High enzyme activity UGT1A1 or low activity UGT1A8 and UGT2B4 genotypes increase esophageal cancer risk

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Abstract. Esophageal cancer (EC) has a globally increasing incidence with poor curative treatment options and survival rates. Environmental and dietary factors have crucial roles in esophageal carcinogenesis. Polymorphisms in the UGT genes, a superfamily of enzymes essential for the detoxification of carcinogens, may alter enzyme activity and subsequently may play a role in EC etiology. Rather than solely establishing differences in genotype distribution, we investigated whether functional polymorphisms in UGT genes that can predict enzyme activity in vivo, may influence EC risk. A case-control study including 351 Caucasian EC patients and 592 Caucasian controls was conducted and polymorphisms in seven UGT genes were determined, using the polymerase chain reaction. On the basis of allelic in vitro enzyme activity measurements, genotypes were categorized according to their predicted in vivo enzyme activity into high, medium and low categories. Predicted enzyme activity groups were combined and compared between patients and controls. The UGT1A1 and UGT1A8 predicted high enzyme activity genotypes were significantly more (OR=1.62; 95% CI, 1.02-2.56) and less frequent (OR=0.36; 95% CI, 0.15-0.84) among patients with esophageal squamous cell carcinoma (ESCC), respectively. High (OR=0.42; 95% CI, 0.22-0.84) and medium (OR=0.25; 95% CI, 0.12-0.52) activity UGT2B4 genotypes were significantly less often present in ESCC patients. No association was detected between UGT genotypes and esophageal adenocarcinoma (EAC) risk. Polymorphisms in UGT genes, resulting in altered enzyme activity genotypes, do not seem modifiers of EAC risk. However, the predicted high activity UGT1A1 genotype, associated with low serum levels of the antioxidant bilirubin, was associated with an increased ESCC risk. The UGT1A8 and UGT2B4 genotypes associated with decreased predicted enzyme activities, were significantly associated with an increased risk of ESCC, probably by a decreased detoxification of carcinogens.

Introduction

Esophageal cancer (EC) is the eighth most common neoplasm in the world with poor 5-year survival rates of 16% in the USA and 10% in Europe (1). Esophageal squamous cell carcinoma (ESCC) is more prevalent in Asia, whereas esophageal adenocarcinoma (EAC) is predominantly seen in the Western world (2). Known risk factors for ESCC are the use of alcohol, tobacco or local dietary habits (3), whereas obesity and gastro-esophageal reflux disease as a result of a Western lifestyle are risk factors for EAC (4). Differences in genetic predisposition can also influence the individual risk profile. Genetic polymorphisms in detoxification enzymes may influence the process of carcinogenesis by altering the enzyme activity and subsequently influence the degree of exposure to carcinogens.

Detoxification occurs through phase I and phase II biotransformation reactions. A major phase II reaction is glucuronidation, catalyzed by the UDP-glucuronosyltransferases (UGTs) (5). This superfamily of detoxification enzymes catalyzes the glucuronidation of small lipophilic agents into more water soluble compounds which are subsequently secreted via bile or urine (5).

Human UGTs consist of two main gene families, UGT1 and UGT2. Xenobiotics such as phenolic compounds, flavones and amines are substrates for the UGT1A family, whereas UGT2B enzymes prefer endogenous substrates including steroids, opioids and bile acids (5,6). In the human esophagus at least seven UGT enzymes of the UGT1A and UGT2B family are expressed (7).

Lacko et al found that polymorphisms resulting in higher activities of UGT1A1 were associated with an increased risk of head and neck cancer (8). Furthermore, Zheng et al concluded
that low-activity UGT1A7 genotypes were associated with an increased orolaryngeal cancer risk, especially in smokers (9). Vogel et al described that the UGT1A1*3 allele, exhibiting reduced carcinogen detoxification activity, was significantly associated with proximal gastrointestinal cancer (10). This last study was probably flawed since the over-representation of the UGT1A1*3 allele was due to PCR-dependent bias (11,12).

There is a gap in the literature with respect to UGT polymorphisms and the risk for EC. Given the fact that head and neck cancer and esophageal cancer share identical risk factors (13), it may be highly relevant to investigate whether polymorphisms in UGT genes that are associated with head and neck cancer, are also associated with esophageal cancer risk.

Rather than to solely compare polymorphism distribution between patients and controls, we set out to examine whether UGT genotypes, associated with altered enzyme activity, modify EC risk. We conducted a case-control study and determined functional polymorphisms in seven UGT genes.

Materials and methods

Patients and controls. The study was approved by the Medical Ethical Review Committee, region Arnhem-Nijmegen (CMO 2002/114). Informed consent was obtained from all participants. Blood or tissue samples from 351 Caucasian patients with esophageal cancer were collected in the period October 2002 to March 2011 from four different hospitals, localized within 30 km distance in the South-East area of the Netherlands (14). Only patients with a diagnosis of esophageal carcinoma as confirmed by a pathologist were included in the study. As a source of DNA, in 92 cases tissue biopsies of normal esophagus or stomach from EC patients was collected after surgery, whereas in the other 259 cases EDTA blood was collected. Blood and tissue samples were frozen at -20°C and -80°C, respectively. DNA isolation was performed by usage of the High Pure PCR Template Preparation Kit (Roche, Mannheim, Germany) according to the instructions of the manufacturer. Post extraction DNA was stored at 4°C. Caucasian healthy controls (n=592) were recruited from the same geographical area of the Netherlands, after advertisement in local papers, as described by Kristinsson (14). Controls were matched with the EC patients for age, ethnicity and gender.

Genotyping methods and allelic in vitro enzyme activity. The selection of UGT enzymes was based upon either esophageal expression or relevance to head and neck carcinoma, as esophageal cancer shares some of the relevant risk factors. Expression in the esophagus of the UGT1A6, 1A7, 1A8 and UGT2B4 enzymes has been detected (5,15,16), while UGT1A1, UGT2B7 and UGT2B17 are known to be highly expressed in liver and intestine and thus may indirectly modify esophageal cancer risk (16).

UGT1A6. The T181A (rs2070959) and R184S (rs1105879) polymorphisms in exon 1 of the UGT1A6 gene were studied by PCR followed by restriction fragment length polymorphism (PCR-RFLP) analysis (18). These polymorphisms express an enzyme with a lower catalytic activity (19). Only the frequencies for the T181A mutation are shown. The R184S SNP corresponds to >90% with these frequencies. However, because of separate analyses for these SNPs, the "1'2 genotype could not be determined.

UGT1A7. The polymorphisms UGT1A8*2 (rs1042597) and UGT1A8*3 (rs17863762) were determined using PCR-RFLP analysis, as described before (22). The two polymorphisms resulted in three allelic variants of the UGT1A8 gene (23). Since the UGT1A8*1 and '2 alleles differ little in function and the UGT1A8*3 allele displays no catalytic activity (23), genotypes were stratified into high ("1'1, '1'2, '2'2) and medium/low activity ('1'3, '2'3, '3'3) genotypes for analyses.

UGT2B4/UGT2B7. A dual-colour allele-specific assay was used for genotyping the polymorphisms at codon 458 of the UGT2B4 gene (rs1319049) and codon 268 of the UGT2B7 gene (rs7439366) PCR was performed on the iCycler iQ Multicolour Real-Time Detection System (Bio-Rad Laboratories BV; Hercules CA) and by PCR-RFLP for detection of the W208R (rs11692021) polymorphism, as described elsewhere (20). The identified UGT1A7*1, *2, *3 and *10 alleles were categorized in enzyme activity categories as described by Guillemette et al (21).

Statistical analyses. The selected functional polymorphisms are known to produce alleles expressing differential in vitro enzyme activity. On the basis of this in vitro enzyme activity, the various genotypes were categorized into three groups of predicted in vivo enzyme activity: high, medium and low. In our study, for the purpose of increasing the power, the combined group of low and intermediate activity was used as reference in the comparison between patients with ESCC or EAC and controls.
Haplotypes were generated using the PLEM program (31). The haplotype with none of the mutations was set as a reference in the comparison between cases and controls. Only participants with complete genotypes were included in the haplotype analyses.

The independent samples t-test was applied for the differences in continues variables between characteristics of patients and controls. The $\chi^2$ test was used for analyzing nominal variables of patient characteristics and to test for differences of frequencies in predicted enzyme activity genotypes between two groups. Odds ratios (OR) with 95% confidence interval (95% CI) were calculated. Stratified analyses were performed according to tumor histology. All P-values were two-sided and a probability level of P<0.05 was considered to be significant. All analyses were performed with the software SPSS for Windows, version 16.0 (SPSS Inc., Chicago, IL, USA).

Table I. Characteristics of patients with esophageal cancer and controls.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>ESCC</th>
<th>EAC</th>
<th>Total</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. (% of total)</td>
<td>85 (24.2)</td>
<td>260 (74.1)</td>
<td>351 (100)*</td>
<td>592</td>
</tr>
<tr>
<td>Age (years; mean ± SD)</td>
<td>63.7±10.3</td>
<td>65.3±11.1</td>
<td>65.0±10.9</td>
<td>63.4±11.9</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>56 (65.9)</td>
<td>221 (85.0)</td>
<td>282 (80.3)</td>
<td>478 (80.7)</td>
</tr>
<tr>
<td>Female</td>
<td>28 (32.9)</td>
<td>39 (15.0)</td>
<td>68 (19.4)</td>
<td>114 (19.3)</td>
</tr>
</tbody>
</table>

*Note that for 6 patients the exact tumor type was not mentioned in the pathology report, whereas for 1 patient the gender is unknown. ESCC, esophageal squamous cell carcinoma; EAC, esophageal adenocarcinoma.

Table II. UGT gene distribution stratified in predicted enzyme activity in patients with esophageal cancer and controls.

<table>
<thead>
<tr>
<th>UGT isozymes</th>
<th>UGT genotypes*</th>
<th>Predicted enzyme activity in vivo</th>
<th>ESCC (n=85) [n (%)]</th>
<th>EAC (n=260) [n (%)]</th>
<th>Controls (n=592) [n (%)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>UGT1A1</td>
<td>'1'</td>
<td>High activity</td>
<td>50 (58.8)</td>
<td>122 (46.9)</td>
<td>276 (46.6)</td>
</tr>
<tr>
<td></td>
<td>'1' '28</td>
<td>Medium activity</td>
<td>30 (35.3)</td>
<td>102 (39.2)</td>
<td>256 (43.2)</td>
</tr>
<tr>
<td></td>
<td>'28' '28</td>
<td>Low activity</td>
<td>5 (5.9)</td>
<td>33 (12.7)</td>
<td>56 (9.5)</td>
</tr>
<tr>
<td>UGT1A6</td>
<td>'1'</td>
<td>High activity</td>
<td>42 (49.4)</td>
<td>119 (45.8)</td>
<td>272 (45.9)</td>
</tr>
<tr>
<td></td>
<td>'1' '2</td>
<td>Medium activity</td>
<td>37 (43.5)</td>
<td>109 (41.9)</td>
<td>249 (42.1)</td>
</tr>
<tr>
<td></td>
<td>'2' '2</td>
<td>Low activity</td>
<td>6 (7.1)</td>
<td>31 (11.9)</td>
<td>71 (12.0)</td>
</tr>
<tr>
<td>UGT1A7</td>
<td>'1', '1' '2', '2' '2'</td>
<td>High activity</td>
<td>33 (38.8)</td>
<td>101 (38.8)</td>
<td>228 (38.5)</td>
</tr>
<tr>
<td></td>
<td>'1', '1' '4', '1' '10', '2' '3'</td>
<td>Medium activity</td>
<td>43 (50.6)</td>
<td>110 (42.3)</td>
<td>274 (46.3)</td>
</tr>
<tr>
<td></td>
<td>'3', '3' '4', '3' '10', '4' '4'</td>
<td>Low activity</td>
<td>9 (10.6)</td>
<td>49 (18.8)</td>
<td>90 (15.2)</td>
</tr>
<tr>
<td>UGT1A8</td>
<td>'1', '1' '2', '2' '2'</td>
<td>High activity</td>
<td>77 (90.6)</td>
<td>247 (95.0)</td>
<td>565 (95.4)</td>
</tr>
<tr>
<td></td>
<td>'1', '3', '2' '3'</td>
<td>Medium activity</td>
<td>7 (8.2)</td>
<td>9 (3.5)</td>
<td>16 (2.7)</td>
</tr>
<tr>
<td></td>
<td>'3' '3'</td>
<td>Low activity</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>3 (0.5)</td>
</tr>
<tr>
<td>UGT2B4</td>
<td>'1'</td>
<td>High activity</td>
<td>50 (58.8)</td>
<td>139 (53.5)</td>
<td>320 (54.1)</td>
</tr>
<tr>
<td></td>
<td>'1' '2'</td>
<td>Medium activity</td>
<td>21 (24.7)</td>
<td>100 (38.5)</td>
<td>233 (39.4)</td>
</tr>
<tr>
<td></td>
<td>'2' '2'</td>
<td>Low activity</td>
<td>14 (16.5)</td>
<td>21 (8.1)</td>
<td>38 (6.4)</td>
</tr>
<tr>
<td>UGT2B7</td>
<td>'1'</td>
<td>High activity</td>
<td>18 (21.2)</td>
<td>59 (22.7)</td>
<td>133 (22.5)</td>
</tr>
<tr>
<td></td>
<td>'1' '2'</td>
<td>Medium activity</td>
<td>42 (49.4)</td>
<td>128 (49.2)</td>
<td>298 (50.3)</td>
</tr>
<tr>
<td></td>
<td>'2' '2'</td>
<td>Low activity</td>
<td>24 (28.2)</td>
<td>73 (28.1)</td>
<td>161 (27.2)</td>
</tr>
<tr>
<td>UGT2B17</td>
<td>'1'</td>
<td>High activity</td>
<td>54 (63.5)</td>
<td>174 (66.9)</td>
<td>353 (59.6)</td>
</tr>
<tr>
<td></td>
<td>'1' '2'</td>
<td>Medium activity</td>
<td>22 (25.9)</td>
<td>62 (23.8)</td>
<td>179 (30.2)</td>
</tr>
<tr>
<td></td>
<td>'2' '2'</td>
<td>Low activity</td>
<td>9 (10.6)</td>
<td>24 (9.2)</td>
<td>54 (9.1)</td>
</tr>
</tbody>
</table>

*The genotypes were classified into the three activity categories according to the observed allelic activity in vitro, as described in the Materials and methods.
Results

Demographics and genotype distribution. We included a total of 351 patients with esophageal cancer and 592 controls in our study. There was no statistical difference between the two groups regarding race, age and gender (Table I). However, the gender distribution differed significantly with females being more frequent in the ESCC group in comparison to the EAC group (32.9 vs. 15.0%; P<0.001). Genotype frequencies of UGT1A8 G→A in controls (P<0.001) and the UGT2B17 deletion polymorphism in controls (P<0.001) were not distributed according to the Hardy-Weinberg equilibrium.

Table II displays the distribution of the genetic polymorphisms in UGTs, along with the genotype distribution and the predicted enzyme activities, for controls and patients with ESCC and EAC, as well as for all cancer patients. Due to PCR bias not all genotypes could be generated.

Enzyme activities and haplotype distribution. Table III and Fig. 1 illustrate that none of the high activity UGT genotypes modified EAC risk in our population. Additionally Fig. 2 demonstrates that the high activity genotypes of UGT1A1 and UGT1A8, respectively, increase and decrease ESCC susceptibility. The frequency of the predicted high enzyme activity UGT1A1 genotype was significantly higher in the ESCC patients in comparison to the predicted low and medium enzyme activity genotypes (OR=1.62; 95% CI, 1.02-2.56) (Table III). The UGT1A8 predicted high activity genotype was significantly less frequent among ESCC patients than in controls (OR=0.36; 95% CI, 0.15-0.84). Furthermore, the high
enzyme activity $UGT2B4$ genotype did not modify ESCC risk when set off against the combined low and intermediate activity group. However, the high (OR=0.42; 95% CI, 0.22-0.84) and medium (OR=0.25; 95% CI, 0.12-0.52) activity $UGT2B4$ genotypes when set off against the low enzyme activity genotypes, were significantly less often present in ESCC in comparison to controls.

Tables IV and V show the $UGT1$ and $UGT2$ haplotype distribution between EAC or ESCC patients and controls, respectively. Setting the haplotype with no mutations as a reference, no $UGT1$ haplotype modified EAC or ESCC risk. Regarding the $UGT2$ haplotypes, the combination of mutations in the $UGT2B7$ and $UGT2B17$ genes (011) was significantly associated with a decreased ESCC risk (OR=0.52; 95% CI, 0.29-0.94). Similarly, only a mutation in the $UGT2B4$ gene (100) decreased risk of ESCC (OR=0.60; 95% CI, 0.34-1.05). However, the latter correlation was not significant.

### Discussion

This study detected the predicted high activity $UGT1A1$ genotype to be associated with an increased ESCC risk. The $UGT1A1^{*}28$ polymorphism in the promoter region of $UGT1A1$ is well studied in relation to glucuronidation of bilirubin (17), in its protective role in coronary artery disease (32) and in the risk of head and neck cancer (8). Nevertheless a potential role of the low activity $UGT1A1^{*}28$ allele in the risk of esophageal cancer has not been investigated before. Our results correspond with those obtained by Lacko et al. in a case control study with head and neck cancer patients (8), where an inverse correlation between the low activity $UGT1A1^{*}28$ genotype and the risk of squamous cell carcinoma was found. The low activity genotype may be associated with lower risk for ESCC, due to the lower levels of glucuronidation, resulting in higher serum bilirubin levels (8,17). This is most likely a systemic effect, as

### Table IV. $UGT1$ haplotypes with corresponding odds ratios (OR) for patients with ESCC and EAC compared to controls.

<table>
<thead>
<tr>
<th>$UGT1$ haplotypes</th>
<th>ESCC n=170 [n (%)]</th>
<th>OR (95% CI)</th>
<th>EAC n=520 [n (%)]</th>
<th>OR (95% CI)</th>
<th>Controls n=1184 [n (%)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1111100</td>
<td>34 (20.0)</td>
<td>0.72 (0.43±1.21)</td>
<td>141 (27.1)</td>
<td>1.20 (0.86±1.66)</td>
<td>287 (24.2)</td>
</tr>
<tr>
<td>0000010</td>
<td>36 (21.2)</td>
<td>0.94 (0.56±1.57)</td>
<td>111 (21.4)</td>
<td>1.16 (0.82±1.63)</td>
<td>234 (19.8)</td>
</tr>
<tr>
<td>0000000</td>
<td>32 (18.8)</td>
<td>Ref</td>
<td>80 (15.4)</td>
<td>Ref</td>
<td>195 (16.5)</td>
</tr>
<tr>
<td>0001000</td>
<td>26 (15.3)</td>
<td>0.99 (0.57±1.73)</td>
<td>82 (15.8)</td>
<td>1.25 (0.86±1.81)</td>
<td>160 (13.5)</td>
</tr>
<tr>
<td>0001010</td>
<td>6 (3.5)</td>
<td>0.65 (0.26±1.64)</td>
<td>17 (3.3)</td>
<td>0.74 (0.41±1.35)</td>
<td>56 (4.7)</td>
</tr>
<tr>
<td>0001100</td>
<td>8 (4.7)</td>
<td>0.94 (0.41±2.16)</td>
<td>21 (4.0)</td>
<td>0.98 (0.56±1.74)</td>
<td>52 (4.4)</td>
</tr>
<tr>
<td>0111100</td>
<td>10 (5.9)</td>
<td>1.35 (0.62±2.96)</td>
<td>20 (3.9)</td>
<td>1.08 (0.60±1.95)</td>
<td>45 (3.8)</td>
</tr>
<tr>
<td>1001100</td>
<td>1 (0.6)</td>
<td>0.24 (0.03±1.86)</td>
<td>9 (1.7)</td>
<td>0.88 (0.39±1.96)</td>
<td>25 (2.1)</td>
</tr>
<tr>
<td>0001101</td>
<td>5 (2.9)</td>
<td>2.03 (0.69±5.98)</td>
<td>7 (1.4)</td>
<td>1.14 (0.45±2.89)</td>
<td>15 (1.3)</td>
</tr>
<tr>
<td>0110000</td>
<td>2 (1.2)</td>
<td>0.94 (0.20±4.35)</td>
<td>1 (0.2)</td>
<td>0.19 (0.02±1.46)</td>
<td>13 (1.1)</td>
</tr>
<tr>
<td>0011000</td>
<td>1 (0.6)</td>
<td>0.51 (0.06±4.04)</td>
<td>6 (1.2)</td>
<td>1.22 (0.44±3.36)</td>
<td>12 (1.0)</td>
</tr>
</tbody>
</table>

Haplotypes are in the following order: $UGT1A1$, $UGT1A6$ (T181A), $UGT1A6$ (R184S), $UGT1A7$ (N129K/R131K), $UGT1A7$ (W208R), $UGT1A8$ (A173G) and $UGT1A8$ (C277Y). 0, no mutation; 1, mutation.

### Table V. $UGT2$ haplotypes with corresponding odds ratios (OR) for patients with ESCC and EAC compared to controls.

<table>
<thead>
<tr>
<th>$UGT2$ haplotypes</th>
<th>ESCC n=170 [n (%)]</th>
<th>OR (95% CI)</th>
<th>EAC n=520 [n (%)]</th>
<th>OR (95% CI)</th>
<th>Controls n=1184 [n (%)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>010</td>
<td>61 (35.9)</td>
<td>1.04 (0.69±1.57)</td>
<td>147 (28.3)</td>
<td>1.03 (0.77±1.37)</td>
<td>351 (29.7)</td>
</tr>
<tr>
<td>000</td>
<td>47 (27.7)</td>
<td>Ref</td>
<td>115 (22.1)</td>
<td>Ref</td>
<td>282 (23.8)</td>
</tr>
<tr>
<td>100</td>
<td>19 (11.2)</td>
<td>0.60 (0.34±1.05)</td>
<td>90 (17.3)</td>
<td>1.16 (0.83±1.62)</td>
<td>190 (16.1)</td>
</tr>
<tr>
<td>011</td>
<td>16 (9.4)</td>
<td>0.52 (0.29±0.94)</td>
<td>75 (14.4)</td>
<td>0.99 (0.70±1.40)</td>
<td>185 (15.6)</td>
</tr>
<tr>
<td>110</td>
<td>16 (9.4)</td>
<td>1.30 (0.70±2.42)</td>
<td>45 (8.7)</td>
<td>1.49 (0.97±2.29)</td>
<td>74 (6.3)</td>
</tr>
<tr>
<td>001</td>
<td>9 (5.3)</td>
<td>0.95 (0.44±2.04)</td>
<td>29 (5.6)</td>
<td>1.25 (0.76±2.05)</td>
<td>57 (4.8)</td>
</tr>
<tr>
<td>101</td>
<td>1 (0.6)</td>
<td>0.17 (0.02±1.28)</td>
<td>14 (2.7)</td>
<td>0.98 (0.51±1.89)</td>
<td>35 (3.0)</td>
</tr>
<tr>
<td>111</td>
<td>1 (0.6)</td>
<td>0.60 (0.08±4.80)</td>
<td>5 (1.0)</td>
<td>1.23 (0.41±3.67)</td>
<td>10 (0.8)</td>
</tr>
</tbody>
</table>

Haplotypes are in the following order: $UGT2B4$, $UGT2B7$ and $UGT2B17$. 0, no mutation; 1, mutation.
the UGT1A1 enzyme is not expressed in the esophagus (5). Bilirubin acts as an antioxidant by inhibiting cellular damage induced by alcohol and smoking related oxidative stress (32,33). So the hypothesis that serum bilirubin protects against ESCC by inhibiting damage induced by reactive oxygen species (ROS), is further encouraged because the correlation is not found in patients with EAC. The latter histological subtype has different risk factors (4).

Genetic polymorphisms in the UGT1A6 gene have been studied regarding the association with aspirin use in relation to colorectal adenomas or carcinoma (34), as aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) are reported to have protective effects on colorectal- or esophageal tumorigenesis (35). Zheng et al reported UGT1A7*3 to be a risk allele in patients with orolaryngeal cancer (9), although controversy still exists as Lacko et al reported different results (20). For both UGfs however, correlations with esophageal cancer risk were not previously explored, but this study did not demonstrate correlations between UGT1A6 or UGT1A7 genotypes and EAC or ESCC risk.

Our analyses illustrates a significantly more common occurrence of the low/medium activity UGT1A8 genotypes in ESCC patients in comparison to controls. This identifies UGT1A8*3 as a high risk allele in the etiology of esophageal SCC, although homozogosity for this allele was not found in patients. The genotype distribution in the controls was not according the Hardy-Weinberg (HW) equilibrium. This may be a by chance finding due to rarity of the UGT1A8*3 polymorphism. Non-random mating, selection or migration within the control population may be also responsible for the HW disequilibrium. Furthermore, although linkage disequilibrium exists within the UGT1 gene as the UGT genes are closely grouped on the same chromosome, the UGT1 haplotypes illustrated that linkage had limited influence on the UGT1A8 correlation. Another explanation for this correlation may be the high substrate diversity of the UGT1A8 isozyme (36). An alteration in its function can highly manipulate the detoxification rate of carcinogenic compounds in the gastrointestinal tract. A key example is the glucuronidation of PhIP, an amine formed in cooked meat and fish and metabolized by phase I biotransformation cytochrome P450 enzymes (37). Heterocyclic amines are associated with colorectal carcinoma, breast cancer and bladder carcinoma (38-40). Another essential group of carcinogens found in red meat and tobacco smoke, are the N-nitroso compounds (NOC), also eliminated by the UGT1A8 isozyme (41), which could further explain the role of the low activity UGT1A8*3 allele in esophageal SCC risk. Furthermore, our results showed no differences in the distribution of the high activity UGT2B4 genotype between patients and controls, when set off against the combined low and medium group. However, the low and medium activity genotypes were more and less frequent in ESCC patients in comparison to controls, respectively. This was not confirmed by the histology based stratified analyses. The lower frequency of the UGT2B4 medium activity genotype in ESCC patients, was confirmed by the haplotype analyses as the 100 haplotype had a tendency to decrease ESCC risk (OR=0.60; 95% CI, 0.34-1.05). The comparison methodology, which obliges the combination of the low and the medium groups, nullifies the original difference in distribution of the UGT2B4 genotypes between ESCC patients and controls. Indeed, the high (OR=0.42; 95% CI, 0.22-0.84) and the medium (OR=0.25; 95% CI, 0.12-0.52) activity UGT2B4 genotypes were significantly less present in ESCC patients when set off against the low activity genotype. This establishes the low activity UGT2B4’ allele to be a risk factor for ESCC. The UGT2B4 isozyme is involved in eliminating eicosanoids (42), compounds derived from fatty acid oxygenation. This is a reaction by which ROS are released and which is catalyzed by cyclooxygenases and lipoxygenases to produce prostanoids and leukotrienes, respectively. These metabolites play an important role in cell proliferation, inflammation and angiogenesis, all vital processes for the development of neoplasms (43,44). One could postulate that modifications in the detoxification process of eicosanoids can shift the tissue equilibrium towards carcinogenesis in the esophagus. However, this also may be a systemic process, since esophageal epithelium probably does not express the UGT2B4 isozyme (5,16).

Oddly we could not verify the significant correlation for EAC patients, given the UGT2B4 involvement in the conjugation of bile acids (42). Capello et al suggested that bile acids can provoke an inflammatory reaction in Barrett’s epithelium (45). Although the UGT2B4’ allele does not seem to play an important part in the etiology of EAC, its role in Barrett’s esophagus is yet to be examined. One could argue that the less active allele could negatively influence the bile acid detoxification process and could stimulate the development of Barrett’s epithelium.

Lastly, our results show that polymorphisms in UGT2B7 and UGT2B17 do not seem implicated in the etiology of esophageal cancer. Although the UGT2B7 isozyme has a significant role in the metabolism of frequently used drugs (28,46,47), there is an expected minor difference in predicted activity between the two alleles. For UGT2B17 however, there was a tendency for the high enzyme activity genotype to be more present in the EAC patients in comparison to controls (OR=1.29; 95% CI, 0.98-1.70). Furthermore, several studies reported a gender difference in the expression of the UGT2B17 deletion allele associated with a lower catalytic activity, resulting in a significant correlation with increased lung adenocarcinoma risk in women due to a decreased NNAL (metabolite of the tobacco-specific nitrosamine carcinogen NNK) glucuronidation rate (48,49). We could not confirm such an outcome. After stratifying for gender, no differences in predicted enzyme activity were found between the groups.

In conclusion, the notion that variations in genes of detoxification enzymes can influence carcinoma risk, may contribute to the elucidation of the EC etiology. In this study predicted high activity UGT1A1 genotype, low activity UGT1A8 and low and medium UGT2B4 genotypes were found to increase ESCC risk. However, these polymorphisms in UGT genes do not associate with EAC risk, which may be due to a different etiological mechanism. Unfortunately, the small size of our ESCC population, since ESCC is rare in the Netherlands, and the multiple testing due to numerous enzyme analyses, disable us from firmly establishing the above findings. Moreover, multivariate analyses that take dietary and lifestyle related factors into account should also be performed. An aim of this study is, to help create a genetic
profile that can predict severe risk and subsequently could
guide the implementation of potential surveillance programs
in order to detect tumors at an early stage, as early stage
diagnosis would dramatically increase the overall survival of
esophageal cancer patients.

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