# Polymorphisms of Microsomal Epoxide Hydrolase Gene and Severity of HCV-Related Liver Disease

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**Factors influencing the progression of liver disease and the development of hepatocellular carcinoma (HCC) in chronic hepatitis C virus (HCV) infection are poorly understood. Inherited variations of drug-metabolizing enzyme (DME) activities may affect liver damage and cancer risk by modifying individual susceptibility to endogenous or exogenous toxic compounds. We investigated the association of liver disease severity with common alleles of microsomal epoxide hydrolase (mEH), an enzyme involved in the metabolism of highly reactive epoxide intermediates. Three polymorphisms (Tyr113His, His139Arg, and −613C/T) were analyzed by polymerase chain reaction (PCR) restriction fragment length polymorphisms (RFLPs) in 394 patients at different stages of disease, including 92 asymptomatic carriers, 109 patients with chronic hepatitis, 100 patients with cirrhosis, and 93 patients with HCC. Reference allele frequencies were obtained from 99 healthy blood donors. Allele distributions between categories were compared using the $\chi^2$ test; odds ratios (ORs) and 95% CI were calculated to express relative risks. Allele frequencies among 99 healthy controls were as follows: 15.1% for 113His/His, 4.0% for 139Arg/Arg, and 46.5% for −613C/T. mEH 113His/His homozygotes were overrepresented in advanced stages of disease, in particular among HCC patients (27.9%; $P = .03$; OR, 2.2; 95% CI, 1.0-4.6). Differences were more pronounced among men and between extreme patient categories. When mEH genotypes were combined to express a metabolic phenotype, very slow metabolizers were highly prevalent among cirrhotic and HCC patients (18% vs. 3.3% in carriers; $P < .001$). In conclusion, mEH gene polymorphisms were significantly associated with HCV-related liver disease severity and HCC risk. Men were at higher risk than women; this might be explained by hormonal regulation of gene expression or by differential exposure to environmental toxins.** (HEPATOLOGY 2002;36:195-201.)

The worldwide burden of deaths caused by liver diseases and liver cancer, which usually arises in the setting of cirrhosis and chronic viral hepatitis, is estimated to be approximately 1.3 million/yr.[1] Chronic hepatitis C virus (HCV) infections account for over 80% of cases of cirrhosis and hepatocellular carcinomas (HCC) in Italy,[2] for which over 2 million anti-HCV–positive cases are estimated.[3] HCV-related liver disease displays a multiplex phenotype in which environmental and viral factors are likely to act in concert with individual susceptibility to induce liver damage. Overall penetration of disease expression is low, because less than 20% of infected individuals will develop cirrhosis over a 20- to 30-year period.[4]

Determinants of HCV pathogenesis are barely known and include age at infection,[5] disease duration,[4] sex,[6] modes of transmission,[7] immunogenetic variables,[8] and viral heterogeneity,[9] but these factors account for only a small part of the clinical variability of the disease. Dietary factors, inherited metabolic defects, such as hemochromatosis,[10] and alcohol consumption[11] can also modulate chronic hepatitis C progression. The identification of genetic determinants of susceptibility to HCV-induced

*Abbreviations: HCV, hepatitis C virus; HCC, hepatocellular carcinoma; DME, drug metabolizing enzyme; mEH, microsomal epoxide hydrolase; HBV, hepatitis B virus; RFLP, restriction fragment length polymorphism; OR, odds ratio.*

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chronic liver damage would assist in predicting individual and population-associated risks of disease progression and would help to clarify gene-environment interactions relevant to this process.

The liver has a complex detoxification system in which several enzymes participate in the metabolism of an extraordinary number of nonpeptidic chemical compounds of both endogenous and exogenous source. Many of these enzymes display functional polymorphisms in the population, which have been extensively characterized at the protein and gene levels, and simple DNA tests have been developed to predict their phenotype. Liver detoxifying enzymes have been originally investigated in the setting of pharmacogenetic studies aimed at clarifying adverse drug reactions; therefore, they are usually referred to as "drug-metabolizing enzymes" (DMEs). Associations of DME polymorphisms with disease susceptibility have been mainly explored in models of environmental carcinogenesis,[12] but several examples suggest that variation in DME function might also influence susceptibility to damage by endobiotic compounds or infectious agents.

The epoxide hydrolases are a family of enzymes that catalyze the irreversible hydration of highly reactive epoxide intermediates to yield metabolites of lower reactivity, which can be readily conjugated and excreted.[13,14] Five classes of mammalian epoxide hydrolases have been characterized that are structurally and antigenically distinct and show different substrate specificity.[15] Microsomal epoxide hydrolase (mEH) is expressed in all tissues, with the highest levels in liver, kidney, and testis,[13,16] and it is primarily involved in the metabolism of xenobiotics with a wide substrate specificity. There is evidence for polymorphic mEH expression in humans,[16-19] and allelic variants of mEH have been identified.[20,21] Two point mutations in the coding sequence lead to amino acid changes, Tyr113His and His139Arg, which affect mEH activity by influencing protein stability.[20,22,23] More recently, 7 polymorphic loci have been identified within the mEH 5′-noncoding promoter region,[24] but their functional significance is controversial.

Clinical studies have documented an association between specific mEH alleles and adverse reactions to hepatotoxic drugs,[25] fetal hydantoin syndrome,[26] risk of emphysema,[27,28] and lung cancer[29] in smokers and susceptibility to liver cancer in hepatitis B virus (HBV)-infected subjects.[30] The role of mEH polymorphism in HCV-related liver disease has never been specifically investigated. In the present study, we explored whether polymorphisms in both the structural and the regulatory region of the mEH gene have any bearing on the severity of HCV-related liver disease and the risk of HCC.

## Patients and Methods

***Patients.*** Four separate cohorts of anti-HCV–positive white subjects, all of Italian descent, were considered for the study: (1) 92 asymptomatic carriers with persistently normal alanine transaminase (ALT) levels as assessed on the basis of monthly or bimonthly ALT determination for at least 1 year (40 men, age 57 ± 12 years), (2) 109 subjects with chronic hepatitis (70 men, age 52 ± 12 years), (3) 100 subjects with cirrhosis without HCC (55 men, age 61 ± 9 years), and (4) 93 subjects with cirrhosis and HCC (64 men, age 68 ± 14 years).

One hundred fifty-one (38%) patients (22 carriers, 67 with hepatitis, 39 with cirrhosis, and 23 with HCC) had a history of transfusion. Mean disease durations estimated according to the date of transfusion were 22 years for carriers, 17 years for hepatitis patients, 22 years for cirrhotic patients, and 29 years for HCC patients.

Chronic hepatitis grade and stage were assessed by liver biopsy in all subjects; cirrhosis was diagnosed by clinical signs of portal hypertension or liver biopsy, and HCC diagnosis was performed by histology or high-resolution contrast computerized tomography (CT). Ninety-nine anti-HCV–negative, ethnically matched controls were included in the study, referred from a local blood bank.

***Viral Marker Detection and HCV Genotyping.*** Anti-HCV RNA antibodies were tested by a second-generation enzyme-linked immunosorbent assay (Ortho Diagnostic System Inc., Raritan, NJ) and in selected cases by immunoblot assay (RIBA 2; Chiron Corporation, Emeryville, CA). HCV RNA was detected by nested reverse transcription polymerase chain reaction (RT-PCR) using conserved primers in the 5′-noncoding region of the viral genome. HCV typing was performed by amplification of core region sequences with universal and 5 subtype-specific primers using a modified type 2a-specific primer, as previously described.[31]

***Detection of mEH DNA Polymorphisms.*** DNA was isolated from 3 to 5 mL of peripheral blood using a commercial kit, according to the instructions of the manufacturer (Roche Diagnostic, Mannheim, Germany). DNA polymorphism was studied by PCR amplification and restriction enzyme digestion. Three different polymorphisms were studied. (1) For exon 3 Tyr113His polymorphism, primers used for the amplification reaction were EH1 5′-GCA TTA CAC CAG AGG ATC GAT AAG and EH2 5′-CCT CAA TCT TAG TCT TGA AGT GAC GGT, yielding a 184-base-pair fragment; PCR products were digested with AspI (New England Biolabs, Beverly, MA) and analyzed by electrophoresis on a 4% agarose-nusieve gel.[20] (2) For exon 4

**Table 1. Distribution of mEH Genotypes Among Carriers, Chronic Hepatitis Patients, Cirrhotic Patients, HCC Patients, and Healthy Controls**

| | Number of Individuals (% of Group) | | | Frequency of Variant Allele | OR (95% CI)* | OR (95% CI) | |
|---|---|---|---|---|---|---|---|
| | Homozygous Wild Type | Heterozygous | Homozygous Variant | | | Males† | Females‡ |
| Exon 3 polymorphism | | | | | | | |
| Controls (n = 99) | 47 (47.5) | 37 (37.4) | 15 (15.1) | 0.34 | REF | REF | REF |
| Carrier (n = 92) | 40 (43.5) | 45 (48.9) | 7 (7.6) | 0.32 | 0.5 (0.2-1.3) | 0.3 (0.1-1.9) | 0.6 (0.1-2.1) |
| Hepatitis (n = 109) | 53 (48.6) | 41 (37.6) | 15 (13.8) | 0.33 | 0.9 (0.4-2.1) | 1.0 (0.3-3.7) | 0.8 (0.2-2.9) |
| Cirrhosis (n = 100) | 44 (44.0) | 36 (36.0) | 20 (20.0) | 0.38 | 1.4 (0.6-3.1) | 1.2 (0.3-4.4) | 1.5 (0.5-5.8) |
| HCC (n = 93) | 29 (31.2) | 38 (40.1) | 26 (27.9) | 0.48 | 2.2 (1.0-4.6) | 2.9 (0.9-9.6) | 1.9 (0.6-6.6) |
| Exon 4 polymorphism | | | | | | | |
| Controls (n = 99) | 60 (60.6) | 35 (35.4) | 4 (4.0) | 0.22 | REF | | |
| Carrier (n = 92) | 57 (61.9) | 31 (33.7) | 4 (4.3) | 0.21 | 1.1 (0.2-5.8) | | |
| Hepatitis (n = 109) | 74 (67.9) | 30 (27.5) | 5 (4.6) | 0.18 | 1.2 (0.2-5.9) | | |
| Cirrhosis (n = 100) | 63 (63.0) | 35 (35.0) | 2 (2.0) | 0.19 | 0.5 (0.1-3.4) | | |
| HCC (n = 93) | 67 (72.0) | 22 (23.7) | 4 (4.3) | 0.16 | 1.1 (0.2-5.9) | | |
| −613 Polymorphism | | | | | | | |
| Control (n = 99) | 3 (3.0) | 50 (50.5) | 46 (46.5) | 0.72 | REF | | |
| Carrier (n = 92) | 7 (7.6) | 30 (32.6) | 57 (61.9) | 0.78 | 1.8 (0.9-3.2) | | |
| Hepatitis (n = 109) | 2 (1.8) | 40 (36.7) | 67 (61.5) | 0.80 | 1.8 (1.0-3.3) | | |
| Cirrhosis (n = 100) | 5 (5.0) | 38 (38.0) | 57 (57.0) | 0.76 | 1.7 (0.9-2.9) | | |
| HCC (n = 93) | 5 (5.4) | 34 (36.6) | 54 (58.1) | 0.76 | 1.6 (0.9-3.0) | | |

NOTE. OR calculated for homozygous variant vs. other genotypes.

*Exon 3 polymorphism, all patients: $P$ for total distribution, .004, $P_c$ = .012, $\chi^2$ analysis.

†Exon 3 polymorphism, male patients: $P$ for total distribution, .03, $P_c$ = NS, $\chi^2$ analysis.

‡Exon 3 polymorphism, female patients: $P$ for total distribution, .11, $\chi^2$ analysis.

His139Arg polymorphism, primers used for the amplification reaction were EPO-3 5′-ACA TCC ACT TCA TCC ACG T and EPO-4 5′-ATG CCT CTG AGA AGC CAT, yielding a 210-base-pair fragment; PCR product were digested with RsaI (New England Biolabs).[20] (3) 5′-Flanking region polymorphism at positions −613C/T and −699C/T were analyzed; primers used for the amplification reaction were EPX 5 5′-GTC AAG TTT GAT GAG TTG TGG C and EPX 6 5′-TCC TTC TTC TTG CTG CAG GC; PCR products were digested with NheI (New England Biolabs) for the −613C/T mutation and with DdeI (New England Biolabs) for the −699C/T mutation.[24]

***Statistical Analysis.*** Associations between groups and mEH genotypes were analyzed for significance by the two-tailed $\chi^2$ test. Odds ratios (ORs) and 95% CI were calculated to assess strength of associations. Healthy controls were used as the reference category.

Multiple logistic regression was used to model the relation between mEH genotypes/phenotypes, age (<60 years vs. ≥60 years), sex, and liver disease severity. It was performed using the statistical package STATA (Stata Statistical Software, release 5.0; Stata Corporation, College Station, TX). Results were expressed as ORs and 95% CI. Statistical significance was set at $P < .05$; significant $P$ values were adjusted by the number of tested alleles ($P_c$) according to Bonferroni.

## Results

***Exon 3 Tys113His Polymorphism.*** Genetic variability in mEH exon 3 was studied analyzing the Tyr113His substitution, which has been demonstrated to reduce the activity of the "*in vitro*"-expressed protein by 40%.[20,22] Variant allele frequency in healthy reference subjects (34%) was in agreement with published data for whites. Observed genotype frequencies were in Hardy-Weinberg distribution; observed/expected values were Tyr/Tyr 0.475/0.438, Tyr/His 0.374/0.447, and His/His 0.151/0.114 ($P = .14$).

Frequencies of alleles and genotypes among different patient categories are detailed in Table 1. The distribution of 113His/His homozygous variant subjects varied considerably in relation to liver disease severity, being increasingly represented in advanced stages of disease ($P = .004$, $P_c = .012$ for total distribution). Differences were especially evident in comparing groups at the extremes of the disease spectrum, carriers and cirrhotic or HCC patients. mEH 113His/His homozygosity was, in fact, associated with a 3-fold risk of cirrhosis (OR, 1.4 vs. 0.5 for carriers; $P = .01$, $P_c = .04$ by $\chi^2$ analysis) and a 5-fold risk of HCC (ORs, 2.2 vs. 0.5; $P < .001$, $P_c = .001$ by $\chi^2$ analysis).

The presence of the exon 3 variant allele, either in homozygous or heterozygous form, was increased overall in HCC patients (68%) compared with cirrhotic (56%)

**Table 2. Distribution of mEH Phenotypes in Controls and Disease Groups**

| | Number of Individuals (%) With Phenotype | | | | Odds Ratio (95% CI)* | Odds Ratio (95% CI)† |
|---|---|---|---|---|---|---|
| | Normal | Fast | Slow | Very Slow | | |
| Control (n = 99) | 43 (43.4) | 21 (21.2) | 22 (22.2) | 13 (13.1) | REF | REF |
| Carrier (n = 92) | 43 (47.6) | 15 (16.3) | 31 (33.6) | 3 (3.3) | 1.1 (0.6-2.0) | 0.2 (0.1-0.9) |
| Hepatitis (n = 109) | 43 (39.4) | 22 (20.2) | 31 (28.4) | 13 (11.9) | 1.3 (0.8-2.3) | 0.8 (0.4-2.1) |
| Cirrhosis (n = 100) | 40 (40.0) | 16 (16.0) | 34 (34.0) | 10 (10.0) | 1.5 (0.8-2.7) | 0.7 (0.3-1.9) |
| HCC (n = 93) | 24 (25.8) | 12 (12.9) | 40 (43.0) | 17 (18.3) | 2.8 (1.5-5.3) | 1.5 (0.6-3.4) |

*Risk of slow/very slow phenotypes vs. all other phenotypes: $P < .003$, $P_c = .006$, $\chi^2$ analysis.
†Risk of very slow phenotype vs. all other phenotypes: $P < .02$, $P_c = .038$, $\chi^2$ analysis.

or cirrhosis plus hepatitis patients (54.6%; $P < .02$, $P_c = .034$). These differences were more pronounced in male patients, HCC patients (73.1%), cirrhotic patients (49.1%, $P < .02$, $P_c = .036$), and cirrhosis plus hepatitis patients (53.6%, $P < .02$, $P_c = .038$). No anticipation of age at diagnosis for cirrhosis or HCC was observed according to the presence of the 113His/His genotype (cirrhosis 61.1 ± 6.6 vs. 60.9 ± 12.1 years, HCC 66.9 ± 8.2 vs. 65.1 ± 8.2 years; $P$ = NS).

***Exon 4 His139Arg Polymorphism.*** Exon 4 variability was explored by testing for substitution His139Arg, which induces a 30% increase in enzymatic activity compared with the wild type.[20,22] Frequency of the variant allele was 22% among control subjects, confirming previous data obtained from whites. Hardy-Weinberg analysis of genotype frequencies did not show any significant departure from expected values: His/His 0.606/0.608, His/Arg 0.354/0.343, Arg/Arg 0.040/0.048 ($P$ = .68). His139Arg allele frequency or genotype distribution did not show any significant difference between patient categories, also accounting for sex (Table 1).

***5′ Flanking Region Polymorphism.*** Seven different polymorphic sites have been identified in the upstream region of the mEH gene, occurring in 2 linkage disequilibrium groups, −200 and −600.[24] We chose to analyze the −613C/T polymorphism, which in *in vitro* experiments was seen to modify the transcription of a reporter gene by 30%. Given its strong linkage with the −699C/T polymorphism, this was also analyzed for control in 50 subjects. As expected, the 2 mutations were in strict linkage disequilibrium (data not shown); however, the detected frequencies markedly differred from those previously reported (72% vs. 32.1% for the −613C/T allele).[24]

The frequency distribution of the −613C/T alleles was analyzed by groups, but it did not show any significant difference between patients and controls or between patient categories. The analysis of combinations of 5′-flanking region and coding region alleles did not improve discrimination between categories (data not shown).

***Enzymatic Phenotype.*** Exon 3 and exon 4 genotypes were combined to express a metabolic phenotype as previously described.[27,29] Briefly, 1 or 2 copies of exon 3 low-activity variant allele defined a slow or very slow phenotype, respectively, unless balanced by the presence of a corresponding number of exon 4 variant high-activity allele. Four different enzymatic phenotypes were thus considered: normal, fast, slow, and very slow activity.

Frequencies in control subjects showed an increased representation of the very slow phenotype (13.1%) compared with previous studies (5%). Significant differences in phenotype distribution were observed among patient categories. The normal and fast phenotypes were less well represented among patients with advanced liver disease compared with HCV carriers and controls (63.9% and 64.6%, respectively, compared with cirrhosis, 56% and HCC, 38.7%). On the contrary, as anticipated from the distribution of genotypes, the frequency of the very slow phenotype was markedly overrepresented among HCC patients (18% vs. 3.3%, $P = .001$, $P_c = .004$; OR, 6.55; 95% CI, 1.78-35.9) compared with HCV carriers. An impaired mEH phenotype (slow or very slow subjects) increased the risk of liver cancer by 3-fold compared with controls and by 7-fold compared with HCV carriers (Table 2). Associations between mEH genotypes/phenotypes and liver disease severity were also confirmed in a multivariable analysis model, which compared HCV carriers and patients with cirrhosis with or without HCC and HCC-only patients, accounting for the possible confounding effect of age and sex of the patients (Table 3).

## Discussion

mEH activity *ex vivo* exhibits a relatively large interindividual variation,[16-19] which might be explained, at least in part, by differences in mEH gene sequences. Genotype/phenotype correlations, however, are imperfect, indicating an effect of induction/inhibition of enzyme expression possibly because of posttranscriptional/posttranslational mechanisms.[23,24]

**Table 3. Multiple Logistic Regression (LR) Analysis of Variables Associated With Liver Disease Severity (Carriers vs. Cirrhotic Patients With or Without HCC and vs. HCC-Only Patients)**

| | Cirrhosis With/Without HCC | | | | HCC | | | |
|---|---|---|---|---|---|---|---|---|
| Variable | OR | 95% CI | LR Test | *P* | OR | 95% CI | LR Test | *P* |
| Exon 3 homozygous mutant | 3.67 | 1.24-10.9 | 6.46 | .0111 | 4.36 | 1.34-14.2 | 6.82 | .0090 |
| Exon 4 homozygous mutant | 0.58 | 0.12-2.84 | 0.45 | NS | 1.36 | 0.22-8.31 | 0.11 | NS |
| −613 Homozygous mutant | 0.84 | 0.46-1.50 | 0.36 | NS | 0.96 | 0.47-1.99 | 0.01 | NS |
| Very slow phenotype | 2.61 | 1.06-3.51 | 4.59 | .0321 | 1.77 | 0.83-3.75 | 2.05 | NS |
| Male sex | 2.08 | 1.17-3.71 | 6.34 | .0118 | 2.59 | 1.27-5.28 | 7.05 | .0079 |
| Age 60 years | 2.84 | 1.60-5.04 | 12.9 | <.001 | 3.49 | 1.70-7.15 | 12.3 | <.001 |

Several lines of evidence indicate that mEH genetic variability can influence individual susceptibility to the development of cancer and chronic diseases. McGlynn et al.[30] observed an association between low-activity exon 3 alleles and occurrence of HCC in 2 HBV-infected populations exposed to aflatoxin B1, the effect of which was attributed to a reduced disposal of the carcinogen. Low-activity alleles have also been convincingly implicated in the risk of emphysema[27] because of the impaired metabolism of toxic compounds of tobacco smoke. Conversely, high-activity alleles have been associated with increased risk of tobacco-related airways cancers,[27,29] implying the involvement of mEH also in the potentiation of carcinogenesis by metabolic conversion of procarcinogenic polycyclic aromatic hydrocarbons.[32]

Few studies have addressed the issue of DME activity variation and chronic liver disease, despite the obvious relevance of these enzymes in liver physiology and the evidence linking a large number of toxic compounds or drugs to liver disorders. It is reasonable to anticipate that the same events involved in acute toxic liver damage in genetically susceptible individuals[25,26] might also influence the response to repeated, subclinical insults, both exogenous, such as alcohol or drugs, or endogenous, such as metabolites produced by oxidative stress in chronic infections.[33,34] In fact, although exposure to xenobiotic epoxide can occur directly, most instances are generated within the cell by various oxidative enzymes.[35]

Our study considered carefully at the clinical level a large number of subjects representative of all stages of HCV-related liver disease. We addressed 2 different but strictly related issues, progression of chronic hepatitis C and HCC development.

The data showed a significant and independent association between polymorphisms in the mEH coding region and disease severity. Both mEH genotypes and phenotypes associated with reduced enzyme activity were overrepresented among subjects with advanced liver disease compared with uninfected healthy controls and with HCV-infected patients with mild, nonprogressive forms of liver damage (asymptomatic carriers). Interestingly, the frequency of subjects with impairment of enzyme activity was progressively higher in advanced stages of disease (Table 1). At variance with mEH coding sequence polymorphisms, variation in the 5′-flanking region of the gene was not associated with disease expression, either singly or in combination, and no linkage disequilibrium was observed between promoter and coding region alleles. The collected data, therefore, argue against a functional role of 5′-noncoding region polymorphisms. We have no explanation for the relevant discrepancies between the frequencies that we detected in −699 alleles and those reported by Raaka et al.[24] The analysis of these polymorphisms in different populations might be useful to rule out variations related to ethnicity.

It was not unexpected that patient categories at either end of the disease spectrum (carriers and HCC patients) were differentiated more clearly, whereas subjects with intermediate disease severity were more similar to controls. With extreme conditions, in fact, the inability to account for infection duration or viral variables is less likely to limit the interpretation of disease courses, allowing the contribution of genetic traits to emerge more clearly. Male subjects provided the main contribution to differences between patient categories, suggesting that the effect of mEH genetic variation is greater in men. It is known that mEH activities are higher in men[36] and that chronic hepatitis C is more progressive in men than in women, irrespective of other confounding variables.[5] Hormonal regulation of mEH expression or differential exposure of sexes to environmental agents or toxic compounds might account for the observed differences.

The only study that explored mEH variability in patients with chronic liver disease is that by Wong et al.,[37] who found an association of the high-activity allele in exon 4 and the risk of liver disease and HCC in alcoholic patients. Their findings, however, were unexpected and unexplained and admittedly limited by small sample size, variability of disease expression, and choice of controls. These arguments and the different etiological and clinical settings explored make comparisons with our study difficult. A more interesting parallel can be drawn with a re-

cent study indicating an effect of low-activity mEH alleles and the rapid decline of lung function in patients with emphysema.[28] Analogously to our results, in fact, differences were evidenced in the long-term outcome and the timing of disease evolution, as would be expected for the effect of a modifier trait of chronic tissue damage in diseases characterized by prolonged courses, variable clinical expression, and multiple causes.

The comparison between cirrhotic patients with or without HCC indicated that mEH low-activity alleles exerted an independent effect on the risk of cancer, although this was essentially restricted to men. This is in agreement with previous observations performed in HBV-infected subjects.[30] It must be noted, however, that it is difficult to dissect the contributions of this trait to carcinogenesis and disease progression, because, in HCV-infected patients, HCC develops essentially in cirrhosis. The independent role of mEH polymorphisms in cancer risk might suggest the reduced disposal of specific classes of compounds, as previously shown for aflatoxin B1,[30] rather than the generic lack of detoxifying activity.

Environmental factors act in concert with individual and viral traits to cause liver damage in chronic hepatitis C patients, but identification of these factors is difficult when the involved relative risks are low (ORs of 1.5 or even 2). Causal relations may emerge more clearly if the etiologic research is focused on specific subgroups with heightened susceptibility.[38] Therefore, our data might help to target the research for carcinogenetic molecules involved in HCC development in the West, which are virtually unknown. Given the relevance of HCC as the leading cause of death in HCV-infected patients with progressive liver disease and in view of the magnitude of ORs observed between HCC and other patient categories (over 7-fold risk compared with carriers), our results might also be of clinical interest.

Cross-sectional association studies are a crude means of assessing the role of low-penetration genetic traits in the progression of complex diseases,[39] because they are biased by the lack of control for disease duration, disease expression, and timing of diagnosis. Therefore, the present study should be considered exploratory and preliminary, and it certainly warrants confirmation in independent, specifically designed, controlled cohorts after correlation with environmental variables.

Multiple DME genes can modulate the effect of toxic compounds, because they participate in different metabolic pathways or possess complementary activities, and interactions between different genes can result in synergistic effects on risk. We are presently exploring the variability of other phase I and phase II enzymatic activities involved in the process of liver detoxification to reconstruct complex DME haplotypes and to model their contribution in liver disease expression or susceptibility to specific risk factors.

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