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.

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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 gastroesophageal 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 *UGT*s 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 *UGT1A7*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 *UGT1A7*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 *et al* (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, UBT2B7 and UGT2B17 are known to be highly expressed in liver and intestine and thus may indirectly modify esophageal cancer risk (16).

UGT1A1. The microsatellite polymorphism of the TATA box in the promoter region of the *UGT1A1* gene (*UGT1A1*28*, rs8175347) was analyzed using the polymerase chain reaction (PCR) followed by polyacrylamide gel electrophoresis as described before (8). The TA repeat polymorphism created the *28 allele associated with a low enzyme activity (8,17).

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 SNP's, the *1*2 genotype could not be determined.

UGT1A7 alleles were genotyped for the polymorphisms at codon 129 (rs17868323) and 131 (rs17868324) by melting curve analysis with fluorescence resonance energy transfer (FRET) probes on the iCycler (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).

UGT1A8. 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 (rs13119049) and codon 268 of the *UGT2B7* gene (rs7439366) PCR was performed on the iCycler iQ Multicolour Real-Time Detection System (Bio-Rad Laboratories) as describe before (24,25). Genotypes were assigned using the iCycler iQ Optical System Software version 3.1. At each PCR run (in 96-well plates) in several wells sterile H_2O instead of genomic DNA was added as negative controls for amplification. The *UGT2B4* polymorphism may be responsible for differences in substrate specificity and catalytic activity (26,27). Furthermore, although the H268Y amino-acid alteration creating the *UGT2B7*2* allele does not produce a significant difference in enzyme activity (28), we still categorized the *UGT2B7* genotypes in predicted activity groups with the premise that the mutated allele produces a lower activity.

UGT2B17. The 150-kb deletion in *UGT2B17* was detected as described by Wilson *et al* (29). It has been demonstrated that due to the *UGT2B17* deletion polymorphism, genotypes with at least one null allele (*UGT2B17*2*) produces a lower level of glucuronidation (30).

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.

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

Characteristics	Patients			Controls
	ESCC	EAC	Total	
No. (% of total)	85 (24.2)	260 (74.1)	351 (100)[a]	592
Age (years; mean ± SD)	63.7±10.3	65.3±11.1	65.0±10.9	63.4±11.9
Gender				
Male	56 (65.9)	221 (85.0)	282 (80.3)	478 (80.7)
Female	28 (32.9)	39 (15.0)	68 (19.4)	114 (19.3)

[a]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.

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

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

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 χ^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 III. *UGT* genotypes and associated predicted enzyme activity with corresponding odds ratios (OR) for patients with ESCC, EAC and EC compared to controls.

UGT isozymes	Predicted enzyme activity	ESCC n=85 [n (%)]	OR (95% CI)	EAC n=260 [n (%)]	OR (95% CI)	Controls n=592 [n (%)]
UGT1A1	Low/medium	35 (41.2)	Ref	135 (51.9)	Ref	312 (52.7)
	High	50 (58.8)	1.62 (1.02-2.56)	122 (46.9)	1.02 (0.76-1.37)	276 (46.6)
UGT1A6	Low (*2*2)	6 (7.1)	Ref	31 (11.9)	Ref	70 (11.8)
	High (*1*1)	40 (47.1)	1.8 (0.74-4.47)	112 (43.1)	0.99 (0.61-1.59)	256 (43.2)
UGT1A7	Low/medium	52 (61.2)	Ref	159 (61.2)	Ref	364 (61.5)
	High	33 (38.8)	1.01 (0.64-1.62)	101 (38.8)	1.01 (0.75-1.37)	228 (38.5)
UGT1A8	Low/medium	7 (8.2)	Ref	9 (3.5)	Ref	19 (3.2)
	High	77 (90.6)	0.37 (0.15-0.91)	247 (95.0)	0.92 (0.41-2.07)	565 (95.4)
UGT2B4	Low/medium	35 (41.2)	Ref	121 (46.5)	Ref	271 (45.8)
	High	50 (58.8)	1.21 (0.76-1.92)	139 (53.5)	0.97 (0.73-1.30)	320 (54.1)
UGT2B7	Low/medium	66 (77.6)	Ref	201 (77.3)	Ref	459 (77.5)
	High	18 (21.2)	0.94 (0.54-1.64)	59 (22.7)	1.01 (0.72-1.44)	133 (22.5)
UGT2B17	Low/medium	31 (36.5)	Ref	86 (33.1)	Ref	233 (39.4)
	High	54 (63.5)	1.15 (0.72-1.84)	174 (66.9)	1.34 (0.98-1.82)	353 (59.6)

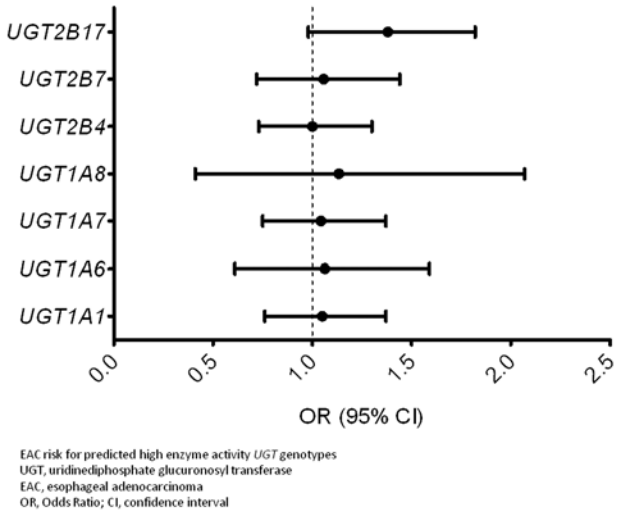

Figure 1. High activity *UGT* genotypes and EAC risk.

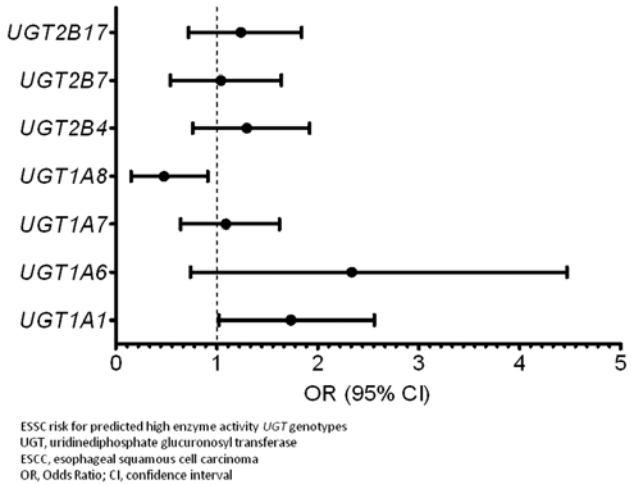

Figure 2. High activity *UGT* genotypes and ESCC risk.

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 *UGT*s, 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

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

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

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.

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

Haplotypes are in the following order: *UGT2B4*, *UGT2B7* and *UGT2B17*. 0, no mutation; 1, mutation.

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*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

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 *UGT*s 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 homozygosity 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*2* 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*2* 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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