Research Article

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Chemokine *RANTES* Promoter Dimorphisms and Hepatocellular Carcinoma Occurrence in Patients with Alcoholic or Hepatitis C Virus–Related Cirrhosis

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Abstract

Background: This study explores the influence of two functional genetic polymorphisms in the regulated on activation in normal T-cell expressed and secreted *(RANTES)* promoter on the risk of hepatocellular carcinoma (HCC) occurrence in patients with alcoholic or Hepatitis C Virus (HCV)-related cirrhosis.

Methods: *RANTES C-28G* and *G-403A* promoter dimorphisms and RANTES serum levels were assessed in 243 HCV-infected patients and 253 alcoholic patients, included at the time of diagnosis of cirrhosis and prospectively followed-up.

Results: During a mean follow-up time of 76 months, 137 (27.6%) patients developed HCC and 170 (34.2%) died or were transplanted. During follow-up, patients with alcoholic cirrhosis and bearing two copies of the *RANTES G-403* variant (*2G-403* genotype, $n = 156/253$) had a higher rate of HCC occurrence compared with patients carrying at least one *RANTES A-403* allele (26.3% vs. 8.2%, $P = 0.0004$). The *RANTES 2G-403* genotype was a risk factor for HCC occurrence [HR = 3.0 (1.3–5.8); first quartile time to HCC occurrence: 60 vs. 120 months; LogRank = 0.007] and death [HR = 1.4 (1.0–2.0); median time to death: 55 vs. 79 months; LogRank = 0.01] in this subgroup. Carriage of the *RANTES 2G-403* genotype was not associated with HCC development or death in patients with HCV-related cirrhosis. The *RANTES C-28G* dimorphism did not influence the occurrence of death or HCC in either cohort of patients.

Conclusion: This study suggests an influence of the chemokine *RANTES G-403A* dimorphism on the occurrence of HCC in patients with alcoholic cirrhosis.

Impact: Our findings provide clues for future studies on *RANTES* gene in relation to HCC susceptibility.
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Introduction

Hepatocellular carcinoma (HCC) is a tumor that slowly develops on a background of chronic inflammation, usually as a consequence of exposure to infectious agents, such as hepatitis C or B viruses, or to chronic excessive ethanol consumption. Differences in the mechanisms of hepatocarcinogenesis according to the cause of liver disease are becoming more clearly known, and a common denominator of the origin of this cancer is the perpetuation of a wound-healing response triggered by parenchymal cell death and inflammation (1).

Chemokines are chemotactic cytokines that bind to G-protein-coupled receptors (2). Chemokines and their receptors are involved in the initial phase of inflammation in the course of acute or chronic liver disease (3–6). Furthermore, a role for chemokines in the promotion of tumor growth, invasion, and metastasis has been also suggested (7, 8), including lymphocyte recruitment in the liver of patients with HCC (9).

A member of the β-chemokine family, the CC-chemokine regulated upon activation in normal T-cell expressed and secreted (RANTES)/CCL5 is a T-cell chemoattractant and an immunoregulatory molecule. Several polymorphisms of genes encoding chemokines have been described, and some are associated with human diseases, including cancers (10). Two *RANTES* promoter polymorphisms (*C-28G* and *G-403A*) have been shown to modify promoter activity, resulting in increased RANTES expression for the *G-28* and *A-403* alleles, respectively (10, 11). Clinical studies have suggested that the *RANTES C-28G* or *G-403A* promoter polymorphisms may genetically influence the course of various cancer diseases in humans such as prostate, pancreas, or oral tumors (12–14). About liver diseases,

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the *RANTES A-403* allele has been associated with lesser portal inflammation in Hepatitis C Virus (HCV)-infected patients, suggesting a possible role for this single-nucleotide polymorphism (SNP) in their prognosis (15, 16).

Given their putative implications in liver inflammation and the carcinogenesis process, the aim of this study was to investigate whether the two most studied functional *RANTES* genetic polymorphisms (namely, the *RANTES C-28G* and *G-403A* promoter dimorphisms) were associated with increased risk of HCC occurrence in two large cohorts of prospectively followed-up patients with cirrhosis.

Patients and Methods

Patients

In the present study, we compiled data from all new patients who were consecutively referred to our liver unit for diagnosis and management of cirrhosis between January 1, 1995 and December 31, 2005, and who fulfilled the following inclusion criteria: (i) biopsy-proven cirrhosis; (ii) no infection by the human immunodeficiency virus or hepatitis B virus; (iii) no evidence of HCC at the time of inclusion, as judged by negative ultrasonographic findings, and a serum α-fetoprotein (AFP) level < 50 ng/mL; (iv) residence in France; (v) acceptance of a regular follow-up and periodical HCC screening; (vi) Caucasian origin; and (vii) written informed consent for the use of frozen DNA.

These patients were divided into two distinct cohorts according to the etiology of their liver disease. The first cohort included patients with alcoholic cirrhosis, who were defined as (i) daily alcohol intake > 80 grams per day, and (ii) no infection by HCV, as defined by negative serum HCV antibodies. The second cohort included patients with HCV-related cirrhosis, who were defined as (i) absence of daily alcohol intake, and (ii) chronic infection by HCV defined by positive serum HCV–RNA. In this study, no patients with mixed alcoholic and HCV-related cirrhosis were enrolled.

For each patient, the date of inclusion was the date of the first liver biopsy showing cirrhosis. Gender, age, presence of ascites or hepatic encephalopathy, serum bilirubin, albumin and prothrombin levels, serum alanine-aminotransferase (ALT) activity, and serum aspartate-aminotransferase (AST) activity, were recorded at inclusion. Daily alcohol intake was recorded by interviewing all patients. Virus genotype was assessed in patients with HCV-related cirrhosis.

All patients were prospectively evaluated at least every 6 months by a physical examination, a liver ultrasonography, and serum AFP levels were measured. When these investigations suggested a possible diagnosis of HCC, computed tomodensitometry, and/or magnetic-resonance imaging, and/or a guided liver biopsy were carried out according to the recommendation of the Barcelona Conference (1). HCC was diagnosed according to one of the following criteria: histologic evidence or convergent demonstration of a focal lesion more than 2 cm in size, and arterial hypervascularization, as assessed by two different imaging techniques, or a combination of one imaging technique that showed this morphologic aspect plus a serum AFP level of 400 ng/mL or more.

The two main end-points were the occurrence of HCC, and the occurrence of death or liver transplantation. Follow-up ended at the date of death or liver transplantation, or at the last recorded visit (or information taken) within the last 6 months before August 31, 2010. This was set as the final time limit for upgrading the patients' file using our computerized data-base, or departmental certificates for patients who died outside our liver unit, or by reaching patients, their relatives or their general practitioner. In patients with HCV-related cirrhosis, antiviral treatment, and sustained virological response (SVR) were recorded.

DNA Extraction, Amplification, and RANTES Genotyping

Genomic DNA was extracted from each patient's peripheral blood mononuclear cells using a MagNA Pure Compact Instrument (Roche Diagnostics).

We genotyped the *RANTES C-28G* (dbSNP: rs 2280788) and *RANTES G-403A* (dbSNP: rs 2107538) polymorphisms by allelic discrimination using fluorogenic probes and the 5′ nuclease (TaqMan) assay. The *RANTES C-28G* and *G-403A* dimorphisms were genotyped using the TaqMan SNP genotyping products C___15874407_10 and C___15874396_20, respectively (Applied Biosystems, Foster City). PCR reactions (25 μl) consisted of 1x TaqMan Universal PCR master mix (Applied Biosystems), 1× assay mix, and 20 ng genomic DNA. Real-time PCR was carried out on a Step One Plus PCR system (Applied Biosystems) using a protocol consisting of incubation at 50°C for 2 minutes and 95°C for 10 minutes, followed by 40 cycles of denaturation at 92°C for 15 seconds, and annealing/extension at 60°C for 1 minute. The FAM and VIC fluorescence levels of the PCR products were measured at 60°C for 1 minute, resulting in the clear identification of all genotypes of *RANTES* on a two-dimensional graph.

ELISA assay

All tests were carried out using frozen serum collected at fasting and stored at –80°C. RANTES sera levels were determined on blood samples collected at inclusion. ELISA assays were conducted in untreated patients without evidence of systemic infection at the time of blood-sample collection, using a Quantikine kit (R&D). The intraassay or interassay CV was 4.8% and 5% for RANTES, respectively.

Statistical analyses

Qualitative variables were compared using Fischer's exact test, the χ^2 test, or the χ^2 trend test with 1 degree of freedom, whereas quantitative variables were compared

Table 1. Characteristics of the studied population according to the cause of liver disease

	Patients with HCV-related cirrhosis $N = 243$ (49%)	Patients with alcoholic cirrhosis $N = 253$ (51%)
Age (years)[a]	53.2 ± 0.8	55.0 ± 0.6
Male gender[b]	132 (54.3%)	185 (73.1%)
Child Pugh score[a]	5.3 ± 0.1	7.3 ± 0.1
Prothrombin activity (%)[a]	82.0 ± 1.0	62.3 ± 1.1
Bilirubin (μmol/L)[a]	15.9 ± 0.6	52.8 ± 4.4
Albumin (g/L)[a]	43.0 ± 5.0	36.8 ±5.3
AST (ULN)[a]	2.2 ± 0.1	2.3 ± 0.1
ALT (ULN)[a]	3.0 ± 0.2	1.4 ± 0.05
RANTES sera levels (pg/mL)[a]	46448 ± 5947	34400 ± 3409
C-28G dimorphism		
CC	230 (94.6%)	243 (96.0%)
CG	13 (5.4%)	10 (4.0%)
GG	0 (0.0%)	0 (0.0%)
G-403A dimorphism		
GG	165 (67.9%)	156 (61.7%)
GA AA	66 (27.1%) 12 (5.0%)	86 (34.0%) 11 (4.3%)
AA	12 (5.0%)	11 (4.3%)
Mean time of follow-up (months)[a]	94.0 ± 2.3	58.8 ± 2.5
HCC[b]	88 (36.2%)	49 (19.4%)
Death[b]	76 (31.2%)	94 (37.1%)
HCC-related	50	37
Liver-related	20	53
Other	6	4

[a]Mean ± SD.
[b]Number (percentage) of patients.

using the nonparametric Wilcoxon test. Multivariate analysis (analysis of variance) was also conducted to compare more than two means. The Kaplan–Meier method was used to estimate the occurrence of HCC for each parameter noted at enrolment; death was considered as an outcome in the experiment. The distribution of death and HCC were compared with the log-rank test. A significant level below 0.10 was used to select the variables in Cox's proportional hazards model, using a stepwise backward procedure with a threshold of $\alpha = 0.05$. Variables associated with the risk of death or HCC based on knowledge and findings from previous studies were also selected. Statistical analyses used the SAS System Package version 8.02 (SAS Institute, Cary, NC). All reported *P* values are two-tailed. Associations were first considered statistically significant at a two-tailed α of 0.05. Bonferroni adjustment was also applied to correct for the number of primary outcomes tested (i.e., for 10 primary outcomes, $\alpha = 0.005$). All reported *P* values are not corrected.

Results

Characteristics of the study population

A total of 496 patients were enrolled in the present study (HCV-related cirrhosis: 243; alcoholic cirrhosis: 253). Their initial characteristics are displayed in Table 1. During a mean follow-up time of 76 months, 137 (27.6%) developed HCC and 170 (34.2%) died or underwent liver transplantation ($n = 26$). Causes of death or transplantation were occurrence and subsequent development of HCC in 87 cases, liver failure in 73 cases, or extrahepatic cause in 10 cases. Genotype distribution analyses revealed that only a few patients [23 (4.6%)] were carriers of two copies of the *RANTES A-403* variant and none were *RANTES 2G-28* homozygotes.

Influence of *RANTES G-403A* dimorphism on the risks of hepatocellular carcinoma occurrence and death

All genotype distributions were within Hardy–Weinberg equilibrium expectations (Table 2 and Table 3). Because only 23 patients were *RANTES 2A-403* homozygotes (Table 1), we pooled those patients with *RANTES GA-403* heterozygotes (namely, *RANTES 1-* or *2A-403* genotype) into a single subgroup for further analyses (Table 2 and Table 3). Baseline characteristics that estimated the severity of liver disease as well as demographic data were similar among the carriers of each genotype. We did not observe any association between the *RANTES G-403A* dimorphism and the baseline chemokine sera

Table 2. Characteristics and outcome of 243 patients with HCV-related cirrhosis classified according to their G-403A genotypes

	RANTES 2G-403 genotype *N* = 165 (67.9%)	RANTES 1 or 2A-403 genotype *N* = 78 (32.1%)	*P*
Age (years)[a]	56.1 ± 1.0	56.6 ± 1.4	0.9
Male gender[b]	86 (52.1%)	46 (58.9%)	0.3
Child Pugh score[a]	5.2 ± 0.1	5.3 ± 0.1	0.4
Ascites[b]	2(1.2%)	2 (2.5%)	0.4
Prothrombin activity (%)[a]	82.4 ± 1.1	81.8 ± 1.8	0.9
Bilirubin (μol/L)[a]	15.8 ± 0.7	16.0 ± 0.9	0.8
Albumin (g/L)[a]	42.1 ± 0.4	41.6 ± 0.6	0.5
AST (ULN)[a]	2.2 ± 0.1	2.1 ± 0.1	0.3
ALT (ULN)[a]	3.1 ± 0.2	2.6 ± 0.1	0.1
RANTES sera levels (pg/mL)[a]	33223 ± 2100	34465 ± 4819	0.3
HCV Genotype 1[b]	125 (75.7%)	57 (73.0%)	0.7
Anti-viral treatment[b]	117 (70.9%)	50 (64.1%)	0.3
Sustained virological response[b]	37 (22.4%)	16 (20.0%)	0.7
HCC[b]	56 (33.9%)	32 (41.0%)	0.3
Death[b]	45 (27.3%)	31 (39.7%)	0.03

[a]Mean ± SD.
[b]Number (percentage) of patients.

levels ($n = 207$ and 203 for HCV-infected and alcoholic cirrhotic patients, respectively).

Patients with hepatitis C virus–related cirrhosis

During follow-up, the same proportion of patients with HCV-related cirrhosis achieved sustained virological response (SVR) whatever their *RANTES GA-403* genotypes (Table 2). Although a slightly higher proportion of HCC was observed in patients bearing at least one *RANTES A-403* allele, this difference was not statistically significant. In addition, using the Kaplan–Meier method, the *RANTES G-403A* dimorphism did not influence the risk of HCC occurring (Fig. 1A). When considering the number of deaths, this same subgroup of patients had a higher rate of death (Table 2), but this association was not confirmed by the Kaplan–Meier

Table 3. Characteristics and outcome of 253 patients with alcoholic cirrhosis classified according to their G-403A genotypes

	RANTES 2G-403 genotype *N* = 156 (61.7%)	RANTES 1 or 2A-403 genotype *N* = 97 (38.3%)	*P*
Age (years)[a]	57.2 ± 0.9	55.6 ± 1.1	0.5
Male gender[b]	113 (72.4%)	72 (74.2%)	0.7
Child Pugh score[a]	7.6 ± 0.2	8.1 ± 0.3	0.2
Prothrombin activity (%)[a]	64.1 ± 1.5	59.1 ± 2.0	0.04
Bilirubin (mol/L)[a]	51.5 ± 5.7	55.1 ± 7.0	0.4
Albumin (g/L)[a]	35.9 ± 0.6	34.6 ± 0.6	0.1
AST (ULN)[a]	2.3 ± 0.1	2.2 ± 0.1	0.6
ALT (ULN)[a]	1.5 ± 0.1	1.4 ± 0.1	0.9
RANTES sera levels (pg/mL)[a]	37252 ± 2672.0	31549 ± 3035.0	0.3
Ascites[b]	99 (63.5%)	62 (63.9%)	0.9
Alcohol abstinence[b]	73 (46.9%)	52 (53.1%)	0.4
HCC[b]	41 (26.3%)	8 (8.2%)	0.0004
Death[b]	65 (41.6%)	29 (29.9%)	0.1

[a]Mean ± SD.
[b]Number (percentage) of patients.

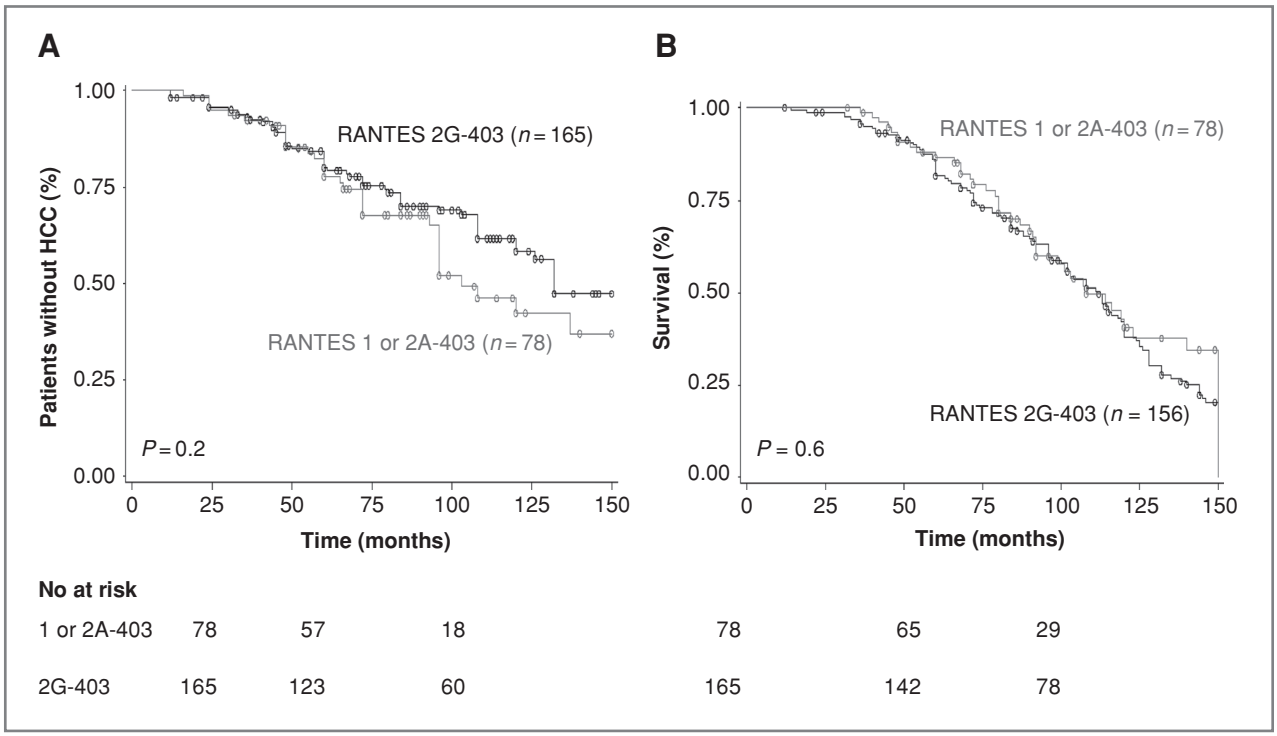

Figure 1. Influence of *RANTES G-403A* dimorphism on the risk of HCC and occurrence of death according to the Kaplan–Meier method in patients with HCV-related cirrhosis. (A) The *RANTES G-403A* dimorphism did not influence the risk of HCC occurrence: HR = 1.3 (0.8–2.0); first quartile time to HCC occurrence: 66 vs. 79 months; LogRank = 0.2. (B) Similarly, the *RANTES G-403A* dimorphism did not influence the risk of death: HR = 0.8 (0.6–1.1); first quartile time to HCC occurrence: 72 vs. 80 months; LogRank = 0.3.

method when considering all causes of death [HR = 0.8 (0.6–1.1); LogRank = 0.3, Fig. 1B], or death caused by liver failure [HR = 0.8 (0.3–2.0)]; LogRank = 0.6) without HCC. Also, RANTES sera levels did not differ according to *RANTES G-403A* dimorphism, and did not influence the risks of HCC or death occurring in this cohort.

Patients with alcoholic cirrhosis

During follow-up, patients bearing two copies of the *RANTES G-403* variant (*2G-403* homozygotes) had a higher rate of HCC occurrence when compared with patients bearing at least one *RANTES A-403* allele ($P = 0.0004$, Table 3). In addition, using the Kaplan–Meier method, the *RANTES 2G-403* genotype was a risk factor associated with the occurrence of HCC [HR = 3.0 (1.3–5.8); LogRank = 0.007, Fig. 2B]. Similarly, the same subgroup of patients also had nonsignificant higher rates of death (41.6% vs. 29.9%, $P = 0.06$), which were, however, statistically relevant when considering their occurrence over time using the Kaplan–Meier method [HR = 1.4 (1.0–2.0); LogRank = 0.01, Fig. 2B]. When taking into account the specific causes of death, there was no association between the *RANTES G-403A* dimorphism and the probability of death related to liver failure without HCC [HR = 1.1 (0.6–1.9), LogRank = 0.7]. As observed in patients with HCV-related cirrhosis, RANTES sera levels did not differ according to *RANTES G-403A* dimorphism and were not associated with HCC or death during follow-up.

In multivariate analyses, the *RANTES 2G-403* genotype, in patients with alcoholic cirrhosis, remained an independent risk factor for HCC along with older age and male gender (Table 4). Conversely, this genetic trait was not selected for by the Cox model in patients with HCV-related cirrhosis, in which clinical and virological features were the only variables associated with HCC (Table 4).

Lack of influence of *RANTES C-28G* dimorphism on the risks of occurrence of hepatocellular carcinoma and death

Genotype distributions were within Hardy–Weinberg equilibrium expectations. As no patients included in this study were *RANTES 2G-28* homozygotes (Table 1), all comparisons were made between *RANTES 2C-28* homozygotes and *RANTES CG-28* heterozygotes. Baseline characteristics that estimated the severity of liver disease, as well as the demographic data or chemokine sera levels, were similar among carriers of each genotype in both cohorts.

When considering patients with HCV-related cirrhosis, the rates of HCC and death occurrence were similar in

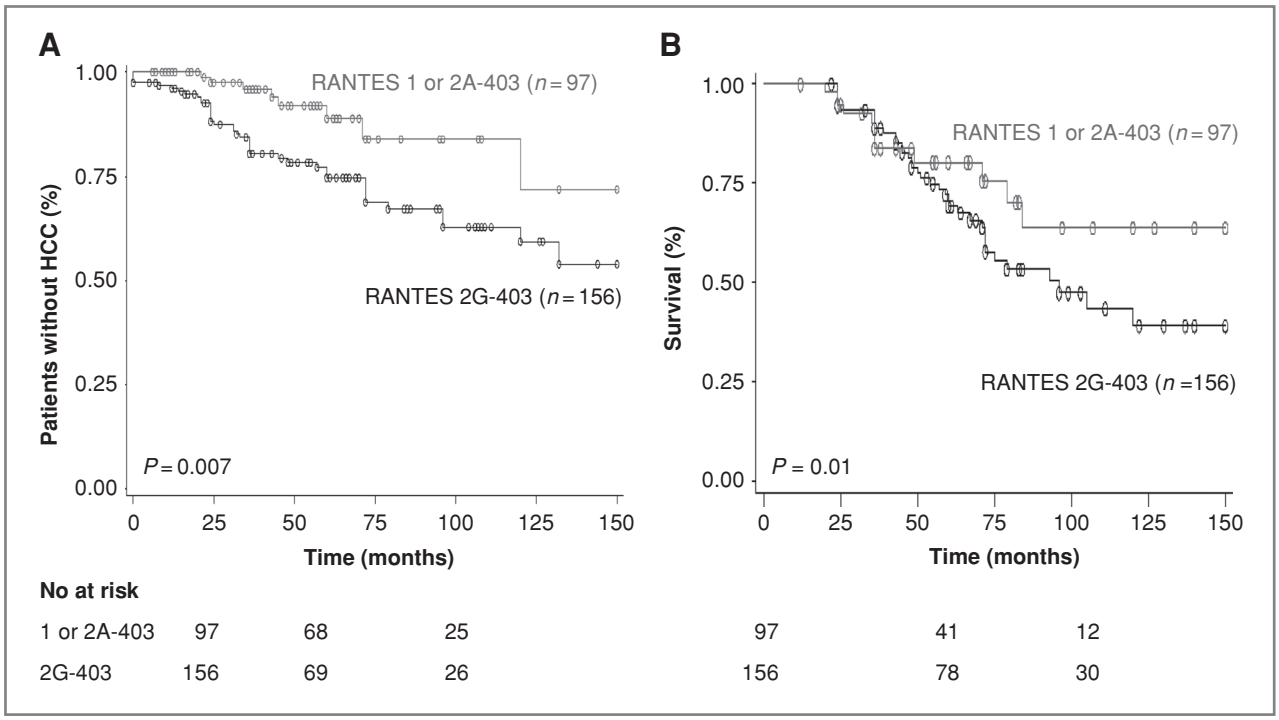

Figure 2. Influence of the *RANTES G-403A* dimorphism on the risk of HCC and occurrence of death according to the Kaplan–Meier method in patients with alcoholic cirrhosis. (A) Compared with having one or two *A-403 RANTES* allele(s), the *RANTES 2G-403* genotype was a risk factor for the occurrence of HCC: HR = 3.0 (1.3–5.8); first quartile time to HCC occurrence: 60 vs. 120 months; LogRank = 0.007. (B) Compared with having one or two *RANTES A-403* allele(s), the *RANTES 2G-403* genotype was also a risk factor for death: HR = 1.4 (1.0–2.0); median time to death: 55 vs. 79 months; LogRank = 0.01

RANTES 2C-28 homozygotes and *CG-28* heterozygotes. The Kaplan–Meier method confirmed the lack of influence of the *RANTES C-28G* dimorphism on the risk of HCC occurrence (LogRank = 0.8) or death during follow-up (LogRank = 0.5).

In addition, the rates of HCC or death did not differ according to *RANTES C-28G* dimorphism in alcoholic patients. Using the Kaplan-Meier method, there were no differences in the occurrence of HCC ($P = 0.7$) or death ($P = 0.2$) according to this SNP.

Table 4. Features associated with the risk of HCC occurrence according to Cox's proportional hazards model

	Features associated with the risk of HCC occurrence in patients with alcoholic cirrhosis		Features associated with the risk of HCC occurrence in patients with HCV-related cirrhosis	
	Cox univariate analyses	**Cox multivariate analyses**	**Cox univariate analyses**	**Cox multivariate analyses**
Age	1.06 (1.03–1.09) $P < 0.0001$	1.06 (1.04–1.09) $P < 0.0001$	1.03 (1.01—1.05) $P < 0.0001$	1.04 (1.02–1.06) $P < 0.0001$
Male gender	6.3 (1.9–20.3) $P = 0.004$	7.7 (2.3–25.0) $P = 0.0007$	1.5 (1.0–2.4) $P = 0.04$	2.3 (1.5–3.7) $P = 0.002$
Platelet count	1.001 (0.998–1.005) $P = 0.4$	_____	0.996 (0.992—1.000) $P = 0.02$	_____
Child-Pugh score	0.9 (0.8–1.1) $P = 0.8$	_____	1.2 (1.0–2.4) $P = 0.04$	_____
SVR	NA	NA	0.4 (0.2–0.8) $P = 0.008$	0.4 (0.2–0.9) $P = 0.03$
RANTES 2G-403 genotype	2.7 (1.2–5.8) $P = 0.007$	3.3 (1.5–7.1) $P = 0.002$	1.3 (0.8–2.0) $P = 0.1$	_____

Abbreviation: NA, not applicable

Discussion

The decision to consider homogenous groups of patients was made according to previous results that showed discrepancies in predictive factors for HCC according to the nature of the underlying liver disease (17–21). Our study was thus restricted to the two main causes of cirrhosis in Western countries, namely excessive alcohol consumption and HCV infection. Our results are consistent with an influence of genetic heterogeneity modulating RANTES chemokine expression for the risk of HCC occurring in Caucasian patients with cirrhosis. Indeed patients bearing two *G-403* alleles had higher incidences of HCC and death, although this association was only significant in patients with alcoholic cirrhosis (Fig. 1 and Fig. 2).

RANTES/CCL5, secreted either from tumoral or stromal cells, may act in an autocrine or a paracrine manner on cancer cells to enhance their motility and invasion (22–24). Nevertheless, controversy still exists about the role of RANTES in tumor development. Indeed, Manes and colleagues reported that RANTES induced transcriptional activation of the tumor suppressor p53 and its target genes (8). Although the respective contribution of cancerous cells or tumor microenvironment in its synthesis remains unclear, RANTES production restricted tumor growth by inducing expression of functional p53 in tumor cells, in a CCR5-dependent manner (8). In addition, some human studies have reported that increased RANTES expression may be associated with favorable outcomes in some cancer diseases (25, 26). About the role of RANTES in HCC development, animal experiments reported a contribution of the RANTES G-protein coupled receptor, CCR1, to the growth and progression of HCC (27). Our team has also previously shown that RANTES can promote metastasis and invasion of the HCC cell line, Huh7, through CCR1 (28). According to the aforementioned studies, the fact that patients who carried two copies of the *G-403* alleles had higher incidences of HCC is quite surprising. Indeed transient transfection of human cell lines with reporter vectors driven by the *A-403* variant of the *RANTES* promoter resulted in 8-fold higher constitutive transcriptional activity as compared with that of the *G-403* promoter (16). A counterintuitive role of RANTES in the development of gastric tumors has also been reported. Indeed, carriage of the A-403 allele has been associated with reduced risk of gastric cancer in women, whereas the chemokine secreted by gastric-cancer cell lines augmented their proliferation and migration through an autocrine manner (25, 29). In line with our data, these controversial results highlight the complex involvement of this chemokine in cancer development.

Another challenging issue is the observed interaction between alcohol-related liver disease and the genetic heterogeneity modulating RANTES expression. Similarly, the *RANTES G-403A* promoter dimorphism has been reported as a genetic factor influencing the risk of chronic pancreatitis in American cohorts (13), a precancerous condition mainly related to excessive alcohol intake. An evidence of a gene-environment effect is however difficult to address as the quantification of chronic alcohol consumption is usually assessed in such cohorts (including ours) during medical interview, a potential source of major bias. Nevertheless, chronic ethanol consumption is characterized by an increase in the expression of a number of inflammatory cytokines. TNFα-induced protein and mRNA expression of RANTES in human hepatoma cells, possibly suggests that TNF plays a pivotal role in migration of inflammatory cells by RANTES to the liver in patients with alcoholic liver disease (30).

Of note, no association was found between the two *RANTES* polymorphisms under study and the baseline serum levels of RANTES in this population. However, this finding does not rule out the phenotypic consequences of both SNPs. Furthermore, other serum compounds may affect RANTES effects, such as the protease DPP-IV/CD26 (31–33), leading to a truncated chemokine antagonist with altered chemotactic properties. However, cirrhotic patients may not be the most suitable population to study the association of SNPs with serum levels of RANTES and/or DPP-IV, as liver-function impairment may affect circulating levels.

The usual limitations observed in clinical studies that aim to assess putative risk factors for HCC have been avoided by conducting this study in prospectively followed-up cohorts of patients with cirrhosis. A specific link between the occurrence of liver cancer and the *RANTES 2G-403* genotype is further supported when its influence on survival in this cohort is taken into account; indeed, these patients also had a higher probability of death that was not attributable to non-HCC liver-related death, as emphasized by the cause-specific mortality analysis.

In summary, the results of this study suggest that the genetic heterogeneity that modulates RANTES expression may influence alcoholic hepatocarcinogenesis. Further studies are needed to clarify the controversial role of this chemokine in the progression of hepatic injury, its involvement in inflammation and the hepatocarcinogenic process, as well as the genetic or environmental factors that seem to influence its complex regulation.

Disclosure of Potential Conflicts of Interest

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