Analysis of genetic variation in the Aurora B kinase coding region of patients with chronic hepatitis C

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BACKGROUND

Previous studies showed that hepatitis C virus (HCV) induces epigenetic changes through interaction with the Aurora B kinase (AURKB), an enzyme that regulates phosphorylation of Serine10 residue in the histone H3. These epigenetic effects correlate with the inhibition of genes associated with the inflammatory pathway and modulate HCV infectivity [1].

[1] Madejón A et al. J Hepatol 2015, pp. 312-319

AIMS

To analyze the genetic variation of AURKB and to determine its potential clinical value as prognostic marker of liver fibrosis progression.

PATIENTS AND METHODS

We compare patients with chronic hepatitis C (CHC) grouped according the profiles of liver disease progression: 121 non-cirrhotic (F0-F3) *vs* 30 cirrhotic (F4) patients. Initial patients cohort was increased. Results shown correspond to the enlarged cohort. The clinical features of non-cirrhotic *vs* cirrhotic patients were:

Patients		NON-CIRRHOTIC	CIRRHOTIC
Gender (Male/Female)		60/61	18/12
Age		55.11 ± 11.03	57.03 ± 12.20
Race	Caucasian	96.69% (117/121)	100% (30/30)
	Sub-Saharan	3.31% (4/121)	0% (0/30)
HCV genotype	1a	20.66% (25/121)	26.67% (8/30)
	1b	42.97% (52/121)	33.33% (10/30)
	1 (1a+ 1b)	63.64% (77/121)	60.00% (18/30)
	3a	8.26% (10/121)	16.67% (5/30)
	4	9.09% (11/121)	6.67% (2/30)
	Unknown	19.01% (23/121)	16.67% (5/30)

We analyzed different regions containing single nucleotide polymorphisms (SNPs) by bulk sequencing in AURKB [rs76841187 (5´UTR region), rs2241909, rs1059476, both in exonic regions] and IL-28 gene (rs12979860).

Purification of chromosomal target hDNA from whole blood was performed using **PCR Direct-Ultra Fast Sample Prep (ARCIS Biotechnology)**, a novel method for fast DNA extraction. ARCIS-extracted DNA is suitable for immediate use in sensitive downstream applications including qPCR, DNA-seq, arrays, and methylation analysis, from different biological sources like fluids, cell cultures or solid tissue samples.

CONCLUSION

Polymorphisms in the rs2241909 and rs1059476 positions of AURKB, alone or in combination, have a protective effect on liver disease progression in chronic HCV-infected patients. These findings suggest that the analysis of AURKB, which is involved in the control of an epigenetic mark modulated by viral infection (H3Ser10ph), may have clinical relevance as a predictive factor of liver disease progression.

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RESULTS

Significant differences between cirrhotic and non-cirrhotic patients were observed. It was found that the absence of allele T in homozygosis or heterozygosis in rs1059476 seems to be protective against cirrhosis progression in a global analysis. These findings were also observed when the population was stratified by subtypes in HCV-1, particularly in HCV-1b (Fig.1). No significant differences were observed related with the distribution of rs2241909; however, it was found that in HCV-1 and in a global analysis, the presence of heterozygosis tends to be associated to cirrhosis progression (Fig.2). Some of these tendencies are maintained in the combined analysis of both SNPs (rs2241909 and s1059476). However, the main contribution to the statistical weight corresponds to rs1059476 (Fig.3). Graphics in Figure 4 show the percentage of population corresponding to SNPs alleles with significant different distribution between cirrhotic (F4) and non-cirrhotic patients (F0-F3).

Figure 1. **Distribution analysis of rs1059476 alleles**

rs1059476	
Location	chr17:8108331-8108331
Ancestral allele	T
Observed alleles	T/C
Function	Missense variant
Change	M (ATG) → T (ACG)
Described genotype frequencies (General population [Bibliographic data])	T: 26.398% (1322 / 5008)
	C: 73.602% (3686 / 5008)
Observed genotype frequencies (Cohort of patients analyzed in this work)	T: 14.57% (44/302)
	C: 85.43% (258/302)
	TT: 3.31% (5/151)
	TC: 22.52% (34/151)
	CC: 74.17% (112/151)

GENOTYPE	NON-CIRRHOTIC	CIRRHOTIC	p-value
1a	TT: 4.00% (1/25)	TT: 0% (0/8)	NS
	TC: 24.00% (6/25)	TC: 37.50% (3/8)	NS
	CC: 72.00% (18/25)	CC: 62.50% (5/8)	NS
1b	TT: 3.85% (2/52)	TT: 0% (0/10)	NS
	TC: 15.38% (8/52)	**TC: 50.00% (5/10)**	**0,025***
	CC: 80.77% (42/52)	**CC: 50.00% (5/10)**	**0,049***
1	TT: 3.85% (3/78)	TT: 0% (0/18)	NS
	TC: 17.95% (14/78)	**TC: 44.44% (8/18)**	**0,027***
	CC: 78.21% (61/78)	CC: 55.56% (10/18)	NS
3a	TT: 0% (0/10)	TT: 20.00% (1/5)	NS
	TC: 20.00% (2/10)	TC: 20.00% (1/5)	NS
	CC: 80.00% (8/10)	CC: 60.00% (3/5)	NS
4	TT: 9.09% (1/11)	TT: 0% (0/2)	NS
	TC: 27.27% (3/11)	TC: 0% (0/2)	NS
	CC: 63.64% (7/11)	CC: 100% (2/2)	NS
All	TT: 3.31% (4/121)	TT: 3.33% (1/30)	NS
	TC: 19.01% (23/121)	**TC: 36.67% (11/30)**	**0,039***
	CC: 77.69% (94/121)	**CC: 60.00% (18/30)**	**0,048***

Figure 2. **Distribution analysis of rs2241909 alleles**

rs2241909	
Location	chr17:8108339-8108339
Ancestral allele	C
Observed alleles	C/T
Function	Synonymous variant
Change	S (TCC) → S (TCT)
Described genotype frequencies (General population [Bibliographic data])	C: 37.919% (1899 / 5008)
	T: 62.061% (3108 / 5008)
Observed genotype frequencies (Cohort of patients analyzed in this work)	C: 37.75% (114/302)
	T: 62.25% (188/302)
	CC: 11.92% (18/151)
	CT: 51.66% (78/151)
	TT: 36.42% (55/151)

GENOTYPE	NON-CIRRHOTIC	CIRRHOTIC	p-value
1a	CC:12.00% (3/25)	CC: 12.50% (1/8)	NS
	CT: 56.00% (14/25)	CT: 75.00% (6/8)	NS
	TT: 32.00% (8/25)	TT: 12.50% (1/8)	NS
1b	CC: 15.38% (8/53)	CC: 10.00% (1/10)	NS
	CT: 42.31% (22/53)	CT: 60.00% (6/10)	NS
	TT: 42.31% (22/53)	TT: 30.00% (3/10)	NS
1	CC: 14.10% (11/78)	CC: 11.11% (2/18)	NS
	CT: 46.15% (36/78)	CT: 66.67% (12/18)	NS
	TT: 39.74% (31/78)	TT: 22.22% (4/18)	NS
3a	CC: 10.00% (1/10)	CC: 20.00% (1/5)	NS
	CT: 50.00% (5/10)	CT: 60.00% (3/5)	NS
	TT: 40.00% (4/10)	TT: 20.00% (1/5)	NS
4	CC: 9.09% (1/11)	CC: 0% (0/2)	NS
	CT: 54.55% (6/11)	CT: 50.00% (1/2)	NS
	TT: 36.36% (4/11)	TT: 50.00% (1/2)	NS
All	CC: 12.40% (15/121)	CC: 10.00% (3/30)	NS
	CT: 48.76% (59/121)	CT: 63.33% (19/30)	NS
	TT: 38.84% (47/121)	TT: 26.67% (8/30)	NS

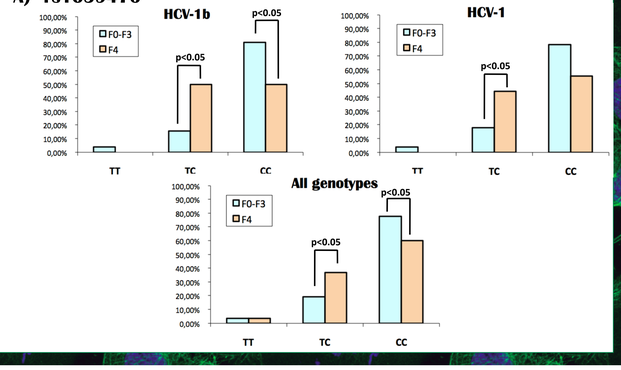
Figure 3. **Combined analysis of rs2241909 and rs1059476 allelic distribution**

GENOTYPE	NON-CIRRHOTIC	CIRRHOTIC	p-value
1a	CC TT: 4.00% (1/25)	CC TT: 0% (0/8)	NS
	CC TC: 4.00% (1/25)	CC TC: 0% (0/8)	NS
	CC CC: 4.00% (1/25)	CC CC: 12.50% (1/8)	NS
	CT TC: 20.00% (5/25)	CT TC: 37.50% (3/8)	NS
	CT CC: 36.00% (9/25)	CT CC: 37.50% (3/8)	NS
	TT CC: 32.00% (8/25)	TT CC: 12.50% (1/8)	NS
1b	CC TT: 3.85% (2/52)	CC TT: 0% (0/10)	NS
	CC TC: 3.85% (2/52)	CC TC: 10.00% (1/10)	NS
	CC CC: 7.69% (4/52)	CC CC: 0% (0/10)	NS
	CT TC: 11.54% (6/52)	**CT TC: 40.00% (4/10)**	**0.046***
	CT CC: 30.77% (16/52)	CT CC: 20.00% (2/10)	NS
	TT CC: 42.31% (22/52)	TT CC: 30.00% (3/10)	NS
1	CC TT: 3.85% (3/78)	CC TT: 0% (0/18)	NS
	CC TC: 3.85% (3/78)	CC TC: 5.56% (1/18)	NS
	CC CC: 6.41% (5/78)	CC CC: 5.56% (1/18)	NS
	CT TC: 14.10% (11/78)	**CT TC: 38.89% (7/18)**	**0.038***
	CT CC: 32.05% (25/78)	CT CC: 27.78% (5/18)	NS
	TT CC: 39.74% (31/78)	TT CC: 22.22% (4/18)	NS
3a	CC TT: 0% (0/10)	CC TT: 20.00% (1/5)	NS
	CC TC: 10.00% (1/10)	CC TC: 0% (0/5)	NS
	CC CC: 0% (0/10)	CC CC: 0% (0/5)	NS
	CT TC: 10.00% (1/10)	CT TC: 20.00% (1/5)	NS
	CT CC: 40.00% (4/10)	CT CC: 40.00% (2/5)	NS
	TT CC: 40.00% (4/10)	TT CC: 20.00% (1/5)	NS
4	CC TT: 9.09% (1/11)	CC TT: 0% (0/2)	NS
	CC TC: 0% (0/11)	CC TC: 0% (0/2)	NS
	CC CC: 0% (0/11)	CC CC: 0% (0/2)	NS
	CT TC: 27.27% (3/11)	CT TC: 0% (0/2)	NS
	CT CC: 27.27% (3/11)	CT CC: 50.00% (1/2)	NS
	TT CC: 36.36% (4/11)	TT CC: 50.00% (1/2)	NS
All	CC TT: 3.31% (4/121)	CC TT: 3.33% (1/30)	NS
	CC TC: 3.31% (4/121)	CC TC: 3.33% (1/30)	NS
	CC CC: 5.79% (7/121)	CC CC: 3.33% (1/30)	NS
	CT TC: 15.70% (19/121)	**CT TC: 33.33% (10/30)**	**0.029***
	CT CC: 33.06% (40/121)	CT CC: 30.00% (9/30)	NS
	TT CC: 38.84% (47/121)	TT CC: 26.67% (8/30)	NS

The first alleles correspond to rs2241909 and the second alleles belong to rs1059476.

Figure 4. **Graphics from the most significant allelic distributions**

A) rs1059476



B) Combined analysis of rs2241909 and rs1059476