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Interaction between immunoglobulin allotypes and NK receptor genes in diabetes post-hepatitis C virus infection

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Abstract

Genetic interactions between natural killer cells (NK) immunoglobulin-like receptor (KIR) genes and immunoglobulin allotypes have been previously reported in type 2 diabetes mellitus (DM) patients. Puerto Rican Americans with a history of intravenous drug use who developed DM following HCV infection (n=32) were compared to individuals infected with HCV without diabetes (n=121) and to DM non infected individuals (n=95). Subjects were genotyped for KIRs and immunoglobulin allotypes. We found interactions of immunoglobulin allotypes KM3/KM3 with NK inhibitory receptors 2DL3/2DL3, 2DL1 in the absence of 2DS4 associated with susceptibility to DM in HCV infected individuals. These data suggest the possibility that a subset of patients with HCV could have an immune-mediated component contributing to the development of DM.

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Keywords

Genetic susceptibility; HCV infection; Immunoglobulin allotypes; KIR receptors; Type 2 diabetes

Introduction

HCV infection affects over 170 million of people worldwide and it is estimated that 3 to 4 million individuals are newly infected each year (Alter et al., 1999). In the United States alone, 40,000 new cases are diagnosed annually (Corey et al., 2006). Several components of the immune system are involved in the response to the HCV, including NK cells, T cells and macrophages (Rehermann, 2009; Negro and Alaei, 2009).

The KIRs are a family of cell surface receptors that recognize MHC class I molecules as ligands and are predominantly expressed on NK cells. KIR proteins react with one or two epitopes at amino acid position 80 on HLA-Cw molecules (Uhberg et al., 1997). In humans, KIRs are encoded by a cluster of genes, located in the leukocyte receptor complex on chromosome 19q13.4. They comprise eight inhibitory receptors (KIR2DL1, 2DL2, 2DL3, 2DL4, 2DL5, 3DL1, 3DL2, and 3DL3), six activating receptors (KIR 2DS1, 2DS2, 2DS3, 2DS4, 2DS5 and 3DS1) and two pseudogenes (KIR2DP1, 3DP1) (McQueen et al., 2007; Khakoo et al., 2004; Moretta et al., 2009; Romero V., et al., 2008a).

The most common outcome of HCV infection is chronic viremia, manifested by clinical chronic hepatitis (85%), leading to cirrhosis and hepatocellular carcinoma. Percentages and severity of the latter complications vary widely, depending on host-related factors (Negro and Alaei, 2009). Approximately 10% of infected patients will undergo spontaneous viral clearance (SC). KIR genotypes that includes KIR2DL3 in combination with homozygosity for HLA-C1 group are thought to be involved in SC (Khakoo et al., 2004; Romero V., et al., 2008b), possibly secondary to a loss of NK cell inhibition (Parham, 2004). NK cell activity is not only important in innate immunity, but also plays a role in autoimmunity, i.e., pancreatic islet cell autoimmunity (Alba et al., 2008). The association between HCV infection and diabetes has been documented by several epidemiological studies in different ethnic groups, including a variety of endocrine abnormalities, cytokine production, insulin receptor insensitivity, liver injury, and autoimmune mediated mechanisms (Negro and Alaei, 2009). Since we had reported the interaction between KIR 2DL3/2DL3, 2DL1/2DL1, 2DS4 and GM immunoglobulin allotype f/z and z/z (ff-) with type 2 diabetes (T2D) (Romero V et al., 2008a; Zuñiga et al., 2006), our main aim was to investigate such genetic interactions in the presence of HCV in diabetes complicating HCV infection.

Materials and Methods

Patients

Between 1999 and 2004, 153 Hispanic patients with HCV infection attending a primary care clinic in western Massachusetts were recruited for this study. All participants signed an informed consent to have their blood tested for HCV and genetic markers approved by a local Institutional Review Board. HCV infection was determined by the presence of anti-HCV IgG, using enzyme immunoassay (EIA) and recombinant immunoblot assay (RIBA). Patients with positive serology for HCV were studied for the presence in plasma of HCV-RNA using RT-PCR (Amplicor, Roche Molecular Systems Inc, Pleasanton, CA, USA). HCV genotypes were determined by restriction fragment length polymorphism analysis of a reverse transcription-polymerase chain reaction (RT-PCR) as previously described (Davidson et al., 1995). These patients were also evaluated for the presence of diabetes mellitus (DM) at the entry in this study and regularly during the follow up period. DM was

diagnosed based on criteria proposed by the American World Health Organization (WHO, 2006). Thirty-two out of 153 subjects (22.5%) who had been infected with HCV developed DM, hyperglycemia with fasting glucose of >140 mg/dl or >200mg/dl at 2-hour post-challenge glucose tolerance test. A group of 95 uninfected diabetic patients was also included. We were unable to document the date of initial exposure to HCV infection in most patients.

HLA class I typing

Typing was performed by PCR with primer sequences to amplify the HLA-C region and sequence-specific oligonucleotide probes (SSOP, HLA quick-type kits, Lifecodes, Stamford, CT, USA). To solve ambiguous PCR-SSOP typing, we performed PCR-SSP (sequence-specific primer amplifications; Unitrax SSP Pel-Freeze; Milwaukee, WI, USA) according to the manufacturer's instruction.

KIR typing

The presence or absence of KIR genes was investigated using PCR-SSP method as described recently (Romero V., et al., 2008b) which permits the detection of all known KIR genes.

Immunoglobulin GM genotyping

DNA samples were typed for G3M b/5 and g/21 alleles by a PCR-RFLP-method as previously described (Balbin et al., 1994). For the determination of G1M f/3 and z/17 alleles, the CH1 region of the γ 1 chain was amplified by PCR, using the primers as described (Balbin et al., 1991), and the purified double-stranded PCR product was subjected to automatic DNA sequencing on an ABIPrism 377 sequencer. Kappa light chain determinants KM1 and 3 were characterized by a PCR-RFLP using primers: 5'TAGGGGGAAGCTAGAAGAAA3' and 5'AAAAAGGGTCAGAGGCCAAA3'. After digestion of the amplified product (538 bp) with the restriction enzyme *AccI*, three genotypes were detected: KM1 (538 bp), KM1/3 (538 bp, 390 bp, 148 bp), and KM3 (390 bp, 148 bp).

Short tandem repeat genotyping

Fifteen autosomal short tandem repeat (STR) markers (CSF1PO, FGA, THO1, TPOX, VWA, D3S11358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11, D19S433, and D2S1338) were typed as previously described. Allele calls were made in Genotype 3.7 by comparison with kit allelic ladders (Applied Biosystems).

Population genetics and stratification analyses

The population genetics analyses of STR's in Puerto Ricans were conducted in three parts. First, the ARLEQUIN v2.1 population genetics software was used to calculate the STR allele frequencies and the possible deviations from Hardy-Weinberg expectations (HWE) were tested by the homozygosity test. Second, allele frequencies of STRs were used to calculate the percent contribution of ancestral populations in Puerto Rican Americans by the maximum likelihood method, considering parental populations Caucasian Europeans, Amerindians and Africans. Gene frequency on the three parental populations was obtained from previously published data (Sanz et al., 2003; Gonçalves et al., 2002; Barrot et al., 2005). Third, to test the presence of population stratification, we analyzed by chi-square the association statistics for alleles of STR markers with DM as previously reported (Romero V et al., 2008b; Zúñiga et al., 2006).

Statistical analysis

Analyses were performed with the Statistical Package for the Social Sciences, SPSS for Windows, release 11.0 (SPSS Inc., Chicago, IL, USA). Comparative analyses used t-tests for continuous variables and chi-square. The distribution of KIR and MHC genes between groups were analyzed by chi-square using 2×2 contingency tables and Fisher's exact test and Bonferroni correction. We also used multiple logistic regression analyses to compare study groups.

Results

Baseline characteristics

Ninety three DM patients, 32 HCV-DM patients and 121 HCV infected controls were included in the study. The mean age of the DM group was higher than that of the HCV-DM group and that there were more females in the T2D group than in the HCV-DM group. However, the gender and the body mass index (BMI, kg/m²) were not statistically different (Table 1).

Of the 153 HCV infected subjects, 124 (81%) developed chronic viremia (CV). The most frequent HCV genotype was 1a (53.4%), 1b (15.3%), followed by the 2 (2a plus 2b, 12.7%), 3a (6.8%) and 4a (5.7%). The frequencies of the genotypes between the HCV and HCV-DM were statistically comparable for the three most frequent genotypes in HCV group: 1a (55/101 or 54.5%), 1b (16/101 or 15.8%) and 2 (12/101 or 11.9%); HCV-DM: 1a (11/23 or 48%), 1b (4/23 or 17.4%) and 2 (3/23 or 13%).

HCV infection influences the development of diabetes

Among 153 HCV-infected subjects, 32 (20.9%) developed DM after HCV infection. Eight of those (25.0%) followed spontaneous clearance (SC) and 24/32 with CV. Three individuals with CV developed post-IFN treatment DM and were included in the HCV control group without DM.

Association of DM with age, sex and BMI in the HCV-exposed population

Logistic regression analysis of the association of age, sex and BMI with the development of DM in HCV infected individuals, according to the viral outcome, revealed a positive association between DM and age, regardless of the viral outcome. The mean age of HCV non-infected (60 years) was greater than the mean observed in both groups, HCV-DM and HCV patients ($p < 0.0001$; 95% CI= 1.09–1.18). No significant differences in BMI and gender were observed between the studied groups, but the BMI of the HCV group was lower compared with the two DM groups.

Admixture estimation and population structure analysis

The tri-hybrid model of admixture revealed an important contribution of European (72.2%), African (17.0%), and American Indians genes (10.7%) in Puerto Ricans. Furthermore, parental contributions in the two separate groups of HCV infection (HCV-DM and HCV-non diabetic individuals) were similar and no statistical differences were detected (HCV-DM: European contribution: 71.7%, African: 16.9% African and Amerindian: 11.3%); (HCV-non-DM: European: 72.8%, African: 17.1%, Amerindian: 10.0%). Bayesian analysis with the software Structure 2.0 also confirmed the lack of population stratification in the studied groups.

Association of KIR genes with DM in HCV-infected patients

Frequencies of the inhibitory KIR genes 2DL1, 2DL2, 2DL3, 2DL4, 2DL5, 3DL1, 3DL2 and 3DL3; stimulatory KIR genes 2DS1, 2DS2, 2DS3, 2DS4, 2DS5 and 3DS1 and the mutant form of 2DS4 (K1D) in the HCV-DM and non-DM groups, demonstrated a significantly higher frequency of the activating KIR 2DS4 in the group of DM than in the HCV-DM group ($pC= <0.03$). In addition, 2DL5 frequency was increased in HCV-DM compared to DM without HCV infection ($pC= 0.05$), (Table 2). The frequencies of KIRs of haplotype A homozygotes, HAH (2DS4, 2DL4, 3DL1, 3DL2, 3DL3, 2DL1, 3DP1 and 2DP1), and other haplotypes negative for 2DS4 in combination with other KIRs such as: 2DS2, 2DP1, 2DL1, 2DL5, 2DS3, 2DS5, 3DS1 and 2DS1 were compared. Interactions between KIR inhibitors of HAH in the presence of 2DS4 with the immunoglobulin allotype GM f/f (–) ($p=0.02$; OR= 0.3; 95% CI= 0.1–0.8) and with allotype KM3/KM3 ($p=0.007$; OR= 6.5; 95% CI= 1.4–29) were significant when the groups of DM and HCV-DM were compared. By contrast, these comparisons in the absence of 2DS4 showed higher frequencies in the patients with HCV-DM, ($p=0.01$; OR= 5.1, 95% CI=1.3–19.6) for f/f (–) and ($p=0.02$; OR=5.6, 95% CI=1.2–24.8) with KM3/KM3. These latter comparisons were higher than non-diabetic infected controls ($p= 0.005$; OR = 3.67, 95% CI = 1.52–8.85) for f/f (–) and ($p= 0.01$ OR=3.55; 95%CI=1.34–9.41) for KM3/KM3. There was an increased frequency of 2DL3/2DL3 ($p= 0.0004$; OR = 5.7, 95% CI = 2.1–14.9) and, more significantly, the interaction of 2DL3/2DL3 with either (f/f–) ($p= 0.00007$, OR = 8.7, 95% CI = 2.9–26); or KM3/KM3 ($p= 0.0002$, OR = 11.73, 95% CI = 2.9–46.8) in the HCV-DM group (Tables 3 a and b). The significance of these genetic associations was confirmed by multivariable logistic regression analysis.

HLA-C allele interactions with NK receptors in DM

The interaction of 2DL3/2DL3, 2DS4 (–) 2DL1 with the HLA-C groups in DM, HCV-DM and HCV non-DM, required classifying the patients with diabetes according to the HLA-C groups, distinguished by lysine (C2) or asparagine (C1) at position 80 of HLA-Cw alleles. Comparing different ligands (C1/C1; C1/C2; C2/C2, the sum of C1/C1+C1/C2 and C2/C2+C2/C2) significant interaction was found in HCV-DM and DM patients of the presence of groups C2 and a less significant interaction comparing HCV-DM with HCV non-DM. The presence of 2DL1 interaction with C2 was important together with C1, the ligand for 2DL3, indicating that both were important in such interactions (Table 4).

Discussion

Several mechanisms are involved in the immunity against HCV including the NK cell receptors (Rehermann, 2009; Parham, 2004).

In the present report, we studied the genetics of NK cell receptors and their interactions with HLA-C ligands and immunoglobulin allotype genes in a group of HCV-infected individuals that developed type 2 diabetes mellitus (DM) post-HCV infection using as contrast groups, HCV infected individuals without DM and HCV uninfected individuals with DM.

Our results indicate that 20.9 % of HCV infected patients developed DM. In addition, the interaction of 2DL3/2DL3, 2DL1 with the allotype KM3/KM3 homozygous in absence of the allotype f/f and KIR2DS4 is associated with the development of DM in HCV infected individuals.

Among the HCV-infected patients reported here, there were only four who developed IFN- α induced DM and they were not included in the analyses. A higher incidence of DM has been described in the patients with chronic HCV infection (Mehta et al., 2003). In our studies, 25% of the HCV-infected population had DM.

The data supporting the association of HCV infection and the development of DM are mainly epidemiological (Negro and Alaei, 2009) and immune mechanisms mediating the development of DM have been suggested before (Tsiavou et al, 2004). Also the tumor necrosis factor (TNF) α has been involved in the development of HCV-DM (Knobler et al., 2003; Sheikh et al., 2008). The complexity of the DM associated to the HCV infection is further increased by the growing number of reports that describe the development of DM in patients with chronic HCV infection as a consequence of IFN alpha treatment (Thuluvath and John, 2003).

In this regard, it is important to mention that previous studies had demonstrated that immunoglobulin GM and KM allotypes are important markers in the production of anti-LKM1 antibodies which in turn attack liver and kidney in patients with HCV (Muratori et al., 2006) and also interactions with HLA-DQA and TNF markers in T2D (Pandey et al., 1999). Also, the majority of the patients with HCV-DM had BMIs consistent with obesity, suggesting the need to study a larger cohort of HCV-DM in order to determine if the interaction described in this report between NK receptors and immunoglobulin allotypes would also be true for non-obese DM patients as described in T2D (Romero V et al., 2008b). Also, there is a need to study the role of leptin receptor polymorphism together with inflammatory cytokines such as TNF α in the case of HCV-DM patients because of their reported influence on insulin resistance, body mass, production of leptin and IL-6 in T2D (Muller et al., 2002) together with the presence of 2DL3/2DL3, 2DL1 NK cell receptors.

The HCV biology, especially in HCV-DM, is not understood, although insulin resistance and autoimmunity have been suggested to be involved. For example, one possible mechanism of how HCV can induce insulin resistance is the oxidative stress that mediates signals involving p38 mitogen activated protein kinase and activation of NF kappa B, which plays a key role in the expression of the cytokines: TNF α , IL-6, IL-8 and TNF β ; particularly TNF α inhibits functions of the insulin receptor leading to insulin resistance (Knobler et al., 2003). Future studies are needed to investigate NK activation and production of cytokines to show differences in immune mechanisms between HCV-DM and T2D with interaction of NK receptors with HLA ligands and/or T cells (Willberg et al., 2003); such NK mediated mechanisms could involve the production of viral peptides by monocytes or Kupffer cells (Racanelli and Manigold, 2007). These viral peptides involved in antigen presentation could also mediate loss of inhibition of KIR-HLA-C interaction, causing an activation of T and NK cells. Cytokine production such as that of IFN γ would follow, thereby increasing MHC class I expression on target cells (Boehm et al., 1997) and thus having role in viral inactivation (Gattoni et al., 2006), (Figure 1). Our study is the first to document a higher incidence of DM in the HCV-exposed population that develops SC. Furthermore, four of the eight HCV-DMs with spontaneous clearance carry the interaction between 2DL3/2DL3, 2DL1 and 2DS4 negative and f/f (-) genes, indicating that chronic viremia measured in plasma is not necessary for the development of DM. It is important to emphasize that the published genetic studies dealing with outcome of viral infection, SC against CV (Moretta et al., 2009; Romero V et al., 2008a; Mehta et al., 2003) did not compare outcome of HCV viral load measurements in PBMCs or liver biopsies (Corey et al., 2006; Dries V et al., Muratori et al., 1994). The same oversight exists in recent reports of the SNP rs12979860 of IFN γ (Mehta et al., 2003; Ge et al., 2009). More importantly, our results demonstrated that HCV antibody measurement is more important than plasma viral loads in diabetes complicating HCV infection. Our results suggest that antibodies of the IgG allotype are involved in HCV infected patients via binding to CD81 (Tseng et al., 2002) in HCV-DM and that the 2DS4 NK activating receptor is replaced by the HCV infection to genetically distinguish a subgroup of T2D from HCV-DM patients by a mechanism of NK dependent ADCC with a loss of inhibition of NK cells. Future studies need to understand also the role of 2DL5 in HCV-DM since was increased in HCV-DM.

In conclusion, susceptibility to T2D is associated with several genetic factors, which include familial, and frequently ethnically clustered, genetic susceptibilities. The interaction of inhibitory KIR 2DL3/2DL3 in absence of the activating KIR2DS4 with the immunoglobulin allotypes KM3/KM3 are susceptibility markers to develop DM in patients with HCV infection. Taken together our results suggest that the development of DM in HCV infected subjects might be associated with immune mechanisms involving NK cell-antibody mediated cytotoxic mechanisms exacerbated by the presence of the HCV in liver. These susceptibility genetic interactions need to be confirmed in different populations to define the effect of ethnicity.

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List of abbreviations

HCV	Hepatitis C virus
DM	Diabetes mellitus
T2D	Type 2 diabetes
NK	Natural killer
KIR	Killer immunoglobulin receptor
MHC	Major histocompatibility complex
HLA	Human leukocyte antigen
SC	Spontaneous clearance
CV	Chronic viremia
PCR	Polymerase chain reaction

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Figure 1. Possible mechanisms involved in the development of DM in HCV infection
Mechanisms by which HCV and its interaction between NK receptors and immunoglobulin allotypes might mediate the development of diabetes mellitus. Possible antibody mediated including an autoimmune component.

Table 1

Demographics

		A		B		C		
		DM n=93 Mean (SD)		HCV-DM n=32 Mean (SD)		HCV n=121 Mean (SD)		
								<i>P</i> [#] value
Age		60 (12.1)		43.6 (+/- 9.13)		38.2 (+/- 8)		0.002
BMI		32 (+/- 6.2)		32 (+/- 6)		27.5 (+/- 5.6)		ns
Fasting		216 (+/- 85)		168 (+/- 35)		103 (+/- 11)		<0.0001
Glucose, Gender		n (%)	n (%)	n (%)				
Female		57 (61.3)	11 (37.9)	30/121 (24.8)	ns			
Male		36 (38.7)	21 (62.1)	91/121 (75.2)	ns			

Data are %, mean and SD in patients with type II Diabetes (DM); HCV-DM: Hepatitis C infected patients with type 2 Diabetes and patients only infected with HCV (HCV). Abbreviations; BMI: body mass index; ns: not significant; p Statistical differences were determined using Student's unpaired t test.

P^{*} *P* value calculated between columns A and B

P[#] *P* value calculated between columns B and C.

Table 2

KIR Frequencies in patients with HCV, HCV-DM and DM.

KIR Genes	DM	HCV-DM	HCV	n (%)	n (%)	n (%)	p* C (OR)	p# C (OR)
2DL1	81 (87.10)	31 (96.88)	99 (81.82)	ns	ns	ns	ns	ns
2DL2	31 (33.33)	10 (31.25)	53 (43.80)	ns	ns	ns	ns	ns
2DL3	85 (91.40)	27 (84.32)	83 (68.60)	ns	ns	ns	ns	ns
2DL4	93 (100.0)	32 (100.0)	121 (100.0)	ns	ns	ns	ns	ns
2DL5	46 (49.46)	10 (31.25)	75 (61.98)	ns	ns	ns	0.051 (0.29)	ns
3DL1	85 (91.40)	27 (84.38)	114 (94.21)	ns	ns	ns	ns	ns
3DL2	93 (100.0)	32 (100.0)	121 (100.0)	ns	ns	ns	ns	ns
3DL3	93 (100.0)	32 (100.0)	121 (100.0)	ns	ns	ns	ns	ns
2DS1	34 (36.56)	9 (28.13)	30 (24.79)	ns	ns	ns	ns	ns
2DS2	35 (37.63)	8 (25.00)	55 (45.45)	ns	ns	ns	ns	ns
2DS3	19 (20.43)	3 (9.38)	15 (12.40)	ns	ns	ns	ns	ns
2DS4	71 (76.34)	16 (50.00)	50 (41.32)	0.03 (3.67)	ns	ns	ns	ns
2DS5	33 (35.48)	10 (31.25)	41 (33.88)	ns	ns	ns	ns	ns
3DS1	39 (41.94)	11 (34.38)	43 (35.54)	ns	ns	ns	ns	ns
2DS4D	52 (55.91)	16 (50.00)	61 (50.41)	ns	ns	ns	ns	ns

DM: type 2 diabetes; HCV-DM: Hepatitis C infected patients with type 2 Diabetes; NS: not significant;

p* P value calculated between columns A and B

p# P value calculated between columns B and C. P values corrected by Bonferroni multiplying by 15 variables.

Table 3 a

Genetic Interactions of KIR and immunoglobulin allotypes in patients with HCV and HCV-DM.

GM Allotypes and KIR genes	DM n=93	HCV-DM n=32	<i>p</i> * (OR; CI)
	n (%)	n (%)	
2DS4+	72 (77)	15 (47)	0.0018 (0.2; 0.1–0.6)
2DS4–	21 (23)	17 (53)	0.0018 (3.9; 1.7–9)
ff+	18 (19)	5 (16)	ns
ff–	75 (81)	27 (84)	ns
KM3/KM3+	55 (59)	16 (50)	ns
KM3/KM3–	38 (41)	16 (50)	ns
2DS4(+) 2DL3/2DL3, 2DL1	42 (45)	8 (25)	ns
2DS4(+) 2DL3/2DL3, 2DL1 ff+	6 (6)	3 (9)	ns
2DS4(+) 2DL3/2DL3, 2DL1 ff–	36 (39)	5 (16)	0.02 (0.3; 0.1–0.8)
2DS4(+) 2DL3/2DL3, 2DL1 3/3+	28 (30)	2 (6)	0.007 (6.5; 1.4–29)
2DS4(+) 2DL3/2DL3, 2DL1 3/3–	14 (15)	6 (19)	ns
2DS4(–) 2DL3/2DL3, 2DL1	10 (11)	13 (41)	0.0004 (5.7; 2.1–14.9)
2DS4(–) 2DL3/2DL3, 2DL1 ff+	4 (4)	1 (3)	ns
2DS4(–) 2DL3/2DL3, 2DL1 ff–	6 (6)	12 (38)	0.00007 (8.7; 2.9–26)
2DS4(–) 2DL3/2DL3, 2DL1 3/3+	3 (3)	9 (28)	0.0002 (11.7; 2.9–46.8)
2DS4(–) 2DL3/2DL3, 3/3–	7 (8)	4 (13)	ns

DM: Type 2 diabetes; HCV-DM: Hepatitis C infected patients with type 2 diabetes; Haplotype A fragment: KIR 2DL3/2DL3, 2DL2 (–), 2DS1 (–), 2DS2 (–) 2DL1, 2DS4;

*p** *p* values analyzed by χ^2 and Fisher two tail exact tests OR; odds ratio; CI; confidence interval; ns; not significant ND: Not determined.

Table 3 b

Genetic Interactions of KIR and immunoglobulin allotypes in patients with HCV and HCV-DM.

GM Allotypes and KIR genes	HCV-DM n=32	HCV n=121	<i>p</i> * (OR; CI)
	n (%)	n (%)	
2DS4+	15 (47)	50(41)	ns
2DS4-	17 (53)	71(59)	ns
ff+	5 (16)	21(17)	ns
ff-	27 (84)	100(83)	ns
KM3/KM3+	16 (50)	56(46)	ns
KM3/KM3-	16 (50)	65(54)	ns
2DS4(+) 2DL3/2DL3, 2DL1	8 (25)	21(17)	ns
2DS4(+) 2DL3/2DL3, 2DL1 ff+	3 (9)	3(2)	ns
2DS4(+) 2DL3/2DL3, 2DL1 ff-	5 (16)	18(15)	ns
2DS4(+) 2DL3/2DL3, 2DL1 3/3+	2 (6)	9(7)	ns
2DS4(+) 2DL3/2DL3, 2DL1 3/3-	6 (19)	12(10)	ns
2DS4(-) 2DL3/2DL3, 2DL1	13 (41)	22(18)	0.01 (3.07; 1.32-7.1)
2DS4(-) 2DL3/2DL3, 2DL1 ff+	1 (3)	5(4)	ns
2DS4(-) 2DL3/2DL3, 2DL1 ff-	12 (38)	17(14)	0.005 (3.67; 1.52-8.85)
2DS4(-) 2DL3/2DL3, 2DL1 3/3+	9 (28)	12(10)	0.01 (3.55; 1.34-9.41)
2DS4(-) 2DL3/2DL3, 3/3-	4 (13)	10(8)	ns

HCV-DM: Hepatitis C infected patients with type 2 diabetes; HCV Non-DM: Hepatitis C infected patients without type 2 diabetes Haplotype A fragment: KIR 2DL3/2DL3, 2DL2 (-), 2DS1 (-), 2DS2 (-) 2DL1, 2DS4

*p** *p* values analyzed by Fisher two tail exact method; OR= odds ratio; CI; confidence interval; ND: Not determined

Table 4
Genetic Interactions between HLA C group and 2DL1 in patients with HCV, HCV-DM and DM.

	A		B		C	
	DM n (%)	HCV-DM n (%)	HCV n (%)	p* (OR)	p# (OR)	
C1/C1	33 (35.5)	10 (34.5)	41 (33.9)	(1.05)	(1.03)	
C1/C2	40 (43.0)	16 (55.2)	50 (41.3)	(0.61)	(1.82)	
C2/C2	20 (21.5)	6 (20.7)	30 (24.8)	(1.06)	(0.75)	
KIR 2DL3/2DL3, 2DS4 (-), 2DL1	10 (10.7)	11 (37.9)	22 (17.7)	0.001 (5.07)	0.020 (2.83)	
KIR 2DL3/2DL3, 2DS4 (-), 2DL1	6 (6.4)	2 (6.9)	7 (5.6)	(1.07)	(1.23)	
C1/C1						
KIR 2DL3/2DL3, 2DS4 (-), 2DL1	4 (4.3)	7 (24.1)	10 (8.1)	0.004 (7.07)	0.018 (3.62)	
C1/C2						
KIR 2DL3/2DL3, 2DS4 (-), 2DL1	0	2 (6.9)	5 (4.0)	(0.15)	(1.76)	
C2/C2						
KIR 2DL3/2DL3, 2DS4 (-), 2DL1	10 (10.7)	9 (31.0)	17 (13.7)	0.012 (3.73)	0.03 (2.83)	
C1 one or two copies						
KIR 2DL3/2DL3, 2DS4 (-), 2DL1	4 (4.3)	9 (31.0)	15 (12.1)	<0.0001 (10.0)	0.015 (3.27)	
C2 one or two copies						

C1: HLA-C Group 1; C2: HLA-C p: p values analyzed by logistic regression analysis; OR; odds ratio.

p* p value calculated between columns A and B

p# p value calculated between columns B and C. p values were calculated by Fisher two tail exact method.