OR) of >2.0. Further strengths of our study include the population-based design and clear characterization of phenotypes to minimize misclassification bias. Detailed interview data allowed us to control for a range of potential confounding factors, and DNA was available for analysis in almost all subjects.

Table 1. Characteristics of controls, reflux esophagitis, Barrett’s esophagus, and esophageal adenocarcinoma patients

<table>
<thead>
<tr>
<th>Variables</th>
<th>Controls, ( n (%) )</th>
<th>Reflux esophagitis, ( n (%) )</th>
<th>( P (\text{reflux esophagitis vs controls}) )</th>
<th>Barrett’s esophagus, ( n (%) )</th>
<th>( P (\text{Barrett’s esophagus vs controls}) )</th>
<th>Esophageal adenocarcinoma, ( n (%) )</th>
<th>( P (\text{esophageal adenocarcinoma vs controls}) )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>220 (84.6)</td>
<td>189 (82.2)</td>
<td>0.468</td>
<td>185 (82.6)</td>
<td>0.548</td>
<td>192 (84.6)</td>
<td>0.992</td>
</tr>
<tr>
<td>Female</td>
<td>40 (15.4)</td>
<td>41 (17.8)</td>
<td></td>
<td>39 (17.4)</td>
<td>35 (15.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, mean (y)</td>
<td>63.0</td>
<td>61.7</td>
<td>0.219</td>
<td>62.4</td>
<td>0.567</td>
<td>64.2</td>
<td>0.276</td>
</tr>
<tr>
<td>Education</td>
<td>12.0</td>
<td>10.8</td>
<td>&lt;0.001</td>
<td>11.3</td>
<td>&lt;0.001</td>
<td>10.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Gastroesophageal reflux symptoms</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>211 (81.2)</td>
<td>140 (60.9)</td>
<td>&lt;0.001</td>
<td>60 (26.8)</td>
<td>&lt;0.001</td>
<td>117 (51.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ever</td>
<td>49 (18.8)</td>
<td>90 (39.1)</td>
<td></td>
<td>164 (73.2)</td>
<td></td>
<td>110 (48.5)</td>
<td></td>
</tr>
<tr>
<td>Smoking status ^</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>102 (40.2)</td>
<td>109 (48.4)</td>
<td>0.026</td>
<td>87 (39.2)</td>
<td>0.400</td>
<td>45 (20.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ex-smoker</td>
<td>107 (42.1)</td>
<td>68 (30.2)</td>
<td></td>
<td>85 (38.3)</td>
<td></td>
<td>99 (44.8)</td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>45 (17.7)</td>
<td>48 (21.3)</td>
<td></td>
<td>50 (22.5)</td>
<td></td>
<td>77 (34.8)</td>
<td></td>
</tr>
<tr>
<td>Alcohol, mean (g/d)</td>
<td>26.1</td>
<td>22.0</td>
<td>0.151</td>
<td>22.3</td>
<td>0.214</td>
<td>19.2</td>
<td>0.012</td>
</tr>
<tr>
<td>Body mass index, mean (kg/m²)</td>
<td>27.0</td>
<td>27.8</td>
<td>0.047</td>
<td>27.0</td>
<td>0.895</td>
<td>28.7</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

^Symptoms of heartburn or acid reflux experienced at least once a week or >50 times per year, >5 y before the interview date.

^Never smoker” is defined as those subjects who had never smoked, who had smoked <100 cigarettes in their lifetime, or who had smoked <1 cigarette per day for >6 mo. “Ex-smoker” is defined as smokers who had stopped >5 y before the interview date. “Current smoker” is defined as smokers who had smoked at least 1 cigarette per day for >6 mo, 5 y before the interview date.
Our findings are in contrast to results from a Swedish case-control study, which suggested that the XPD Lys751Gln polymorphism was associated with a significantly increased risk of esophageal adenocarcinoma (10). This study also had a population-based design, strict definition of phenotypes, and detailed interview data. However, DNA was available for analysis in only 50% of subjects recruited, major limitations were its hospital-based design and small sample size, including only 56 esophageal adenocarcinoma subjects. Liu et al. also reported an increased risk of esophageal adenocarcinoma in association with the XPD Lys751Gln polymorphism (9). Although this study was a reasonable size, with 183 esophageal adenocarcinoma subjects recruited, major limitations were its hospital-based design and lack of complete case ascertainment. In a Canadian study, Casson et al. reported further conflicting results, suggesting that the XPD Lys751Gln polymorphism was associated with a reduced risk of esophageal adenocarcinoma subjects. Liu et al. also reported an increased risk of esophageal adenocarcinoma in association with the XPD Lys751Gln polymorphism (9). Although this study was a reasonable size, with 183 esophageal adenocarcinoma subjects recruited, major limitations were its hospital-based design and small sample size, including only 56 esophageal adenocarcinoma subjects.

In keeping with our results, all three previous studies have reported no association with the XRCC1 Arg199Gln polymorphism and risk of esophageal adenocarcinoma (9-11). However, in the only previous study to investigate DNA repair polymorphisms in Barrett’s esophagus and reflux esophagitis, Casson et al. reported a reduced risk of these conditions in association with XRCC1 Arg199Gln (11). The limitations of this study have been discussed and it is likely that results from our study are more robust given that we recruited over double the number of study subjects.

We reported no association between the hOGG1 Ser326Cys polymorphism and risk of esophageal adenocarcinoma, Barrett’s esophagus, or reflux esophagitis. No previous study has investigated this polymorphism in relation to these conditions.

However, association studies in esophageal squamous cell carcinoma have produced conflicting results (13, 14). A limitation of our study was inadequate power to detect a modest excess risk (OR < 2.0) or to examine the role of possible gene-environment interactions.

In summary, we found no significant associations between the hOGG1 Ser326Cys, XRCC1 Arg199Gln, and XPD Lys751Gln polymorphisms and the risk of esophageal adenocarcinoma, Barrett’s esophagus, or reflux esophagitis.

References
7. Lunn RM, Langlois RG, Hsieh LL, Thompson CL, Bell DA. XRCCI

Table 2. Frequency of hOGG1, XRCC1, and XPD genotypes and risk of reflux esophagitis, Barrett’s esophagus, and esophageal adenocarcinoma, relative to asymptomatic controls

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Controls (n = 248)</th>
<th>Reflux esophagitis (n = 230)</th>
<th>Barrett’s esophagus (n = 212)</th>
<th>Esophageal adenocarcinoma (n = 209)</th>
</tr>
</thead>
<tbody>
<tr>
<td>hOGG1</td>
<td></td>
<td>Unadjusted OR (95% CI)</td>
<td>Adjusted OR* (95% CI)</td>
<td></td>
</tr>
<tr>
<td>Ser/Ser</td>
<td>141</td>
<td>146</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Ser/Ser</td>
<td>96</td>
<td>71</td>
<td>0.71 (0.49-1.05)</td>
<td>0.73 (0.48-1.22)</td>
</tr>
<tr>
<td>Ser/Cys</td>
<td>11</td>
<td>13</td>
<td>1.14 (0.50-2.63)</td>
<td>1.01 (0.42-2.45)</td>
</tr>
<tr>
<td>Ser/Cys</td>
<td>107</td>
<td>84</td>
<td>0.76 (0.53-1.10)</td>
<td>0.77 (0.51-1.14)</td>
</tr>
</tbody>
</table>

*Adjusted for age, sex, years of full-time education, body mass index, smoking, alcohol, and gastroesophageal reflux symptoms. 95% CI, 95% confidence interval.

† One subject was “undetermined” and excluded from analysis.

‡ Two subjects were “undetermined” and excluded from analysis.
polymorphisms: effects on aflatoxin B1-DNA adducts and glyco-
repair capacity by XPD polymorphisms in lung cancer patients.
9. Liu G, Zhou W, Yeap BY, et al. XRCC1 and XPD polymorphisms and
XPD 795Gln allele is associated with an increased risk for esophageal
adenocarcinoma: a population-based case-control study in Sweden.
DL. Polymorphisms in DNA repair genes in the molecular
pathogenesis of esophageal (Barrett) adenocarcinoma. Carcinogene-
inflammatory drugs and the esophageal inflammation-metaplasia-
13. Xing DY, Tan W, Song N, Lin DX Ser326Cys polymorphism in hOGG1
gene and risk of esophageal cancer in a Chinese population. Int J
base excision repair pathway and their associations with risk of
No Association between hOGG1, XRCC1, and XPD Polymorphisms and Risk of Reflux Esophagitis, Barrett's Esophagus, or Esophageal Adenocarcinoma: Results from the Factors Influencing the Barrett's Adenocarcinoma Relationship Case-Control Study


Cancer Epidemiol Biomarkers Prev 2008;17:736-739.