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DNA-repair and carcinogen-metabolising enzymes genetic polymorphisms as an independent risk factor for hepatocellular carcinoma in Caucasian liver-transplanted patients

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ABSTRACT

We studied polymorphisms of three genes, *UDP-glucuronosyltransferase1A7* (*UGT1A7*), *Glutathione-S-transferaseM1* (*GSTM1*) and *X-Ray Cross Complementing group 1* (*XRCC1*), involved in detoxification of xenobiotics or DNA-repair in a population of 133 liver-transplanted patients, including 56 patients with hepatocellular carcinoma (HCC) and 77 without HCC, and in 89 healthy controls originating from the south of France. Multiple logistic regression analysis showed that, among liver-transplanted patients, interactions between XRCC1-G/G or -G/A and GSTM1-nul polymorphisms were independently associated with hepatocellular carcinoma (p interaction = 0.027) concurrently with increasing age ($p < 0.001$), male sex ($p = 0.037$) and chronic hepatitis B or C virus infection ($p = 0.018$ and $p = 0.001$ respectively). On the contrary, no relationship was observed between UGT1A7 polymorphisms considered alone or in interaction with GSTM1 or XRCC1 polymorphisms and HCC. This suggests that concomitant impaired metabolism of carcinogenic compounds and impaired DNA-repair function play an important role in liver carcinogenesis in high-risk cirrhotic patients originating from the south of France.

1. Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the third cause of cancer-related death worldwide. Considerable geographical differences exist in the incidence as well as in the aetiological factors associated with this cancer.[1] In developing countries, the major aetiological factor for HCC is chronic hepatitis B virus (HBV) infection. In Europe, HCC usually develops in a background of cirrhosis, whatever the aetiology, but mostly following hepatitis C virus infection or excessive alcohol intake.[2–4] However, only a minority of patients at risk develops HCC and it is likely that other risk

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factors such as environmental carcinogenic compounds may contribute to HCC development. In this context, the relationships between HCC and genetic polymorphisms, that could impair the activity of genes involved at different levels in the metabolism of carcinogenic xenobiotics and cellular defense, are of primary research interest.

UDP-glucuronosyltransferases (UGT) are members of a multigene superfamily of coding products that take part in detoxication of xenobiotics such as polycyclic hydrocarbons. *UGT1A7* is expressed in intestinal and pulmonary epithelial cells.[5] *UGT1A7* polymorphisms have been shown to encode enzymes with a lower carcinogen detoxication and relationships between *UGT1A7* polymorphisms and colorectal or pancreatic cancer have been reported.[6–8] Recent studies have suggested a link between UGT1A7 and primary liver cancer.[9–11] Thus in the study from Vogel and colleagues,[9] three *UGT1A7* polymorphisms encoding for a low detoxification protein activity (UGT1A7*2*3 or *4 alleles) were found in 93.2% of HCC patients. However, the actual importance of UGT1A7 polymorphisms in the development of HCC remains controversial since a recent report did not find such association.[12]

Glutathione-S-transferases (GST) are a superfamily of detoxifying enzymes involved in the neutralisation of electrophilic compounds and organic hydroperoxides such as polycyclic aromatic hydrocarbons present in aflatoxin B as well as endogenously generated reactive oxygen species produced during chronic liver disease.[13–15] Relationship between HCC risk and polymorphisms of *GSTM1* and *GSTT1* genes has been reported with conflicting results.[16,17]

Finally, a potential role for DNA-repair genes in hepatocarcinogenesis has been underlined in a recent study showing that transcription of most of these genes was upregulated in cirrhotic liver of HCC-bearing patients.[18] Among DNA-repair genes, *XRCC1* (X-Ray Cross Complementing group 1) plays a pivotal role in base excision repair (BER).[19,20]XRCC1 genetic polymorphisms could be associated with increased risk of various cancers such as head and neck, bladder, breast or lung cancer.[21–26] Few studies have evaluated XRCC1 polymorphisms among HCC patients and it is not clear whether a specific XRCC1 genotype could effectively modulate the risk for HCC.[27–30] No data have been reported about XRCC1 polymorphisms and HCC risk in populations originating from Europe.

In the present work, we studied genetic polymorphisms of *UGT1A7*, *GSTM1* and *XRCC1* genes, in a population of end stage liver disease patients originating from France who underwent liver transplantation. In this population of end stage liver disease, no relationship could be demonstrated between UGT1A7 polymorphisms and HCC while interactions between XRCC1 and GSTM1 polymorphisms were independently associated with an increased risk for HCC.

2. Patients and methods

2.1. Study population

A total of 133 liver-transplanted patients originating from France, consecutively seen for regular follow up after liver transplantation over a 6 month period, were recruited for the study. All patients underwent liver transplantation for end-stage liver disease in the Department of Hepatology and Liver transplantation, la Conception hospital, Marseilles, France. Decision for transplantation was taken for all patients according to commonly used criteria: decompensated liver disease in patients under 60 (men) or 65 (women) without concurrent cardiovascular or pulmonary severe disease, or concurrent extrahepatic malignancy. Before transplantation, all patients were included in a systematic survey programme including clinical and laboratory evaluation every month, abdominal ultrasonography and serum alpha-foeto-protein dosage every 3 months. In case of detection of hepatocellular carcinoma, patients were transplanted only if they met the Milan's criteria.[31] Eighty nine healthy controls (mean age 51 ± 6) were randomly selected from a cohort of healthy subjects originating from the south of France. None of them were known to be affected by chronic liver disease. The study was approved by local ethical committee. All patients and controls gave informed consent for the study.

2.2. Histological examination of explanted livers

For all transplanted patients, systematic examination of explanted liver was performed at time of transplantation. Macroscopic examination of all explanted liver was performed with thin and uniform sectioning of 5 mm each. Liver samples for microscopic examination were collected from nodules larger than 10 mm and all nodules were macroscopically different as compared to the cirrhotic background. The histological slices were stained with haematoxylin and eosin, Picrosirius red and Perls. The diagnostic of hepatocellular carcinoma was based upon usual, previously described, histopathologic criteria (mitotic figures, cell density, nuclear abnormalities, vascular invasion,...).[32]

2.3. Molecular analysis

Genomic DNA was extracted from blood samples using an automated device (Quiagen Bio Robot M48).

2.3.1. Characterisation of UGT1A7 gene polymorphisms

The UGT gene family contains numerous homologous genes whose sequence identity could reach 93% in coding sequences. For this reason, we have developed an Amplification Refractory Mutation System (ARMS) - PCR test[33] to identify UGT1A7 sequence variations with increased specificity. ARMS-PCR employs two primer pairs to amplify respectively the two different alleles of a single nucleotide polymorphism (SNP).

In a first step, sequences of UGT1A7 exon 1 were amplified by classic PCR using forward and reverse primers as previously described.[9] The forward primer was located –61 to –38 upstream of the ATG start codon of *UGT1A7* gene (GenBank accession number: U39570). The use of this primer was required to obtain specific gene amplification because UGT1A7 coding sequences share a high level of homology with other UGT related genes (UGT1A8-10). A 917 bp DNA fragment was amplified in 100 μL containing 20 ng of genomic DNA, 2 μMol/L of primers. Taq polymerase was obtained from Invitrogen (Carlsbad, CA).

In a second step, three SNPs at codons 129 (AAT → AAG), 131(CGA → AAA) and 208 (TGG → CGG) were detected from the first PCR amplification product allowing the determination of the four different UGT1A7 alleles previously described[9]:

UGT1A7*1: wild-type allele.
UGT1A7*2: p.Asn129Glu and p.Ala131Lys.
UGT1A7*3 : p.Asn129Glu, p.Ala131Lys and p.Val208Ala.
UGT1A7*4: p.Val208Ala.

Sequence variations at codons 129 and131 were detected in the PCR product from exon 1 of *UGT1A7* gene using ARMS-PCR: Wild-type forward primer: 5′-TTGCAGGAGTTT-GTTTAATGACGG-3′, Variant forward primer: 5′-ATTG-CAGGAGTTTGTTTAAGGACAA-3′. The sequence of the reverse primer was homologous to all UGT1A7 alleles: 5′-TGCC-GTGACAGGGGTTTGGAG-3′. The presence of the homologous sequences led to the specific amplification of 355 bp DNA fragment (Fig. 1a and b).

Sequence variation at codon 208 was identified by Restriction Fragment Length Polymorphism (PCR-RFLP) using *Csp 61 (RsaI)* restriction enzyme. The cleavage of UGT1A7 fragment gave a 236 bp and 681 bp fragment in the variant sequence that was not observed in the wild-type sequence (Fig. 1c).

Regularly, data obtained by ARMS-PCR and RFLP-PCR were verified by sequencing the UGT1A7 PCR fragment using a CEQ™ 8000 DNA sequencing device (Beckman-Coullter Inc., Fullerton, CA).

2.3.2. *Characterisation of XRCC1 gene polymorphisms*

The DNA polymorphism CGG → CAG (p.Arg399Gln) of *XRCC1* gene (GenBank accession number: M36089) was detected by sequencing amplified PCR fragments using primers described in http://snp500cancer.nci.nih.gov. Sequence analysis was performed on a CEQ 8000 Beckman sequencing device.

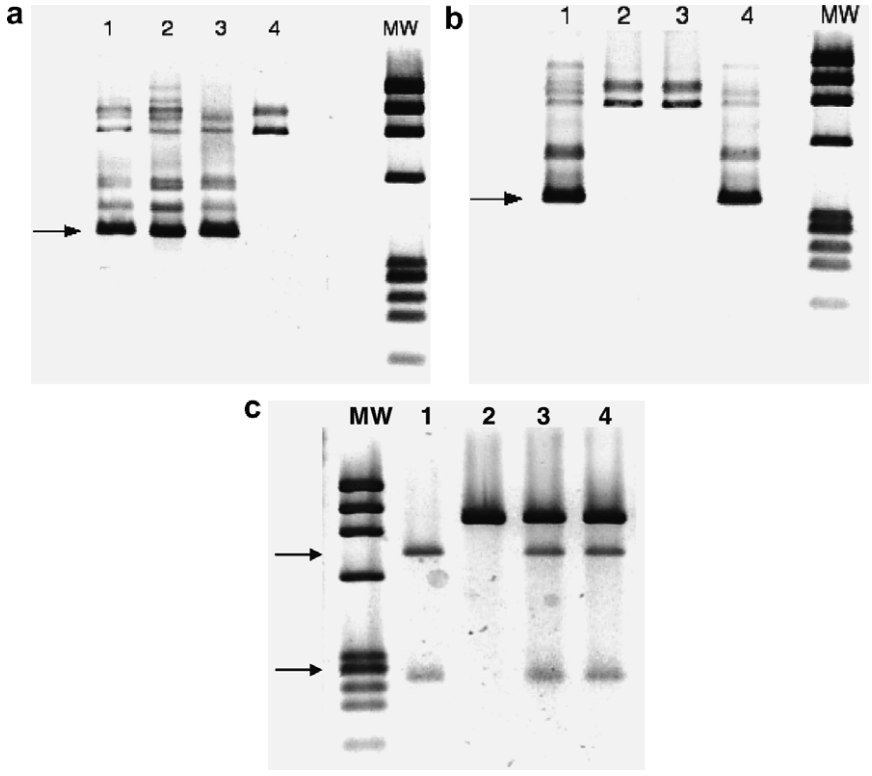

Fig. 1 – Detection of UDP-glucuronosyltransferase 1A7 (UGT1A7) polymorphisms in four patients. (a and b): Agarose gel electrophoresis showing the specific amplification of UGT1A7 polymorphic variants at codons 129/131 using ARMS-PCR. The PCR fragment from exon 1 of UGT1A7 gene was further amplified using specifics primers for the wild type (w) or the variant (m) at codons 129 and 131. Two direct primers, whose 3′ ends hybridised to the wild (w) or the variant (m) sequences were designed and used with a common reverse primer. Only perfectly matching direct-primer allowed amplification of a DNA fragment. (a): PCR amplifications using 5′primers for the 'wild' sequence. (b): PCR amplification using 5′ primers for the 'variant' sequence. The expected size of the amplified fragment is indicated by an arrow. (c): Detection of sequence variant at codon 208. DNA fragment from exon 1 of *UGT1A7* gene was amplified by PCR and cleaved with *RsaI* restriction enzyme. The variant allele (m) creates a *RsaI* restriction site that is absent in the wild (w) allele. MW: molecular weight markers: ϕX Hae III RF DNA. Determination of UGT1A7 genotype is determined by the combination of the different detected alleles as described in Patients and Methods. The UGT1A7*2*4 and UGT1A7*1*3 genotypes, genetically not distinguishable, were classified as UGT1A7*1*3 genotype. In this example, results are as follows: Patient 1: UGT1A7*3*4 (129/131 w/m - 208 m/m). Patient 2: UGT1A7*1*1 (129/131 w/w - 208 w/w). Patient 3: UGT1A7*1*4 (129/131 w/w - 208 w/m). Patient 4: UGT1A7*2*3 (129/131 m/m - 208 w/m).

2.3.3. Detection of GSTM1 gene deletion

The deletion of *GSTM1* gene (GenBank accession number: AY510272) was assessed by PCR using the following primers: 5′-GAACTCCCTGAAAAGCTAAAGC-3′ and 5′-GTTGGGCTC-AAATATACGGTGG-3′. The presence of homozygous deletion resulted in the absence of a 215bp DNA fragment.

2.4. Statistical analysis

All data analysis was performed using SPSS version 10.1. Chi square test was used to test for association. To identify the potential factors that were associated with HCC, we used multiple logistic regression analysis. To investigate possible interaction between two factors, we included the corresponding product terms in the modelling process. Calibration of the logistic model was assessed using the Hosmer–Lemshow goodness-of-fit test.

3. Results

3.1. Clinical characteristics of patients

The 133 liver transplanted patients were predominantly of male sex (75%). At time of transplantation, the mean age of patients was 51 ± 7 years. According to CHILD–PUGH classification, twenty-six patients were A, 55 were B and 52 were C. Excessive alcohol consumption was found in 50 patients (37%), including 12 with concomitant hepatitis virus infection. Chronic viral hepatitis C was found in 41 patients (31%), chronic viral hepatitis B in 17 (13%), and both infections in four patients (3%). Other aetiologies (cryptogenetic hepatitis, autoimmune hepatitis, primary biliary cirrhosis and metabolic liver disease) were found in 33 patients (23%).

HCC was found in explanted liver from 56 patients (HCC patients). Fifteen were CHILD A, 22 were B, 19 were C. HCC was known before liver transplantation (42 patients) or incidentally found during histological examination of explanted liver (incidental HCC, 14 patients). No HCC was found in the 77 remaining patients (non-HCC patients).

Characteristics of HCC and non-HCC patients are presented in Table 1. HCC patients were significantly older with a higher male to female sex ratio than non-HCC patients. The proportion of HCV or HBV infection was also significantly increased in HCC patients. The severity of the underlying liver disease was similar in HCC and non-HCC patients.

Table 1 – Characteristics of liver-transplanted patients presenting with or without HCC at time of transplantation

Patients characteristics	Transplanted patients		*p* trend HCC *versus* non-HCC
	HCC (*n* = 56)	Non-HCC (*n* = 77)	
Age at transplantation	54.4 ± 7	48 ± 10	<0.01
Sex M/F	49 / 7	52 / 26	0.005
Child score	8 ± 3	8.9 ± 2	0.08
Alcohol	21 (37%)	29 (38%)	0.98
HCV infection	24 (44%)	21 (27%)	0.044
HBV infection	17 (31%)	4 (5%)	0.001

3.2. UGT1A7 genetic polymorphisms in HCC and non-HCC patients and in healthy subjects

The distribution of four UGT1A7 polymorphisms (*1, *2, *3 and *4)[9] was studied in transplanted patients with or without HCC and healthy subjects (Table 2).

The most frequently found allele in the healthy subjects in our study was the *1 allele (39%) and the most frequently found genotype was the *1*3 (31%). The UGT1A7 *4*4 genotype was never found in our population.

No difference was observed when considering UGT1A7 polymorphisms between transplanted patients with and without HCC. Thus, in both groups, the most frequent allele was the *3 allele (42% and 41%). The frequency of *2*3 genotype, previously reported to be highly associated with HCC, was similar in HCC and non-HCC patients.

It is known that UGT1A7 polymorphisms are associated with different enzymatic activities. The *1 allele is related to higher enzymatic activity than are *2, *3 or *4 alleles. We therefore designated *1 allele as H (higher activity) and the others as L (lower activity) and categorised UGT1A7 genotypes in three different groups according to the enzymatic activity H/H, H/L, and L/L. No difference could be seen when considering categorised genotypes (H/H, H/L and L/L) between patients with and without HCC.

Interestingly, when considering healthy subjects as compared to non-HCC patients or the overall population of transplanted patients significant differences could be seen in UGT1A7 polymorphisms distribution. Thus, the frequency of H/L genotype was significantly lower in non-HCC and the whole transplanted patients as compared to healthy subjects [OR 0.50 (0.28–0.90) $p = 0.01$ and OR 0.43 (0.22–0.84) $p = 0.007$, respectively for transplanted and non-HCC patients]. An increase in L/L genotype was also noted in transplanted patients [OR 1.68 (0.92–3.08) $p = 0.06$]. There was a tendency for an increase in *2*3 genotype in transplanted patients as compared to healthy controls [OR 1.72 (0.76–3.97) $p = 0.158$].

3.3. GSTM1 genotype and XRCC1 genetic polymorphisms in transplanted patients with and without hepatocellular carcinoma and in healthy subjects

The distribution of XRCC1 and GSTM1 polymorphisms in transplanted patients and healthy subjects are detailed in Table 3.

No difference could be seen in GSTM1 polymorphisms between HCC and non-HCC patients. When considering only alcoholic patients, a significant increase in GSTM1 del/del genotype was noted in HCC patients [OR = 3.46 (0.89–13.95) $p = 0.04$]. This association was not observed in HCV positive patients. There was a tendency for an increase in the frequency of the GSTM1 del/del genotype in healthy controls as compared to HCC patients [OR 1.78 (0.86–3.70) $p = 0.093$].

When considering XRCC1 polymorphisms, the most frequent genotype was the A/G in non-HCC patients and healthy controls and G/G in HCC patients. Thus, the G/G genotype was more frequent in HCC patients versus healthy controls [OR 2.3

Table 2 – Distribution of UGT1A7 polymorphisms in liver-transplanted patients and healthy subjects

Polymorphisms	Transplanted patients ($n = 133$)		HCC *versus* non HCC Odds Ratio (95% CI)	Healthy subjects ($n = 89$)	HCC versus healthy subjects Odds Ratio (95% CI)
	HCC ($n = 56$)	non HCC ($n = 77$)			
UGT1A7 genotype					
*1/*1	6 (11%)	14 (18%)	0.55 (0.17–1.67)	9 (10%)	1.07 (0.31–3.54)
*1/*2	9 (16%)	9 (12%)	1.47 (0.49–4.41)	22 (25%)	0.58 (0.23–1.48)
*1/*3	17 (30%)	20 (26%)	1.26 (0.55–2.90)	28 (31%)	0.95 (0.43–2.08)
*1/*4	0	0	–	2 (2%)	–
*2/*2	3 (5%)	4 (5%)	0.98 (0.16–5.51)	4 (4%)	1.20 (0.2–6.7)44
*2/*3	12 (21%)	14 (18%)	1.31 (0.51–3.36)	11 (11%)	1.93 (0.72–5.19)
*3/*3	9 (16%)	13 (17%)	0.88 (0.32–2.39)	13 (15%)	1.12 (0.40–3.08)
*3/*4	0	3 (4%)	–	0	
UGT1A7 allele frequency					
*1	38 (34%)	57 (37%)	0.89 (0.52–1.53)	70 (39%)	0.79 (0.47–1.34)
*2	27 (24%)	31 (20%)	1.28 (0.69–2.39)	41 (22%)	1.06 (0.59–1.92)
*3	47 (42%)	63 (41%)	1.01 (0.60–1.71)	65 (36%)	1.26 (0.75–2.10)
*4	0	3 (2%)	–	2 (2%)	–
UGT1A7 categorised genotype					
H/H	6 (11%)	14 (18%)	0.55 (0.17–1.62)	9 (10%)	1.07 (0.31–3.54)
H/L	26 (46%)	29 (38%)	1.46 (0.69–3.12)	52 (58%)	0.62 (0.30–1.28)
L/L	24 (42%)	34 (44%)	0.92 (0.43–1.95)	28 (31%)	1.63 (0.77–3.47)

Table 3 – Distribution of XRCC1 and GSTM1 polymorphisms in liver-transplanted patients and healthy subjects

Polymorphisms	Transplanted patients ($n = 133$)		Odds Ratio (95% CI) HCC *versus* non HCC	Healthy subjects ($n = 89$)	Odds Ratio (95% CI) HCC *versus* healthy subjects
	HCC ($n = 56$)	non HCC ($n = 77$)			
XRCC1					
G/G	27 (48%)	29 (38%)	1.54 (0.72–3.29)	27 (30%)	2.30 (1.01–4.53) $p = 0.03$
A/G	21 (37.5%)	35 (45%)	0.72 (0.34–1.54)	43 (48%)	0.64 (0.31–1.34)
A/A	8 (15%)	13 (17%)	0.82 (0.28–2.34)	19 (21%)	0.61 (0.23–1.64)
XRCC1 allele frequency					
G	77 (68.7%)	93 (60.4%)	1.44 (0.84–2.49) $p = 0.16$	97 (54.5%)	1.84(1.09–3.11) $p = 0.015$
A	35 (31.3)	61 (39.6%)	–	81 (45.5%)	–
GSTM1 deletion					
no del	30 (54%)	33 (43%)	1.54 (0.73–3.26)	35 (39%)	1.78 (0.86–3.70)
del / del	26 (46%)	44 (57%)		54 (61%)	

(1.01–4.53), $p = 0.03$] and, although the difference was not significant, versus non-HCC patients [OR 1.54 (0.72–3.29)]. The frequency of G allele was also higher in HCC patients than in healthy controls [OR 1.84(1.09–3.11) $p = 0.015$].

3.4. *Multivariate analysis of factors associated with HCC in transplanted patients*

We then evaluated factors associated with HCC in transplanted patients using stepwise multivariate logistic regression analysis. Age, sex, HCV infection, HBV infection, UGT1A7 categorised genotype (H/H, H/L or L/L), GSTM1 and XRCC1 genotypes were included. Furthermore, we evaluated potential interactions between UGT1A7, GSTM1 and XRCC1 genotypes. The results are presented in Table 4.

Age, male sex, HCV and HBV infections were independently associated with HCC in our cohort of transplanted patients. When considering genetic polymorphisms, neither UGT1A7, XRCC1 nor GSTM1 polymorphisms alone were associated with HCC. However, when considering interactions between different genetic polymorphisms, combinations between GSTM1 and XRCC1 genotypes were associated with various risk for HCC (p interaction = 0.027). Thus, association of GSTM1 del/del genotype and XRCC1 G/G or G/A genotype

Table 4 – Multivariate analysis of risk factors for hepatocellular carcinoma in liver-transplanted patients

Factors	Odds Ratio (95% CI)	p value
Age	1.13 (1.05–1.21)	<0.001
Male Sex	3.73 (1.08–12.83)	0.037
HCV	3.144 (1.22–8.79)	0.018
HBV	15.38 (3.26–72.53)	0.001
GSTM1 × XRCC1		0.027
del/del × A/G	86.11 (3.2–2291)	0.008
del/del × G/G	54.86 (2.17–1385)	0.015

increased significantly the risk of HCC ($p = 0.015$ and $p = 0.008$ respectively) (Table 4).

4. Discussion

In this work, we studied the distribution of genetic polymorphisms of three genes, UGT1A7, GSTM1 and XRCC1 in a population of liver-transplanted patients from Caucasian origin with or without HCC. Our study demonstrates that interactions between polymorphisms of XRCC1 and GSTM1 genes are independent risk factors for HCC, concurrently with well known risk factors for HCC such as age, male sex and chronic HCV or HBV infections.[34,35]

Different studies suggested that G to A substitution at codon 399 in exon 10 of XRCC1 gene could modulate the risk of various cancers although polymorphisms-related specific variations in XRCC1 enzymatic activities have not been demonstrated.[21–26] A link between XRCC1 polymorphisms and HCC has been suggested in populations with high HBV endemicity and aflatoxin exposure. Yu et al.[27] have reported an increased risk of early onset HCC in HBV infected patients presenting with A/A genotype. On the contrary, Kirk et al.[28] observed a 15 fold increased risk of HBV-related HCC in patients presenting, among other genetic factors, with A/G or G/G XRCC1 genotypes. Our results are in accordance with the latter findings as we observed an increased frequency of XRCC1 G/G genotype in HCC transplanted patients as compared to healthy controls. Furthermore, interactions between G/G or G/A genotypes and GSTM1-nul genotype were found to be an independent risk factor for HCC in liver-transplanted patients.

GSTM1-nul genotype, which results in the absence of GSTM1 protein, was also previously found to modulate HCC risk in populations with high HBV endemicity and aflatoxin exposure.[27,36,37] However, this has not been observed in populations originating from Spain and Italy.[16,17] In these latter studies, interactions between GSTM1 and XRCC1 polymorphisms were not taken into account. In our work, although GSTM1-nul genotype was associated with HCC in alcoholic patients, when considering the whole population of transplanted patients, neither XRCC1 nor GSTM1 gene polymorphisms were independently associated with HCC risk. But, interactions between polymorphisms of these two genes were clearly an independent risk factor for HCC using multivariate analysis although with imprecise estimates. Thus, GSTM1-nul genotype is associated with a lower elimination rate of exogenous toxic compounds as well as endogenously generated reactive oxygen species. The subsequent increase in cellular DNA damage could overcome the capacities in DNA repair conferred by XRCC1 G/G or G/A genotypes. Such positive interactions between XRCC1 and GSTM1 polymorphisms in HCC development have been reported in populations of high HBV prevalence and aflatoxin exposure.[27,28,30] Our study extends these results in a population from the south of France, in an area of low HBV endemicity and without known exposure to specific liver co-carcinogens, raising the question whether so far undetermined environmental toxic compounds might play a significant role in HCC development in Europe.

On the contrary, we did not find a link between HCC and UGT1A7 polymorphisms although such an association has been previously reported in different studies from Germany, Japan and Taiwan.[9–11] It has been recently suggested that a bias in PCR leading to uneven allele amplification could have dampened the informativity of UGT1A7 polymorphism detection. Thus, no relationship between UGT1A7 polymorphisms and HCC has been reported in a population originating from Germany using different probes for alleles detections.[12] Results from our study, in which each UGT1A7 allele was specifically detected using ARMS-PCR, also suggest the absence of relationship between HCC and UGT1A7 polymorphisms either considered alone or in interaction with XRCC1 or GSTM1 genotypes.

Besides these technical pitfalls, the discrepancies between previously published results and the present data could also be related to the population studied. We studied a population of liver-transplanted patients. This may have significant implications. Thus, liver transplantation is in current use only in highly selected, young patients presenting with HCC at an early stage. This population could therefore be different from those studied previously that included older patients, such as in the study from Vogel and colleagues[9], or patients at a more advanced stage. Importantly, our population was particularly well characterised when considering the severity as well as the aetiology of liver disease. Thus, common aetiologies of HCC in Europe such as alcohol, HCV or HBV infection were found in 89% of our patients while none of these three factors could be found in up to 40% of HCC patients in the study from Vogel and colleagues. Moreover, in this latter study, the severity of the underlying liver disease was not described in either HCC patients or in controls. It must be underlined that our study is the first in which the presence or absence of HCC could be ascertained in all patients by histological examination of explanted liver. This is a major point because HCC diagnosis is usually difficult in cirrhotic patients.[38] In our study, in the absence of histological assessment, 15% of cirrhotic patients would have been incorrectly considered as non-HCC patients despite a careful follow up and HCC screening before liver transplantation. This is in agreement with previously published results evaluating the percentage of incidental HCC in liver transplanted patients.[39,40]Moreover, the fact that well known risk factors for HCC such as age, male sex and chronic hepatitis B or C viral infection were associated with HCC suggests that the population studied in our work, at least, shares the same HCC risk factors with the overall population of HCC patients. Finally, in the present study, due to the selection of patients presenting with end-stage liver disease necessitating liver transplantation, no difference could be noted in the severity of cirrhosis between HCC and non-HCC patients. This is an important point as, among cirrhotic patients, HCC risk is clearly related to the severity of the liver disease.[34] Thus, factors associated with progression of cirrhosis could be confounding when considering the risk for HCC in chronic liver diseases. In this context, the observation of different UGT1A7 polymorphism distribution between healthy controls and the overall population of transplanted patients, as well as non-HCC patients, might suggest that UGT1A7 polymorphisms could be associated with the severity of liver disease leading to end-stage liver disease, rather than with an increased risk of HCC by itself.

A specific work aimed at studying this point is therefore necessary.

In conclusion, interactions between XRCC1 and GSTM1 genetic polymorphisms are independently associated with HCC in liver-transplanted patients originating from the south of France concurrently with age, male sex and chronic hepatitis B or C viruses infection. Once more, this raises the fact that both genetic and environmental factors might be involved in HCC development in cirrhotic patients.

Conflict of interest statement

None declared.

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