

## ELECTRONIC LETTER

# Polymorphisms of UDP-glucuronosyltransferase 1A7 are not involved in pancreatic diseases

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**Background:** Xenobiotic mediated cellular injury is thought to play a major role in the pathogenesis of pancreatic diseases. Genetic variations that reduce the expression or activity of detoxifying phase II biotransformation enzymes such as the UDP-glucuronosyltransferases might be important in this respect. Recently, a *UGT1A7* low detoxification activity allele, *UGT1A7\*3*, has been linked to pancreatic cancer and alcoholic chronic pancreatitis.

**Objective:** To investigate whether *UGT1A7* polymorphisms contribute to the risk of pancreatitis and pancreatic cancer.

**Methods:** Genetic polymorphisms in the *UGT1A7* gene were assessed in a large cohort of patients with different types of pancreatitis and pancreatic cancer originating from the Czech Republic (n=93), Germany (n=638), Netherlands (n=136), and Switzerland (n=106), and in healthy (n=1409) and alcoholic (n=123) controls from the same populations. Polymorphisms were determined by melting curve analysis using fluorescence resonance energy transfer probes. In addition, 229 Dutch subjects were analysed by restriction fragment length polymorphism.

**Results:** The frequencies of *UGT1A7* genotypes did not differ between patients with acute or chronic pancreatitis or pancreatic adenocarcinoma and alcoholic and healthy controls.

**Conclusions:** The data suggest that, in contrast to earlier studies, *UGT1A7* polymorphisms do not predispose patients to the development of pancreatic cancer and pancreatitis.

the serine protease inhibitor, Kazal type 1 (*SPINK1*), in patients with hereditary or idiopathic chronic pancreatitis.<sup>6-9</sup> In addition, an increased frequency of *SPINK1* mutations has been reported in patients with alcohol related chronic pancreatitis (ACP).<sup>10 11</sup>

As these variants can only be identified in a minority of patients, it is likely that additional genetic factors modify the susceptibility to the different types of pancreatitis and pancreatic cancer. Xenobiotic mediated cellular injury has been hypothesised to be an important pathogenic mechanism in pancreatic diseases. Thus genetic variations that reduce the expression or activity of detoxifying phase II biotransformation enzymes might represent a risk factor for pancreatitis and pancreatic cancer.<sup>12</sup>

Uridine 5'-diphosphate (UDP)-glucuronosyltransferases (UGTs) catalyse the addition of a glucuronyl group to a wide variety of endogenous and exogenous hydrophobic compounds to form water soluble glucuronides and enhance renal or biliary metabolite excretion. UGTs constitute a major cellular defence mechanism against xenobiotic chemicals and endogenous toxins<sup>13-15</sup> and contribute to the detoxification of known human carcinogens, such as heterocyclic amines and heterocyclic and polycyclic hydrocarbons.<sup>16</sup>

UGTs are classified on the basis of sequence homology into the UGT1A, UGT2A, and UGT2B subfamilies.<sup>17</sup> Subfamily 1A isoforms are derived from a single gene locus on chromosome 2q37.<sup>18</sup> In humans, UGT1A is composed of at least nine functional proteins (UGT1A1, UGT1A3-UGT1A10), which are encoded by five exons. Exons 2 to 5 are shared by all UGT1A proteins. Thus the separate UGT1A forms consist of unique N-terminals and a conserved 246 amino acid C terminus.<sup>19</sup> Each of these UGT1A forms possesses distinctive substrate specificity.<sup>20</sup>

Recently, functional polymorphisms in various genes encoding UGT1 family members have been discovered.<sup>21-25</sup> *UGT1A7* is highly polymorphic and so far at least 11 variants in four different codons have been characterised. These missense variants have been detected in codon 115, 129, 131, 139, and 208. This results in 11 polymorphic alleles (*UGT1A7\*1* \*2, \*3, \*4, \*5, \*6, \*7, \*8 \*9, \*10, \*11). The nomenclature of these allelic variants reflects the chronological order in which they were discovered (fig 1).

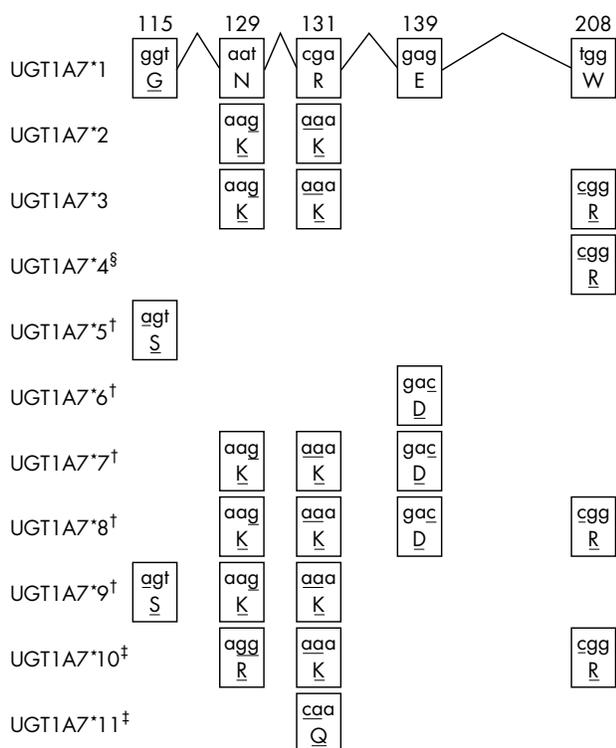
Human *UGT1A7* is an extrahepatic enzyme expressed in pancreas, lung, oesophagus, and stomach. *UGT1A7* catalyses the glucuronidation of phenols, benzo[a]pyrenes, coumarins, and steroid hormones.<sup>16 26</sup> Case-control studies have

**Abbreviations:** ACP, alcohol related chronic pancreatitis; FRET, fluorescence resonance energy transfer; UGT, uridine 5'-diphosphate-glucuronosyltransferase

Chronic pancreatitis and pancreatic adenocarcinoma are common diseases in industrialised countries and are associated with considerable morbidity and mortality. The underlying causes of pancreatic inflammation and cancer are multifaceted, and include environmental as well as genetic factors.<sup>1</sup>

Pancreatic adenocarcinoma is the fifth most frequent cause of cancer related death worldwide.<sup>2</sup> Diabetes, obesity, smoking, several chemicals such as gasoline and related compounds, as well as certain insecticides are known risk factors for pancreatic cancer.<sup>3 4</sup>

Chronic pancreatitis shares risk factors with pancreatic cancer, such as smoking, but having chronic pancreatitis puts the patient at a higher risk for pancreatic adenocarcinoma.<sup>5</sup> The concept of genetic predisposition to pancreatitis is supported by the identification of sequence alterations in the genes encoding cationic trypsinogen (*PRSS1*), the cystic fibrosis transmembrane conductance regulator (*CFTR*), and



**Figure 1** *UGT1A7* polymorphisms: 11 allelic variants of the *UGT1A7* gene. Each block represents a base triplet with the corresponding amino acid. The variants detected so far have been detected in codon 115, 129, 131, 139, and 208 and are indicated on top. *UGT1A7\*1* represents the wild type while the other variants are numbered from *UGT1A7\*2* to *UGT1A7\*11*. The base pair and amino acid that are different from wild type are underlined. For *UGT1A7\*2* to *UGT1A7\*11* only the changed codons are indicated. The different signs indicate: <sup>§</sup>, not found in our study population, <sup>†</sup>, not investigated in this study, <sup>‡</sup>, novel detected alleles.

suggested that polymorphisms leading to a low *UGT1A7* detoxification activity are associated with cancer of the colon, liver, and oral cavity.<sup>27–29</sup> Most notably, the *UGT1A7\*3* allele, which carries the lowest *UGT1A7* detoxification activity, has been reported as a significant risk factor for pancreatic cancer and alcoholic chronic pancreatitis.<sup>30</sup>

These data suggest that *UGT1A7* polymorphisms may contribute to the risk of pancreatitis and pancreatic cancer. We therefore investigated this association in a large cohort of white patients with pancreatitis and pancreatic cancer of Czech, Dutch, German, and Swiss origin.

## METHODS

### Subjects

The study was approved by the local medical ethics review committee at each participating centre and all subjects gave their informed consent.

The study included patients from the Czech Republic (n = 93), Germany (n = 638), Netherlands (n = 136), and Switzerland (n = 106) (table 1). In all, 973 consecutive patients were recruited from the different centres and blood samples were drawn during a routine outpatient clinic visit. Patient information was collected using a structured questionnaire in the native language of the patient.

The ACP group consisted of 318 patients. We also enrolled 266 patients with non-alcoholic chronic pancreatitis (idiopathic or hereditary chronic pancreatitis) in our study. The clinical diagnosis of chronic pancreatitis was based on two or more of the following criteria:

- presence of typical history of recurrent pancreatitis;
- radiological findings such as pancreatic calcification;
- pancreatic ductal irregularities revealed by endoscopic retrograde pancreatography or magnetic resonance imaging of the pancreas.

Hereditary chronic pancreatitis was diagnosed when two first degree relatives or three or more second degree relatives suffered from recurrent acute pancreatitis or chronic pancreatitis without no apparent precipitating factor. Alcoholic chronic pancreatitis was diagnosed in patients who had consumed more than 60 g (women) or 80 g (men) of ethanol a day for more than two years. Patients were classified as having idiopathic chronic pancreatitis when precipitating factors such as alcohol abuse in the amounts described above for alcoholics, trauma, drug use, infection, metabolic disorders, or a positive family history were all absent.

The acute pancreatitis patient group consisted of 153 German patients (101 male, 52 female) with pancreatitis of biliary (n = 63), idiopathic (n = 23), or various (n = 25) origins, as well as patients with an acute attack of alcoholic pancreatitis (n = 42). Acute pancreatitis was defined as acute abdominal pain with a typical clinical picture and serum amylase concentration at least three times the upper limit of normal and typical findings on sonography or computed tomography.<sup>30</sup>

We also included 236 patients with histologically confirmed pancreatic adenocarcinoma of German (n = 201) and Swiss extraction (n = 35). Controls were medical students or staff and participants in the BASE study (Berlin aging study) (German controls), blood donors (Swiss controls), randomly selected healthy newborn infants (Czech controls), or individuals recruited by advertisement in a local paper (Dutch controls). Controls originating from the Czech Republic (n = 319), Germany (n = 432), Netherlands (n = 275), and Switzerland (n = 383) (table 1) were from the catchment populations of the patients. The control individuals volunteered without a set reward and no selection criteria were applied. In addition, we examined two additional control groups consisting of alcoholic subjects without pancreatic disease (n = 123) of German (n = 30) and Dutch (n = 93) descent (table 1) recruited from a detoxification unit which they were attending because of their addiction. We defined alcoholics as people who had consumed more than 60 g (women) or 80 g (men) of ethanol respectively a day for more than two years. None of the alcoholics had chronic pancreatitis or other known end organ damage associated with alcoholism.

### Genotyping

For DNA extraction, blood samples were collected into EDTA tubes and stored at –20°C until use. DNA was isolated from whole blood using the Pure Gene or Qiagen DNA isolation kit according to the instructions from the manufacturers (Gentra Systems, Minneapolis, Minnesota, USA and Qiagen, Hilden, Germany).

We screened for the mutations in codons 129, 131, and 208. Primers flanking the polymorphisms of interest in exon 1 of *UGT1A7* and fluorescence resonance energy transfer (FRET) probes were synthesised on the basis of the published nucleotide sequence (GenBank U39570). Primer sequences (S and A, table 2) used for polymerase chain reaction (PCR) were described by Köhle *et al.*<sup>31</sup> We undertook PCR using 0.5 U AmpliTaq Gold (Applied Biosystems, Foster City, California, USA), 400 μM dNTPs, 1.5 mM MgCl<sub>2</sub>, and 0.1 μM of each primer in a final volume of 25 μl. The reaction mix was denatured at 95°C for 12 minutes followed by 48 cycles of denaturation at 95°C for 20 seconds, annealing at 56°C for 40 seconds, elongation at 72°C for 90 seconds, and

**Table 1** Clinical characteristics of patients with pancreatic diseases and controls

Characteristic	Czech Republic			Germany					Netherlands				
	ACP	ICP/HP	CO	ACP	ICP/HP	AP	PC	CO	Alc CO	ACP	ICP/HP	CO	Alc CO
n	38	55	319	148	136	153	201	432	30	77	59	275	93
Sex													
Male/female	32/6	33/22		134/14*	73/63	101/52*	110/91	184/195	26/4	54/23	30/29	144/131	64/29
Unknown			319					53					
Age (years)													
Mean (SD)	53 (17)	30 (18)		43 (9)	30 (19)*	54 (17)	62 (9)*	56 (14)	42 (8)	50 (9)*	41 (18)	34 (5)	45 (14)
Unknown			319									107	
Smoking													
Yes/no					0/59		0/25	12/35		65/7	26/19	27/65	84/9
Unknown	38	55	319	148	77	153	176	385	30	5	14	183	0
Characteristic	Switzerland				All countries								
	ACP	ICP/HP	PC	CO	ACP	ICP/HP	AP	PC	CO	Alc CO			
n	55	16	35	383	318	266	153	236	1409	123			
Sex													
Male/female	52/3*	9/7	9/16	250/128	272/46*	145/121	101/52	129/107	578/454	90/33			
Unknown				5					377				
Age (years)													
Mean (SD)	35 (8)*	21 (5)*	63 (10)*	47 (13)	45 (10)	32 (20)*	54 (17)	62 (10)*	50 (22)	43 (9)			
Unknown									426				
Smoking													
Yes/no			8/15		65/7	26/78		8/40	39/100	84/9			
Unknown	55	16	12	383	246	162	153	188	1270	30			

\*p<0.05 v control group from the same country.

ACP, alcoholic chronic pancreatitis; Alc CO, alcoholic control subjects; AP, acute pancreatitis; CO, healthy control subjects; ICP/HP, idiopathic chronic pancreatitis/hereditary chronic pancreatitis; PC, pancreatic adenocarcinoma.

a final extension step for two minutes at 72°C in an automated thermocycler. For haplotype analysis, we carried out allele specific PCR in 168 individuals using primers 3/4F and R with an annealing temperature at 60°C (table 2).

Genotyping of *UGT1A7* alleles was carried out by melting curve analysis with FRET probes in the LightCycler (Roche Diagnostics, Mannheim, Germany). For detection of the polymorphisms at codons 129 and 131 we used 5'-LC 640-TTAAGTATTCTACTAATTTTTGTCCCTT-ph as the sensor probe (LC, LightCycler Red attached to 5' terminus; ph, phosphate) and 5'-GGATCGAGAAACTGCATCAAACAACCTCTCC-FL as the anchor probe (FL, 5,6-carboxyfluorescein attached to 3'-O-ribose) (fig 2A). For identification of the W208R polymorphism the sequence of the sensor probe was 5'-TGATGTGGTCCGACTCTCTCCTT-FL and of the anchor probe, 5'-LC 705-AAAGTCATGCCGTCTGAGAACCCTAAG-ph

(fig 2B). Both sensor probes were complementary to the mutant sequences (129K/131K and 208R, respectively). During melting curve analysis, a more stable duplex was formed with the mutant allele than with the wild-type allele, resulting in an allele specific melting curve (N129K/R131K: 58°C v 47°C; W208R: 65°C v 60.5°C). The program for analytical melting was 95°C for 60 seconds, 38°C for 40 seconds, and an increase to 75°C at a 0.1°C/s ramp rate. All FRET probes were designed and synthesised by TIB Molbiol (Berlin, Germany).

Additionally, we carried out RFLP (restriction fragment length polymorphism) analysis in 229 Dutch samples. To detect the variations at codon 129/131, we use the forward primers F1 and F2 and the reverse primer R1 (table 2). F1 detects only the N129K/R131K mutation, F2 detects the N129K/R131K and N129R/R131K mutations. After digestion

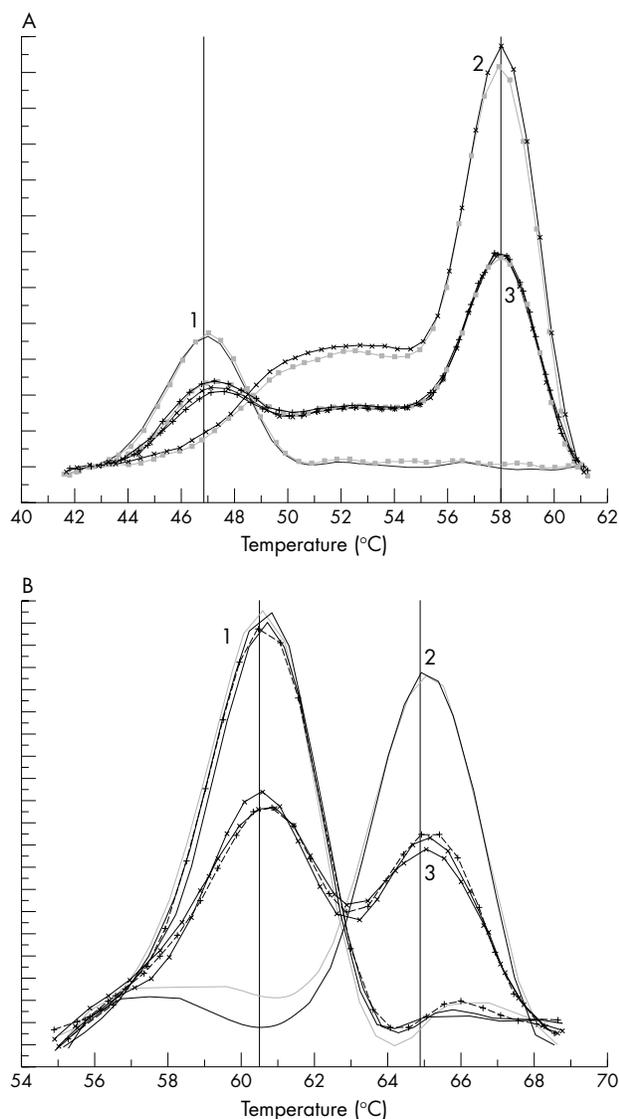
**Table 2** Primers used for polymerase chain reaction amplification of *UGT1A7*

FRET analysis			
All individuals	S	Forward	5'-TGC CGA TGC TCG CTG GAC G-3'
	A	Reverse	5'-ATC TCT ACA GCC ACA CAT CAA TT-3'
Haplotype analysis			
3/4F†	Forward	5'-GGT GGA CTG GCC TCC TTg CA-3'	
	R	Reverse	5'-CCA TAG GCA CTG GCT TTC CCT GAT GAC A-3'
RFLP analysis			
Codon 129 and 131	F1‡	Forward	5'-AAT TGC AGG AGT TTG aTT AA-3'
	F2‡	Forward	5'-AAT TGC AGG AGT TTG aTT A-3'
	R1	Reverse	5'-TTC AGA GGC TAT TTC TAA GA-3'
Codon 208	F3	Forward	5'-ATG CTC GCT GGA CGG CAC CAT TG-3'
	R2	Reverse	5'-TGC CGT GAC AGG GGT TTG GAG A-3'
Allele specific	F4	Forward	5'-ATT GCA GGA GTT TGT TTA AGG ACA-3'
	R1	Reverse	5'-TTC AGA GGC TAT TTC TAA GA-3'

†Bold "g" in the primer sequence denotes mutagenesis for more selective amplification of the *UGT1A7*\*3 and *UGT1A7*\*4 alleles.

‡Bold "a" in the primer sequence denotes site directed mutagenesis for introduction of a *VspI* restriction site into the wild-type allele.

FRET, fluorescence resonance energy transfer.



**Figure 2** Genotyping of *UGT1A7* 129/131 and 208 polymorphisms with FRET probes. Fluorescence signal data were obtained during the melting transition of the fluorophore labelled detection probe from the amplified fragment. Fluorescence data were converted to derivative melting curves by plotting the negative derivative of the fluorescence with respect to temperature against temperature. Genotyping of codons 129/131 and 208 using modified primers published by Köhle *et al.*<sup>31</sup> (A) Curves: 1, homozygous wild-type sample (129N/131R) as found in *UGT1A7\*1* and *UGT1A7\*4*; 2, homozygous mutant sample (129K/131K) as found in *UGT1A7\*2* and *UGT1A7\*3*; 3, heterozygous samples displaying two peaks (129N/131R and 129K/131K). (B) Curves: 1, homozygous wild-type sample (208W) as found in *UGT1A7\*1* and *UGT1A7\*2*; 2, homozygous mutant sample (208R) as found in *UGT1A7\*3* and *UGT1A7\*4*; 3, heterozygous samples displaying two peaks (208W and 208R). FRET, fluorescence resonance energy transfer.

of the PCR product with *VspI* the following fragments can be found: *wild-type*, 315+18 bp; *heterozygotes*, 333+315+18 bp; *homozygotes*, 333 bp.

To detect the W208R alteration, we use the forward primer F3 and reverse primer R2 (table 2). After digestion of the PCR product with *RsaI* the following fragments can be found: *wild-type*, 440 bp; *heterozygotes*, 440+337+103 bp; *homozygotes*, 337+103 bp. To determine whether the N129K/R131K or N129R/R131K and W208R occurred *cis* or *trans*, we used the allele specific primers F4 and R1 (table 2). After digestion of the PCR product with *RsaI* the following fragments would be expected: *\*1/\*3*, 253+79 bp; *\*2/\*4*, 332 bp.

## Statistics

Data were analysed by SAS version 8.0. Differences in the baseline clinical characteristics of patients and control groups were estimated with Fisher's exact test and the *t* test;  $\chi^2$  statistics or Fisher's exact test were used to estimate differences of the *UGT1A7* alleles among the different study groups. Odds ratios (OR) with 95% confidence interval (CI) were calculated by logistic regression analysis for the allele frequencies and genotype distribution in *UGT1A7* taking possible confounding factors as age, sex, and country of origin into account.

The distribution of *UGT1A7* polymorphisms among the control populations was tested to determine whether they were in agreement with the Hardy–Weinberg equilibrium.

## RESULTS

### Characteristics of patients and controls

Table 1 lists the characteristics of the patients with chronic pancreatitis and pancreatic adenocarcinoma, and the healthy controls. There was a small but significant difference in sex distribution between the alcoholic chronic pancreatitis and acute pancreatitis groups and the healthy controls, with relatively more female subjects in the control group ( $p < 0.05$ ). In the German and Swiss population, the mean age of the patients with pancreatic adenocarcinoma was greater than that of the control group ( $p < 0.05$ ). The patients with hereditary chronic pancreatitis were significantly younger than the control subjects, as they develop chronic pancreatitis at a younger age than patients with other pancreatic diseases ( $p < 0.05$ ).

### *UGT1A7* polymorphisms

No statistical differences were observed in the distribution of the *UGT1A7* genotype among patient and control groups as a whole (OR = 0.99 (95% CI, 0.1 to 2.4), table 3), except for the German patients with pancreatic adenocarcinoma. In these, a significant difference was found with respect to *UGT1A7* genotypes, which reflected the relatively high prevalence of the *\*2/\*3* genotype compared with the control group. Likewise, the *UGT1A7* genotype distribution did not differ among the various ethnic control groups ( $p = 0.20$ ). To assess the impact of alcoholism, we compared Dutch and German patients with alcoholic chronic pancreatitis with a control group consisting of alcoholic subjects of similar ethnic origin without pancreatic disease. Again, we observed no significant difference in genotype distribution.

As expected, the ratio of *UGT1A7* allele frequencies was similar in the different patient and control groups (OR = 0.9 (95% CI, 0.8 to 1.1), table 4). In contrast to genotype distribution, the allele frequency was similar in the German pancreatic adenocarcinoma group. Comparison of the allele frequencies in patients with alcoholic chronic pancreatitis with those of alcoholic control subjects or healthy control individuals of Dutch and German origin did not show any difference. We did not detect any individual carrying the *UGT1A7\*4* allele, but found two novel rare alleles: R129-K131-R208 (*UGT1A7\*10*) and N129-Q131-W208 (*UGT1A7\*11*) (fig 1). RFLP analysis of 229 Dutch samples led to similar results and matched those done by FRET analysis.

## DISCUSSION

Glucuronidation catalysed by UGTs is one of the most important mechanisms involved in the host defence against xenobiotic chemicals and endogenous toxins. Xenobiotic mediated cellular injury is thought to represent a major pathogenic factor in pancreatic diseases.<sup>13–15</sup> In the present study, we investigated the relations of pancreatic cancer and pancreatitis with the *UGT1A7* polymorphisms in codons 129, 131, and 208 that have been associated with altered enzyme

**Table 3** Distribution of *UGT1A7* genotypes in patients with pancreatic diseases and healthy controls

Genotype	Czech Republic			Germany				Netherlands					
	ACP (n=38)	ICP/HP (n=55)	CO (n=319)	ACP (n=148)	ICP/HP (n=136)	AP (n=153)	PC (n=201)	CO (n=432)	Alc CO (n=30)	ACP (n=77)	ICP/HP (n=59)	CO (n=275)	Alc CO (n=93)
<i>UGT1A7</i>													
*1/*1	4 (11%)	9 (16%)	38 (12%)	22 (15%)	16 (12%)	20 (13%)	25 (12%)	56 (13%)	0 (0%)	10 (13%)	12 (20%)	38 (14%)	17 (18%)
*1/*2	4 (11%)	10 (18%)	60 (19%)	18 (12%)	23 (16%)	19 (12%)	37 (18%)	63 (15%)	5 (17%)	12 (16%)	10 (17%)	45 (16%)	22 (24%)
*1/*3	12 (32%)	15 (27%)	88 (28%)	43 (29%)	49 (36%)	44 (29%)	45 (22%)	104 (24%)	10 (33%)	25 (33%)	13 (22%)	80 (29%)	23 (25%)
*1/*10	0 (0%)	0 (0%)	3 (0.9%)	0 (0%)	1 (0.7%)	0 (0%)	1 (0.5%)	2 (0.5%)	0 (0%)	1 (1.3%)	1 (1.7%)	2 (0.7%)	0 (0%)
*2/*2	4 (11%)	6 (11%)	12 (4%)	13 (9%)	8 (6%)	11 (7%)	4 (2%)	33 (8%)	2 (7%)	3 (4%)	5 (8%)	19 (7%)	3 (3%)
*2/*3	7 (18%)	3 (6%)	59 (19%)	28 (19%)	19 (14%)	32 (21%)	55 (27%)	79 (18%)	7 (23%)	13 (17%)	6 (10%)	42 (15%)	13 (14%)
*2/*10	0 (0%)	1 (2%)	2 (0.6%)	2 (1.4%)	0 (0%)	0 (0%)	2 (1%)	2 (0.5%)	0 (0%)	0 (0%)	0 (0%)	1 (1%)	0 (0%)
*3/*3	7 (18%)	11 (20%)	50 (16%)	22 (15%)	20 (15%)	25 (16%)	28 (14%)	90 (21%)	6 (20%)	11 (14%)	11 (19%)	48 (18%)	15 (16%)
*3/*10	0 (0%)	0 (0%)	7 (2%)	0 (0%)	0 (0%)	2 (1.3%)	4 (2%)	3 (0.7%)	0 (0%)	2 (2.6%)	1 (1.7%)	0 (0%)	0 (0%)
*3/*11	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
<i>Switzerland</i>													
<i>All countries</i>													
Genotype	ACP (n=55)	ICP/HP (n=16)	PC (n=35)	CO (n=383)	ACP (n=318)	ICP/HP (n=266)	AP (n=153)	PC (n=236)	CO (n=1409)	Alc CO (n=123)			
<i>UGT1A7</i>													
*1/*1	10 (18%)	1 (6%)	1 (3%)	47 (12%)	46 (14%)	38 (14%)	20 (13%)	26 (11%)	179 (13%)	17 (14%)			
*1/*2	9 (16%)	3 (19%)	6 (17%)	55 (14%)	43 (14%)	46 (17%)	19 (12%)	43 (18%)	223 (16%)	27 (22%)			
*1/*3	11 (20%)	6 (39%)	9 (26%)	126 (33%)	91 (29%)	83 (31%)	44 (29%)	54 (23%)	398 (28%)	33 (27%)			
*1/*10	1 (1.8%)	0 (0%)	1 (3%)	2 (0.5%)	2 (0.6%)	2 (0.8%)	0 (0%)	2 (0.8%)	9 (0.6%)	0 (0%)			
*2/*2	4 (7%)	0 (0%)	4 (11%)	23 (6%)	24 (8%)	19 (7%)	11 (7%)	8 (3%)	87 (6%)	5 (4%)			
*2/*3	14 (26%)	4 (25%)	9 (26%)	65 (17%)	62 (19%)	32 (12%)	32 (21%)	64 (27%)	245 (17%)	20 (16%)			
*2/*10	0 (0%)	0 (0%)	0 (0%)	2 (0.5%)	2 (0.6%)	1 (0.4%)	0 (0%)	2 (0.8%)	7 (0.5%)	0 (0%)			
*3/*3	6 (11%)	2 (13%)	5 (14%)	58 (15%)	46 (14%)	44 (17%)	25 (16%)	33 (14%)	246 (17%)	21 (17%)			
*3/*10	0 (0%)	0 (0%)	0 (0%)	4 (1%)	2 (0.6%)	1 (0.4%)	2 (1.3%)	4 (1.7%)	14 (1%)	0 (0%)			
*3/*11	0 (0%)	0 (0%)	0 (0%)	1 (0.3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (0.1%)	0 (0%)			
OR <sup>1</sup>					0.73	0.91	0.95	0.71	0.90	0.93			
95% CI					0.48 to 1.12	0.57 to 1.45	0.54 to 1.68	0.44 to 1.15	0.38 to 2.11	0.51 to 1.68			
OR <sup>2</sup>					1.11	0.95	1.29	1.25	1.60	0.95			
95% CI					0.81 to 1.50	0.67 to 1.36	0.84 to 1.98	0.89 to 1.75	0.86 to 2.97	0.60 to 1.52			

OR<sup>1</sup> is given for the \*3/\*3 v \*x/\*x genotypes; OR<sup>2</sup> is given for \*3/\*x v \*x/\*x genotypes (x=1, 2, 10, or 11). ACP, alcoholic chronic pancreatitis; Alc CO, alcoholic control subjects; AP, acute pancreatitis; CI, confidence interval; CO, healthy control subjects; ICP/HP, idiopathic chronic pancreatitis/hereditary chronic pancreatitis; OR, odds ratio adjusted for age, sex, and country of origin; PC, pancreatic adenocarcinoma.

activity (fig 1).<sup>21 24 32</sup> The frequencies of the different *UGT1A7* alleles in the control subjects were similar to those observed in patients with pancreatic adenocarcinoma and different types of acute and chronic pancreatitis. These findings indicate that individuals carrying less active *UGT1A7* alleles do not run a higher risk of pancreatic diseases. This study controlled for potential confounding variables such as age, sex, and country of origin. However, because of lack of detailed data, we could not control for certain lifestyle factors as smoking, dietary habits, and drug treatment. In prospective studies we recommend that these possible confounders should also be controlled for.

Our results differ from those published recently by Ockenga and colleagues. In that study, the investigators identified the low detoxification allele *UGT1A7\*3* as a risk factor for alcoholic chronic pancreatitis and pancreatic cancer.<sup>30</sup> They analysed 52 patients with pancreatic cancer and 63 with alcoholic chronic pancreatitis, while we investigated a much larger cohort of almost 1000 patients and more than 1500 control subjects originating from four different European countries. The control subjects were recruited from the same population as the patients to minimise confounding effects of ethnic variation and environmental exposures, although the selection of controls was not matched to the cases, which could introduce bias in the populations analysed.

There may be several reasons the different findings. First, the differences between our results and those published earlier can in part be explained by the relatively low frequency of the *UGT1A7\*3* allele in control subjects in the earlier study.<sup>30</sup> We detected a frequency of the *UGT1A7\*3* allele of about 40% in healthy controls, which is in line with

the frequencies of 32%, 36%, and 37% in white control subjects reported by other researchers,<sup>25 31 32</sup> but appreciably higher than the 16%–21% described by the group from Hanover.<sup>27 28 30 33</sup> It is worth noting that in previous studies on the associations between the *UGT1A7\*3* polymorphism and other diseases such as hepatocellular carcinoma or colon cancer, the latter group detected a frequency of the *UGT1A7\*3* allele in their patients that varied between 31% and 44%.<sup>27 28 30 31</sup> Consequently, the association of *UGT1A7\*3* with pancreatic diseases, and perhaps in their studies with hepatocellular and colon cancer as well, might be attributed to the relatively low *UGT1A7\*3* frequency in the control group of those studies.

In contrast to several earlier studies from the same group in which a *UGT1A7\*4* allele frequency up to 11% was found,<sup>27 28 30 33</sup> we failed to detect the *UGT1A7\*4* allele in any of about 2500 white subjects investigated, although we specifically searched for it. These results are in line with other investigators who have also been unable to detect the *UGT1A7\*4* allele in their samples.<sup>31 34</sup> Only one other group has reported a frequency of 1% to 3% for the *UGT1A7\*4* allele,<sup>25 29</sup> but these latter studies did not take into account the novel *UGT1A7\*10* and *UGT1A7\*11* alleles. We suspect that in these studies the *UGT1A7\*10* allele, which occurred in our study populations in a frequency of 1% to 2%, might have been interpreted as being a *UGT1A7\*4* allele.

We surmise that the high frequency of *UGT1A7\*4* alleles found in other recently published studies may reflect dissimilarities in the interpretation of the genotype results. The investigators in those studies described overall *UGT1A7* allele frequencies, but did not provide detailed data on the distribution of the single allele combinations.<sup>30</sup> A reappraisal

**Table 4** Allele frequencies of *UGT1A7* in patients with pancreatic diseases and healthy controls

Alleles	Czech Republic			Germany						Netherlands			
	ACP (n=76)	ICP/HP (n=110)	CO (n=638)	ACP (n=296)	ICP/HP (n=272)	AP (n=306)	PC (n=402)	CO (n=864)	Alc CO (n=60)	ACP (n=154)	ICP/HP (n=116)	CO (n=550)	Alc CO (n=186)
<i>UGT1A7</i>													
*1	0.32	0.39	0.36	0.35	0.39	0.34	0.33	0.33	0.25	0.38	0.41	0.37	0.42
*2	0.25	0.24	0.23	0.25	0.21	0.24	0.25	0.25	0.27	0.20	0.22	0.23	0.22
<b>*3</b>	<b>0.43</b>	<b>0.36</b>	<b>0.40</b>	<b>0.39</b>	<b>0.40</b>	<b>0.42</b>	<b>0.40</b>	<b>0.42</b>	<b>0.48</b>	<b>0.40</b>	<b>0.35</b>	<b>0.40</b>	<b>0.35</b>
10	0.00	0.01	0.02	0.01	0.004	0.007	0.02	0.006	0.00	0.02	0.02	0.01	0.00
*11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Alleles	Switzerland				All countries								
	ACP (n=110)	ICP/HP (n=32)	PC (n=70)	CO (n=766)	AC (n=636)	ICP/HP (n=532)	AP (n=306)	PC (n=472)	CO (n=2818)	Alc CO (n=246)			
<i>UGT1A7</i>													
*1	0.37	0.34	0.26	0.36	0.36	0.39	0.34	0.32	0.35	0.38			
*2	0.28	0.22	0.33	0.22	0.24	0.22	0.24	0.26	0.23	0.23			
<b>*3</b>	<b>0.34</b>	<b>0.44</b>	<b>0.40</b>	<b>0.41</b>	<b>0.39</b>	<b>0.38</b>	<b>0.42</b>	<b>0.40</b>	<b>0.41</b>	<b>0.39</b>			
10	0.01	0.00	0.01	0.01	0.006	0.004	0.007	0.013	0.007	0.00			
*11	0.00	0.00	0.00	0.003	0.00	0.00	0.00	0.00	0.0004	0.00			
OR					0.90	0.95	1.02	0.91	0.95				
95% CI					0.74 to 1.10	0.75 to 1.20	0.78 to 1.34	0.73 to 1.13	0.71 to 1.29				

Odds ratio is given for the \*3 allele v the other alleles.

ACP, alcoholic chronic pancreatitis; Alc CO, alcoholic control subjects; AP, acute pancreatitis; CI, confidence interval; CO, healthy control subjects; ICP/HP, idiopathic chronic pancreatitis/hereditary chronic pancreatitis; OR, odds ratio adjusted for age, sex, and country of origin; PC, pancreatic adenocarcinoma.

of genetic data formerly reported by the same group<sup>27</sup> reveals disequilibrium of *UGT1A7* genotype distribution in their control population. For example, in their report, 20% of 210 control subjects were homozygous for *UGT1A7\*1* (12–14% in our controls) and nearly the same proportion (17%) were compound heterozygous for *UGT1A7\*1/\*4*. In contrast, 8% were homozygous for *UGT1A7\*3* (15–21% in our controls), but none of the controls was compound heterozygous for *UGT1A7\*3/\*4*.<sup>27</sup> These data either suggest a biased selection of control subjects or a methodological problem.

In most of the studies *UGT1A7* polymorphisms have been determined by direct DNA sequencing, which is generally accepted as the gold standard for mutation detection and is thought to be superior to other methods of mutation analysis. However, as sequencing requires PCR amplified DNA as a template, uneven allele amplification during PCR might lead to erroneous findings in the subsequent sequencing reaction. The observed differences in *UGT1A7* gene analysis might be explained by a primer dependent PCR bias for the various alleles. In the present study a primer dependent bias is unlikely because we undertook two different PCR assays, with both RFLP and FRET analysis, and both methods led to the same results. All in all we think that the discrepancies between our study and that of Ockenga *et al* most probably stem from differences in the selection of the control groups, the different sample sizes, or the different methodology used in the two studies.

As the *UGT1A7* gene is expressed exclusively in the extrahepatic tissues of the gastrointestinal tract it could play an important role in first pass metabolism.<sup>21</sup> *UGT1A7* has been detected in the mucosa of the mouth, oesophagus, stomach, lungs, and very recently the pancreas, and therefore seems to be a candidate gene influencing the risk for pancreatic diseases. There are inter-individual variations in the expression of the *UGT1A7*, and other isoforms of UGTs may have overlapping substrate specificity, explaining why individuals bearing a low detoxification activity allele are not at higher risk of pancreatic diseases. Unless the UGT in question is responsible for the exclusive metabolism of a particular drug or chemical, or is the predominant or only UGT present in the cell, it is unlikely that this polymorphism will be of major clinical significance.

## Conclusion

We did not detect any association between pancreatic diseases and *UGT1A7* polymorphisms. The presence of low detoxification activity *UGT1A7* alleles such as *UGT1A7\*3* does not predispose to pancreatic cancer or to any type of acute or chronic pancreatitis.

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