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C4BQ0: a genetic marker of familial HCV-related liver cirrhosis

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Abstract

Background and methods. Host may have a role in the evolution of chronic HCV liver disease. We performed two cross-sectional prospective studies to evaluate the prevalence of cirrhosis in first degree relatives of patients with cirrhosis and the role of two major histocompatibility complex class III alleles BF and C4 versus HCV as risk factors for familial clustering.

Findings. Ninety-three (18.6%) of 500 patients with cirrhosis had at least one cirrhotic first degree relative as compared to 13 (2.6%) of 500 controls, (OR 7.38; CI 4.21–12.9). C4BQ0 was significantly more frequent in the 93 cirrhotic patients than in 93 cirrhotic controls without familiarity (Hardy–Weinberg equilibrium: χ^2 5.76, $P = 0.016$) and in 20 families with versus 20 without aggregation of HCV related cirrhosis (29.2% versus 11.3%, $P = 0.001$); the association C4BQ0-HCV was found almost only in cirrhotic patients with a family history of liver cirrhosis.

Conclusions. Our studies support the value of C4BQ0 as a risk indicator of familial HCV related cirrhosis.

Keywords: C4BQ0; Familial clustering; HCV diffusion; Liver cirrhosis; MHC class III alleles

1. Introduction

There is little data on the familial clustering of cirrhosis and on its relationship with the known etiological factors of the disease.

In a large multicenter case-control study about the risk factors of cirrhosis [1], familiarity of chronic liver disease was found to be significantly more frequent in cirrhotic patients than in controls (141/1058 versus 62/1408; OR 3.02, CI 2.4–4.3, data not published). In a prospective study of the natural history of cirrhosis in progress at our unit, 73 out of 494 consecutive patients with cirrhosis (15%) had at least one first degree relative with cirrhosis [2].

There is evidence that host genetic factors play a major role in determining the outcome of HCV infection. I and II class major histocompatibility complex (MHC) alleles are involved in the clearance of HCV infection and its long term outcome [3–8]. The role of class III MHC alleles is well established in autoimmune hepatitis and primary biliary cirrhosis [9] but there is little data on how they affect the clinical course of HCV infection [10]. We report here two prospective studies. The first, assessing the prevalence of cirrhosis among first degree relatives of cirrhotic patients and the prevalence of class III MHC C4 and BF alleles among patients with familial cirrhosis; the second, investigating the relationship between C4B allele deficit and HCV infection as a risk indicator for familial aggregation.

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2. Methods

2.1. First study: familiarity of cirrhosis

2.1.1. Patient recruitment

Between January 1994 and December 1997, 500 unrelated patients with virus and/or alcohol related cirrhosis and 500 unrelated hospital controls with chronic non-hepatic disease, pair matched for sex, age (±5 years) and time of admission (±6 months), were enrolled at "V. Cervello" Hospital in Palermo. The study was approved by the local ethics committee and all the patients gave their consent to participate. Patients with metabolic causes of cirrhosis were excluded. The diagnosis of cirrhosis was biopsy-proven in 215 patients and based on the presence of ascites and/or oesophageal varices on endoscopy and/or signs of portal hypertension on ultrasound and compatible biochemistry and physical signs in the remaining 285 patients. Child–Pugh classification was as follows: 295 (59%) A, 170 (34%) B and 35 (7%) C. All cirrhotic patients with a diagnosis of hepatocellular carcinoma were excluded. Relevant clinical data and a complete family pedigree including number, age and sex of first degree relatives were recorded on predefined standard forms. Requested information on first degree relatives included (a) survival status and, if deceased, age at death; (b) age and sex of parents, siblings and children; (c) whether a diagnosis of liver cirrhosis had been made in family members. For diagnostic verification secondary cases were traced first by searching in our extensive files of patients admitted before and during the study and in secondary cases not found in our file, the diagnosis of cirrhosis was accepted only when supported by medical records or discharge letters from other hospitals, or by death certificates.

2.1.2. Cirrhosis in relatives

The prevalence of cirrhosis among relatives was calculated for all the exposed relatives and separately for parents, siblings and offsprings. The number of exposed relatives was adjusted according to the Strömgren's method modified by Tillil [11] by age at diagnosis: the distribution of patients according to the age at diagnosis of cirrhosis was obtained from a cohort of 494 consecutive patients included between 1981 and 1984 in a prospective study of the natural history of cirrhosis [12] still in progress at our department. Age groups were compared with the corresponding groups in the Sicilian population in 1996 [13].

2.1.3. Genetic markers

The allele distribution of the two class III MHC genes, C4 and BF, was investigated in all cirrhotic patients with familiarity of cirrhosis (CASE families) and, as controls, in an identical number of cirrhotic patients without familiarity (CONTROL families), pair-matched for sex, age (±5 years) and etiology and in a cohort of 235 healthy blood donors (BD) consecutively observed during the time of the first study. No demographic differences were present between the 93 pair-matched cirrhotic patients without familiarity, used as controls, and the remaining 314 not included in the analysis. No information was available on whether the 235 healthy blood donors had a family history of cirrhosis or not. Allomorphisms of C4 and BF were studied by high-voltage agarose gel electrophoresis and immunofixation [14]. The distribution of C4 null alleles was obtained using the Hardy–Weinberg equilibrium. A step-down multi-variable regression analysis by the logistic model including 13 predefined variables was performed to explore the role of C4 null alleles in familial clustering of cirrhosis, independently from etiology, severity of the disease and demographic characteristics of index cases. The selected variables were: sex, age, age at diagnosis, alcohol abuse, HCV, HBsAg, Child-Pugh score, C4A3, C4AQ0, C4B1, C4BQ0, BFS and BFF.

2.2. Second study: C4B allele deficiency and HCV infection in familial cirrhosis

2.2.1. Family recruitment

Since the first study showed that C4B null allele is a possible risk indicator of cirrhosis mostly HCV related, we investigated the prevalence of this null allele (C4BQ0) and its relationships with HCV infection in two sets of families in which probands had HCV related liver cirrhosis. Only 20 families with at least one cirrhotic and one healthy sibling (CASE families) were found in the first study; these families and, as controls, 20 with at least two healthy siblings (CONTROL families) and no other first degree relative with cirrhosis were included in this second study. These control families are the first 20 families who gave consent to be enrolled in the study.

2.2.2. Diagnostic work-up

To identify first degree relatives with cirrhosis all the participants in the study underwent the following assessment: physical examination particularly aimed at searching for signs of liver disease (liver and spleen size and consistency, spider naevi, palmar erythema, ascites, edema, abdominal wall collateral veins); blood testing for AST/ALT, anti HCV and HBsAg. Anti HCV was determined by a second generation enzyme–linked immunoabsorbent assay (ELISA II- Ortho Diagnostic System, Raritan, NJ). HCV-RNA was tested using reverse transcriptase-polymerase chain reaction (RT-PCR) with primers based on the 5′ untranslated region (UTR) of the genome. Genotyping was performed by RT-PCR with type specific primers according to Okamoto [15]. HBsAg was detected using a commercially available ELISA test (Abbott Diagnostic North Chicago); in HBsAg positive relatives HBV-DNA was detected by quantitative PCR (Amplicor HBV monitor^{TM} test, Roche Diagnostic System). All patients and relatives were also screened for anti nuclear (ANA), anti mitochondria (AMA) and anti smooth muscle autoantibodies (ASMA). Serum ceruloplasmin was determined in subjects aged less than forty years.

Table 1
Familiarity of cirrhosis in patients with cirrhosis and controls ($n = 500$)

	No. of patients	Median age (range)	Male sex (%)	Alcohol ≥80 g/die, ≥5 yrs (%)	Anti-HCV + ve alone (%)	HBsAg + ve (%)	>1 Risk factor (%)[a]
Patients with cirrhosis							
CONTROL-families	407	57 (13–88)	212 (52.0)	56 (13.8)	313 (76.9)	27 (0.7)	51 (12.5)
CASE-families	93	58 (15–87)	51 (54.8)	9 (0.9)	76 (81.7)	6 (0.6)	11 (11.8)
Controls[b]							
CONTROL-families	487	54 (19–85)	285 (58.5)	4 (0.9)	NT[c]	NT	NT
CASE-families	13	55 (16–8)	5 (38.5)	1 (7.7)	NT	NT	NT

[a] Anti-HCV+ve and HBsAg+ve and/or alcohol ≥80 g/day for ≥5 years.
[b] Distribution percentage of chronic diseases among controls: absolute frequencies and (percentages): chronic pulmonary disease: 155 (31), inflammatory bowel disease: 80 (16); cancer: 75 (15); chronic heart disease: 65 (13); metabolic-endocrine diseases: 30 (6); Others (mostly associated diseases): 95 (19).
[c] NT: not tested for anti HCV and HBsAg.

Relatives with physical signs of cirrhosis or with virus hepatitis infection markers and/or hypertransaminasemia were submitted for further laboratory tests (repeat AST/ALT, bilirubin, alkaline phosphatase, prothrombin time, albumin, gamma-globulins, full blood count) and abdominal ultrasound; when liver disease was suspected a liver biopsy was suggested. Allomorphism of C4 genes was studied in all cirrhotic patients to validate the results of the first study.

3. Results

3.1. First study: familiarity of cirrhosis

The principal characteristics of patients and controls are reported in Table 1. Ninety-three (18.6%) of 500 patients with cirrhosis had at least one first degree relative with cirrhosis, as compared to 13 (2.6%) of 500 controls without (OR 7.38; 95% CI 4.21–12.9). Sixteen of the 93 cirrhotic patients with familiarity had more than one first degree relative with cirrhosis so that the total number of first degree relatives with cirrhosis was 123. Three of the 13 controls with first degree cirrhotic relatives had more than one cirrhotic relative. No significant differences in HCV, HBsAg, alcohol abuse, male gender and age were found between cirrhotic patients with and without familiarity of cirrhosis (Table 1).

3.1.1. Disease risk in first degree relatives

Prevalence of cirrhosis among first degree relatives was 2.7% (123/4485) for cirrhotic patients and 0.4% (16/4086) for controls, (OR 6.84; 95% CI 4.0–11.5). First degree cirrhotic relatives of cirrhotic patients were 81/1997 (4.0%) siblings, 36/1000 (3.6%) parents, 6/1488 (0.4%) offspring. First degree relatives with cirrhosis in controls were 8/500

Table 2
Strömgren's method of age correction for cirrhosis

1	2	3	4	5	6	7	8
No.	Age group	a_N	b_N ($\times 10^4$)	$c_N = a_N/b_N$	Relative risk d_N (%)	Cumulative relative risk $e_N = \sum d_N$(%)	Correction factor
1	0–9	2	7.03	0.28	0.15	0.15	0.00075
2	10–14	5	3.88	1.29	0.69	0.84	0.00495
3	15–19	6	4.34	1.38	0.74	1.58	0.0121
4	20–24	12	4.40	2.73	1.46	3.05	0.0231
5	25–29	10	4.40	2.27	1.22	4.26	0.0365
6	30–34	18	3.72	4.84	2.59	6.86	0.0556
7	35–39	38	3.36	11.31	6.07	12.92	0.0988
8	40–44	43	3.16	13.61	7.30	20.22	0.1656
9	45–49	77	2.58	29.84	16.01	36.23	0.2822
10	50–54	89	2.84	31.34	16.81	53.03	0.4463
11	55–59	74	2.74	27.01	14.48	67.52	0.6027
12	60–64	49	2.69	18.22	9.77	77.28	0.7240
13	65–69	28	2.43	11.52	6.18	83.46	0.8038
14	70–74	24	1.39	17.27	9.26	92.72	0.8812
15	75–79	14	1.42	9.86	5.29	98.01	0.9537
16	≥80	5	1.35	3.70	1.99	100.00	0.9901
Total		494	517.2	186.47	100		

Col. 3, a_N, Number of patients with cirrhosis in the age at diagnosis class, given by D'Amico and co-workers [12]. Col. 4, b_N, average age structure of population of Sicily in 1996 [13]. Col. 6, $d_N(\%) = c_N \times 100/\sum c_N$. Col. 8, factor of age correction for relatives in respective age group = mean of two consecutive cumulative relative risks from Col.7, divided by 100.

Table 3
Age-corrected empirical risk estimates of cirrhosis for first degree relatives of probands with cirrhosis and controls

Exposed relatives	No. of first degree relatives with cirrhosis/No. of corrected exposed (observed number of subjects before correction)		
	In the families of cirrhotics	In the families of controls	Odds ratio[a] (95% CI)
Parents	36/806 (1000)	8/760 (1000)	4.8 (0.9 to 9.6)
Siblings	81/1093 (1997)	7/931 (1789)	13.4 (1.7 to 25.0)
Offsprings	6/172 (1488)	1/191 (1297)	11.5 (20.8 to 43.9)
Total	123/2071 (4485)	16/1882 (4086)	8.0 (5.6 to 9.7)

[a] Using the corrected no. of exposed subjects after age correction.

fathers (1.6%) and 8/3586 remaining relatives (0.2%). Sources of validation of the diagnosis of cirrhosis in the secondary cases in the families of index cases and controls were respectively the following: 57 and 7 were traced in our files and were diagnosed according to the criteria used for index cases; 33 and 4 were diagnosed in other hospitals, as reported in medical records or discharge letters and in 34 and 5 the diagnosis was reported in the death certificate. The age corrected risk estimates of cirrhosis for parents, siblings, and offspring was calculated by correction factors for each age class (Table 2), according to the Strömgren's method (Table 3). The age-corrected risk estimates of cirrhosis for first degree relatives of cirrhotic patients were: 81/1093 (7.4%) for siblings, 36/806 (4.5%) for parents and 6/172 (3.5%) for offspring; overall 123/2071 (5.9%). The corresponding figures for the control group are reported in Table 3: overall the age-adjusted prevalence of first degree cirrhotic relatives was 0.8% (16/1882). The age-adjusted OR for familiarity of cirrhotic patients versus controls was 8.0 (95% CI: 5.6–9.7).

3.1.2. Genetic markers in patients with cirrhosis

C4A and BF allele frequencies were found to be similar in the 93 cirrhotic patients with familiarity, 93 cirrhotic patients without familiarity and 235 blood donors. However the distribution of C4B alleles showed a significantly higher prevalence of null alleles among CASE-families as compared to CONTROL families and blood donors. According to the Hardy–Weinberg equilibrium χ^2, was 5.76; $P = 0.016$ (Table 4). A total of 33 out of 93 patients with familiarity had C4B allele deficit (eight were homozygous) and were HCV positive versus 15 out of 93 patients without familiarity (one was homozygous).

C4BQ0 allele distribution was also found to be the only variable independently associated with familial clustering of cirrhosis (OR 2.7; 95% CI = 1.83–3.96) in a step-down multi-variable regression analysis by the logistic model, including the 13 predefined variables reported in Section 2.

3.2. Second study: C4B allele deficiency and HCV infection in familial cirrhosis

The number of first degree relatives in CASE families was 148 (median 7, range 4–15) and 139 in CONTROL families (median 6, range 4–14); 18 first degree relatives in CASE-FAMILIES and 24 in CONTROL families did not participate in the study: 23 of them were residing out of Palermo, and 19 did not consent. A total of 130 subjects in CASE-families (20 case patients and 110 relatives) and 115 in CONTROL-families (20 control patients and 95 relatives), participated in this study.

There were 23 first degree relatives with cirrhosis in the 20 CASE families and obviously no case of cirrhosis in the 20 CONTROL families. Therefore, there were 43 cirrhotic (29 Child–Pugh class A, 8 class B, and 6 class C) subjects in CASE-families and 20 (14 Child-Pugh class A, 4 B, and 2 C) in CONTROL-families. Only one sister in CONTROL-families, with normal AST/ALT, was found to be HBsAg positive and HBV-DNA negative. In all cases, normal levels of ceruloplasmin were found; autoantibodies prevalence was similar in the two groups: 17.3% in case families (ANA 17.1%, ASMA 2.0%) versus 15.0% in control

Table 4
C4B phenotypes observed and predicted by the Hardy–Weinberg equilibrium in cirrhotic patients with (CASE-families) and without (CONTROL-families) first degree relatives with cirrhosis and in blood donors

	Patients with cirrhosis ($n = 186$)				Blood donors ($n = 235$)	
	CASE-families = 93		CONTROL-families = 93			
	Observed n (%)	Expected n (%)	Observed n (%)	Expected n (%)	Observed n (%)	Expected n (%)
Homozygous C4BQO	8 (8.6)	4 (4.8)	1 (1.1)	1 (1.1)	5 (2.1)	3 (1.3)
Heterozygous C4BQ0	25 (26.8)	33 (34.5)	14 (15.1)	15 (16.1)	39 (16.6)	43 (18.6)
No. C4BQ0	60 (64.6)	56 (60.7)	78 (83.8)	77 (82.8)	191 (81.3)	189 (80.1)
χ^2	5.76		0.071		1.94	
P-value	0.016		0.789		0.20	

Table 5
C4BQ0 and HCV in patient with cirrhosis, first degree relatives without cirrhosis and whole families with (CASE-families) and without (CONTROL-families) familial cirrhosis

		HCV+	*P*-value*	C4BQ0 (homozygous)	*P*-value*	HCV+ and C4BQ0	*P*-value*
Patients with cirrhosis							
CASE-families	20 + 23	43	ns	16 (3)	0.01	16	0.0001
CONTROL-families	20	20		2 (0)		1	
Relatives without cirrhosis							
CASE-families	87	16	0.001	22 (4)	0.01	0	ns
CONTROL-families	95	2		11 (0)		1	
Whole families							
CASE-families	130	59	0.0001	38 (7)	0.01	16	0.001
CONTROL-families	115	22		13 (0)		2	

* χ^2 test: only P values ≤ 0.0125 were considered as statistically significant according to Bonferroni's adjustment for multiplicity of tests [16].

families (ANA 15.0%, ASMA 0%), P value not significant. No AMA positive subjects were found in either group.

C4BQ0 and HCV distribution in the two sets of families are reported in Table 5.

3.3. C4BQ0 allele prevalence and familial spreading of HCV

Null C4B alleles were significantly more frequent in CASE than in CONTROL family subjects (38/130 versus 13/115, $P = 0.001$). Overall 59/130 subjects in case families were anti-HCV positive (49 HCV-RNA positive) as compared to 22/115 in CONTROL families (19 HCV-RNA positive): *P*-value for anti-HCV = 0.00001 and for HCV-RNA = 0.0002. All the cirrhotic subjects either in CASE ($n = 43$) or in CONTROL families ($n = 20$) were anti-HCV positive. Among non-cirrhotic subjects anti-HCV positive were 16/87 (13 HCV-RNA positive) in CASE and 2/95 (1 HCV-RNA positive) in CONTROL families: the *P*-value for anti-HCV = 0.0002 and for HCV-RNA = 0.0004.

Genotyping was performed for all 68 HCV-RNA positive subjects: 1b was the only genotype found.

A total of 16/130 (12.3%) subjects in CASE families were anti-HCV positive and had null alleles for C4B as compared to 2/115 (1.7%) in CONTROL families ($P = 0.0009$) and 18/19 subjects with the association of C4BQ0 and anti HCV positivity had cirrhosis; this association was found in only one healthy first degree relative.

Therefore the CASE families had a higher prevalence of C4 null alleles and HCV positivity: both markers were associated almost exclusively in patients with cirrhosis.

4. Discussion

The aim of the present two studies was to investigate the familial clustering of cirrhosis and the role of C4 alleles and HCV as risk factors for this aggregation.

In the first study we found that 18.6% of patients with cirrhosis have at least one first degree relative with cirrhosis, confirming the results of three previous studies from Italy [1,17,18]. However the most important finding of this study was that the age adjusted risk of cirrhosis among the first degree relatives of cirrhotic patients was 5.9% as compared to 0.8% for first degree relatives of controls, corresponding to an odds ratio of 8.0 (CI 5.6 – 9.7). This information was previously unknown and suggests that it would be important to aim at the prevention or at least at the early diagnosis of cirrhosis in the families of cirrhotic patients.

The significantly higher prevalence of C4BQ0 among index cases with familiarity of cirrhosis mostly HCV related, suggests a possible role of this MHC class III antigen as genetic marker of familial aggregation of cirrhosis and leads to the hypothesis of a genetic predisposition to liver cirrhosis.

Many studies on familial spreading of HCV are available from the literature. In a cumulative analysis of Italian studies recently published [19–22] the HCV pooled prevalence among 1796 first degree relatives was 2.7% (range 1.7–3.8%). More recently in a systematic review including 11 controlled studies [23], the pooled prevalence of anti-HCV positivity was 4.0% among adult siblings and no-sexual contacts of patients with HCV-related chronic liver disease, as compared with none in negative controls.

The second study explored this hypothesis in families of patients with HCV related cirrhosis. As expected anti-HCV and HCV-RNA prevalence were significantly higher in families with at least one first degree relative with cirrhosis than in those without, suggesting a somewhat facilitated spreading of the virus infection in the families with more cirrhotic subjects. This suggestion is also supported by the higher prevalence of C4BQ0 found in families with higher prevalence of the disease. It is of special interest in this regard the finding that in the studied families of cirrhotic probands, all but one of the subjects infected with HCV and expressing C4BQ0 were cirrhotic, whereas nearly half of the subjects who were HCV infected and not expressing C4BQ0 were not.

Our finding that HCV infection is associated with the development of cirrhosis in C4BQ0 subjects, supports the

hypothesis of a genetically transmitted impairment of HCV clearance in families with familial cirrhosis.

Lack of C4B could limit the capacity to weed out exogenous and endogenous pathogenic factors and may contribute to explain the association with some infective and immune diseases. It is of interest in this respect that C4BQ0 has an important role in HIV disease: HIV infected patients have a remarkably high prevalence of C4B null alleles [24] and the asymptomatic period is significantly shorter in patients with C4B null alleles [25].

Moreover an association of autoimmune hepatitis and primary biliary cirrhosis with C4AQ0 has been recently demonstrated [10]. There is evidence for a possible role of MHC II genes in the outcome of HCV infection [3,7–9] and several studies show a relationship among certain I-II MHC alleles and the enhanced risk of the progression of HCV infection towards cirrhosis [4–6]. Only one of the studies so far published has analyzed the role of III MHC alleles in HCV clearance, without achieving conclusive results [7].

An additional hypothesis is that C4 alleles have a role in regulating liver inflammation and fibrogenesis: in fact, C4AQ0 has been found to be associated with type 1 and 2 autoimmune hepatitis and primary biliary cirrhosis [10], and our results suggest that C4B deficiency may act as a factor facilitating fibrogenesis independently on the mechanism of impaired HCV clearance. These data further support the hypothesis that chronic liver diseases result from an interplay of environmental factors and genes, each contributing to the susceptibility and the clinical heterogeneity of the disease.

In conclusion there is a considerable risk of familial aggregation of cirrhosis. In HCV related disease, this risk is associated with C4BQ0 alleles. The observation that C4BQ0 alleles are significantly associated with familial clustering of liver cirrhosis is novel and suggests class III MHC alleles as markers for genetic predisposition to chronic liver disease.

Conflict of interest statement

None declared.

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