Introduction

Innate immunity is the first line of defence against pathogens, and operates non-specifically [1]. Natural killer (NK) cells are important effector lymphocytes that participate in this early immune response to pathogens, including virally infected cells, and tumoral cells through the production of cytokines and chemokines [2]. NK cell function is regulated by a network of activating and inhibitory receptors [3], including killer immunoglobulin-like receptors (KIRs) [4].

KIRs, which are members of the CD158 gene family, are clustered in a 160 kilobase (kb) length of the 19q13.4 chromosome region within the leukocyte receptor complex (LRC) [5]. This diverse family of activating and inhibitory receptors modulates the development and activity of NK cells and some subpopulations of CD8+ T cells by interacting with the class I major histocompatibility complex (MHC) [6]. Although this recognition is well established, their interactions have yet to be fully understood. Genetic analyses indicate that KIR variation, in conjunction with polymorphic MHC class I genes, plays a key role in immune defence [7]. Thus, the extensive polymorphisms of HLA and KIR genes and their independent segregation give rise to unusual expression features. KIR receptors for which there is no HLA ligand can be expressed, while conversely an HLA ligand can be expressed for which there is no KIR. Furthermore, the interactions are influenced by peptides that bind to HLA class I and are contacted by KIR [8].

To date, 14 KIR receptors and two pseudogenes have been identified, and on the basis of this variation in gene content, more than 50 KIR haplotypes have been identified [9].

Previous analyses of these haplotypes indicate that they are subdivided into two groups: haplotype A and haplotype B [10]. Group A haplotypes comprise seven genes (KIR2DL1, KIR2DL3, KIR2DL4, KIR3DL1, KIR3DL2, KIR3DL3 and KIR2DS4) and two pseudogenes (KIR2DPI and KIR3DPI). The gene KIR2DS4 is potentially activating but is disabled by a 22-bp frameshift deletion in approximately 75% of A haplotypes and is only functional in a minority of individuals [11]. Moreover, KIR2DL4 encodes a
receptor that has both inhibitory and activating functions [12]. In contrast, Group B haplotypes are composed of varying numbers of KIR genes, including at least one of the following KIRs: KIR2DL2, KIR2DL5, KIR3DS1, KIR2DS1, KIR2DS2, KIR2DS3 and KIR2DS5. Subsequently, these two groups were shown to have distinct associations with several diseases [13,14]. Classic linkage disequilibrium (LD) studies identified two distinct regions in the KIR cluster around the KIR2DL4 gene: a centromeric (cen) region, which seems to be driven by the KIR2DL5 and KIR2DL2/3 loci [15], and a telomeric (tel) region driven by the KIR3D1/S1 locus [10].

Several studies have shown that individuals vary in the number and type of KIR loci they contain [16]. This variability and biomedicai relevance of KIRs make it important to study their organization. There is increasing evidence that receptor–ligand specificity between polymorphic KIRs and polymorphic MHC class I genes is associated with a wide range of infections, such as HIV and hepatitis C, in addition to autoimmune diseases [17], disorders in pregnancy [18], and in bone marrow and solid organ transplantation [19,20,21].

Hepatitis C virus (HCV) is a hepatotropic non-cytopathic positive-strand RNA virus that is a major cause of chronic hepatitis, cirrhosis, and hepatocellular carcinoma, affecting over 170 million people worldwide [22]. Pegylated interferon-alpha (Peg-IFN-α) plus Ribavirin has been the most effective therapy for chronic hepatitis C [23], until the emergence of new directly acting antiviral agents (DAA) like Boceprevir and Telaprevir. Several host and viral factors influence the treatment outcome [24,25] and approximately 50% of patients with HCV genotype 1 achieve a sustained viral response (SVR) [26]. Recently, single nucleotide polymorphisms (SNPs) in the IFNL3 gene (Also known as IL28B) that influence the responsiveness of treatment of HCV infection and the clearance of HCV have been identified [27,28]. The strongest association has been shown with the IFNL3 SNP rs12979860 C/C genotype, which has an SVR ratio over 70% in European-American and Hispanic patients with the HCV genotype 1 [27]. Other host genetic factors associated with the response outcome have been reported, such as KIR [29] and the PD-1.3 polymorphism in the PDCD1 gene, which has recently been described by our group [30].

The aim of this study was to investigate the role of KIR genes and their genotypes on the response to combined therapy in a well-characterised group of patients with chronic HCV infection. We also examined the effect of the different KIR2DL2/2DL3 alleles and KIR haplotypes on the response outcome. Finally, a mathematical model was developed to evaluate the combined effect of IFNL3 polymorphisms and KIR receptors.

Materials and Methods

Study population

A cohort of 811 unrelated HCV genotype 1 infected Caucasian patients was recruited from several Spanish Hospitals: Hospital Universitario Central de Asturias (HUCA), Hospital San Agustin (Aviles), Clinica Universitaria de Navarra (CUN) and Hospital La Princesa (Madrid). Patients included in this study had received their first course of antiviral therapy. The duration of treatment with PEG-IFN-α-2a or α-2b and Ribavirin was established in 48 weeks on the basis of the HCV genotype, according to consensus clinical guidance. Those without HCV RNA in their sera by six months post-treatment were defined as sustained viral responders (SVR, n = 313). The other patients were defined as non-sustained viral responders (NSVR, n = 498) which included non-responders, relapsers and partial responders.

This study was granted ethical approval by the Regional Ethics Committee for Clinical Investigation of all hospitals (Regional Ethics Committee of Clinical Research of Principado de Asturias; Ethics Committee of Clinical Research of Navarra; Regional Ethics Committee of Clinical Research of Madrid). All participants provided written informed consent.

DNA extraction

Genomic DNA was extracted from peripheral blood with the Magatration-Magazorb DNA Common Kit-200 N using the Magatration 12GC system (Precision System Science Co., Ltd., Woerstadt, Germany) and the Maxwell 16 Blood Purification Kit using the Maxwell 16 Instrument (Promega Corporation, Madison, Wisconsin, USA).

IFNL3 genotyping

The rs12979860 SNP was genotyped by amplifying the region containing the polymorphic site and hybridisation with fluorospectral-labelled probes in an RT-PCR based on the melting-curve analysis using the Light-Cycler system (Roche Diagnostics, Mannheim, Germany) [30].

HLA/KIR genotyping

The HLA-B, HLA-C, and KIR genes were typed by using Lifecodes HLA-SSO and KIR-SSO typing kits (Tepnel Lifecodes Corporation, Stamford, UK) based on the Luminex Multi-Analyte Profiling system (xMAP technology) (Lumixen Corp., Austin, TX), following the manufacturer’s instructions. Ambiguities in KIR typing were resolved by PCR-single specific primer (SSP), as defined previously [31]. HLA-Bw4, HLA-Bw6, HLA-C1 and HLA-C2 were assigned on the basis of the amino acid residues of HLA-B and HLA-C alleles [32]. The KIR genotypes were deduced from KIR profiles as previously described [10,33]. KIR gene profiles were classified with respect to the centromeric and telomeric regions of the KIR A and B haplotypes: Cen-A driven by KIR2DL3 while Cen-B by KIR2DL1 and Tel-A driven by KIR3D1/S1.

Finally, a PCR was also designed for KIR2DL3 subtyping to distinguish between expressed and non-expressed variants of this gene, as previously described [34].

KIR2DL2 and KIR2DL3 oligotyping

Based on the results of KIR genotyping, patients with KIR2DL2 and/or with KIR2DL3 genes were genotyped as described elsewhere [35,36] to identify different alleles. 146 NSVR and 103 SVR patients were randomly selected for this study. Different regions of these KIR receptors were selectively amplified by a PCR using locus-specific primers. For SSP hybridisation, amplified DNA was blotted onto nylon membranes and hybridised with a panel of 5 and 13 SSOPs designed to detect unique sequence motifs of known KIR2DL2 and KIR2DL3 alleles, respectively. Alleles were assigned with respect to the reaction patterns of the SSOP, based on the known KIR2DL2 and KIR2DL3 sequences. This method identifies the most common alleles for KIR2DL2 (*001, *002, *003, *004 and *005) and for KIR2DL3 (*001, *002, *003, *004, *005 and *006).

Statistical analysis

Continuous variables were summarised as the mean and standard deviation. Categorical variables were summarised as absolute frequencies and percentages. The Genetic Analysis
Package (GAP) in R (www.r-project.org) was used to compute LD statistics for pairs of alleles. In particular, the D’ statistic based on Cramer’s V was used. The general bootstrap algorithm (gBA) [37] was used to compare global and particular LDs in responders and non-responders. Fisher’s exact test was used to examine the relationship between categorical variables. In addition, where the relationships were statistically significant (p < 0.05) the odds ratios (ORs) and the respective 95% confidence intervals were also reported. Finally, the R package Logit-Reg (available in the CRAN) was used to develop a logic regression (LR) model [38]. A stepwise criterion based on the Akaike interaction was included in this model. LR aims to identify predictors that are Boolean (logical) combinations of the original predictors. In particular, LR looks for an optimal model of the form:

$$\text{logit}(\mathbb{E}(Y)) = \beta_0 + \beta_1 L_1 + \beta_2 L_2 + \ldots + \beta_p L_p$$

where Y is the response (Yes/No), \( \beta_1, \ldots, \beta_p \) are the parameters, and \( L_1, \ldots, L_p \) are Boolean combinations (intersection (AND, ^) unions (OR V) and complement (NOT, \( \neg \)) of the dichotomous variables considered. The \( L_i \)'s are also called logic trees. Our model considered the variables: HLA-Bx. In general, we observed that genotypes that included KIR2DL2 and KIR2DL3 alleles were more frequent in NSVR than in those with NSVR (20.7% vs. 11.2%; p < 0.005). Moreover, KIR2DL2*001 was associated with a poor treatment outcome (OR = 1.92, 95% CI = 1.11–3.31; p < 0.05) and the KIR2DL3*001 allele was associated with SVR (OR = 0.44, 95% CI = 0.26–0.74; p < 0.005). In contrast, KIR2DL3*001 in combination with HLA-C1 ligand was related to a good treatment outcome (OR = 0.37, 95% CI = 0.22–0.65; p < 0.001). No association was found between KIR2DL3*001-C1, KIR2DL2*001-C1 and KIR2DL3*003-C1 (data not shown).

KIR haplotype frequency in patients with chronic HCV infection

In the current study, the KIR genotypes were analysed to determine the KIR haplotypes, based on those characterised in other studies [10,33]. Thirty-five genotypes were identified in this cohort and were resolved into corresponding pairs of haplotypes (Figure 1).

The most common genotype was KIR3DL3-2DL3-2DP1-2DL1-3DP1-2DL4-3DL1-2DS4-3DL2 (Genotype N° 1), corresponding to homozygosity for the major subtype of the previously reported A haplotype (28.1% in SVR and 23.5% in NSVR). Most of these genotypes included the KIR2DL3*001 allele. The other KIR genotypes found included at least one activating locus in addition to KIR2DS4, corresponding to haplotype B. These genotypes are named Bx. In general, we observed that genotypes that included KIR2DL2, especially the *001 allele, were present at a higher frequency in NSVR patients. The most prevalent was KIR3DL3-KIR2DS2-KIR2DL2 2DL3-2DP1-2DL1-3DP1-2DL4-3DL1-2DS4-3DL2 (genotype N° 2), which includes the KIR2DS2 in strong LD with the KIR2DL2 gene. On the other hand, in SVR patients, the predominant genotypes were those that included KIR2DL3, like the aforementioned genotype 1.

The overall patterns of linkage disequilibrium of KIR receptors were similar in two groups of patients (Figure 2). As previously described, the measures showed two distinct regions in the KIR cluster around the KIR2DL4 gene. One was a centromeric region (KIR3DL3, KIR2DS2, KIR2DL2, KIR2DS3, KIR2DP1, KIR2DL1 and KIR3DP1) that appeared to be driven by the KIR2DL3 and KIR2DL2/3 locus; the other was a telomeric region (KIR2DL4, KIR3DL1, KIR2DS1, KIR2DL3, KIR2DS5, KIR2DS1, KIR2DS4 and KIR3DL2), driven by KIR3DL1/S1.
centromeric and telomeric motifs in the two groups, we found that the Cen-A haplotype in homozygous was significantly more common in SVR patients (OR = 0.63, 95% CI = 0.47–0.84; p < 0.005) and the Cen-B haplotype segment in homozygous was more frequent in NSVR patients (OR = 2.04, 95% CI = 1.35–3.03; p < 0.001) (Table 3). Other combinations were not significant. Finally, we analysed the association of the centromeric and telomeric KIR haplotypes with HLA-C and HLA-B. We found a statistically significant association of the Cen-A/A motif and HLA-C with good treatment outcome (OR = 0.62, 95% CI = 0.45–0.84; p < 0.001).

Table 1. KIR gene frequencies, KIR2DL2/3-HLA and KIR2DS2-HLA genotypes distribution in the two groups of patients.

|     | NSVR (%) n = 498 | SVR (%) n = 313 | OR (95% CI) | p     |
|-----|------------------|-----------------|-------------|-------|
| KIR2DS1 | 210 (42.2)   | 130 (41.5)    |             |       |
| KIR2DS2 | 317 (63.7)   | 166 (53)      | 1.55 (1.16–2.07) | <0.005|
| KIR2DS3 | 200 (40.2)   | 116 (37.1)    |             |       |
| KIR2DS4 | 462 (92.8)   | 295 (94.2)    |             |       |
| KIR2DS5 | 176 (35.3)   | 111 (35.5)    |             |       |
| KIR2DP1 | 468 (94)     | 302 (96.5)    |             |       |
| KIR2DL1 | 466 (93.6)   | 302 (96.5)    |             |       |
| KIR2DL2 | 323 (64.9)   | 172 (55)      | 1.51 (1.13–2.02) | <0.005|
| KIR2DL3 | 394 (79.1)   | 278 (88.8)    | 0.48 (0.32–0.72) | <0.001|
| KIR2DL4 | 498 (100)    | 313 (100)     |             |       |
| KIR2DL5A | 212 (42.6)  | 137 (43.8)    |             |       |
| KIR2DL5B | 176 (35.3)  | 97 (31)       |             |       |
| KIR3DL1 | 461 (92.6)   | 290 (92.7)    |             |       |
| KIR3DL2 | 498 (100)    | 313 (100)     |             |       |
| KIR3DL3 | 498 (100)    | 313 (100)     |             |       |
| KIR3DP1 | 498 (100)    | 313 (100)     |             |       |
| KIR3DS1 | 211 (42.4)   | 136 (43.5)    |             |       |
| KIR2DL2/KIR2DL2 | 104 (20.7) | 35 (11.2) | 2 (1.31–3.03) | <0.001|
| KIR2DL2/KIR2DL3 | 219 (44)   | 137 (43.8)   |             |       |
| KIR2DL3/KIR2DL3 | 175 (35.3) | 141 (45)   | 0.66 (0.49–0.88) | <0.01 |
| KIR2DS2-HLAC1 | 255 (51.2) | 128 (40.9) | 1.46 (1.09–1.96) | <0.01 |
| HOMOKIR2DL2-HLAC1C1 | 32 (6.4)    | 13 (4.1)     |             |       |
| HOMOKIR2DL2-HLAC1C2 | 61 (12)     | 14 (4.4)     | 2.85 (1.56–5.26) | <0.001|
| HOMOKIR2DL2-HLAC2C2 | 11 (2.2)    | 8 (2.6)      |             |       |
| KIR2DL2/2DL3-HLAC1C1 | 74 (14.9)   | 49 (15.7)    |             |       |
| KIR2DL2/2DL3-HLAC1C2 | 95 (19)     | 58 (18.5)    |             |       |
| KIR2DL2/2DL3-HLAC2C2 | 50 (10.1)   | 30 (9.9)     |             |       |
| HOMOKIR2DL3-HLAC1C1 | 32 (6.4)    | 51 (16.3)    | 0.34 (0.21–0.54) | <0.001|
| HOMOKIR2DL3-HLAC1C2 | 91 (18.5)   | 60 (19.2)    |             |       |
| HOMOKIR2DL3-HLAC2C2 | 52 (10.5)   | 30 (9.3)     |             |       |

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Table 2. Genotyping and frequencies of KIR2DL2 and KIR2DL3 alleles in relation to treatment outcome.

|     | NSVR (n = 146) | SVR (n = 103) | p     | OR (95% CI) |
|-----|----------------|---------------|-------|-------------|
| KIR2DL2*001 | 61 (41.8)    | 28 (27.2)     | <0.05 | 1.92 (1.11–3.31) |
| KIR2DL2*003 | 38 (26)      | 31 (30.1)     | -     | -            |
| KIR2DL3*001 | 60 (41.1)    | 63 (61.2)     | <0.005 | 0.44 (0.26–0.74) |
| KIR2DL3*002 | 74 (50.7)    | 45 (43.7)     | -     | -            |
| KIR2DL3*001-HLAC1 | 35 (24)    | 47 (45.6)     | <0.001 | 0.37 (0.22–0.65) |
| KIR2DL3*002-HLAC1 | 60 (41.1)   | 37 (35.9)     | -     | -            |

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In contrast, the association of the Cen-B/B motif and HLA-C combination was significant (OR = 2.5, 95% CI = 1.5 – 3.96; p < 0.001) in relation with non-response. The Tel-HLA combinations were not found to be associated with treatment outcome.

### Analysis of KIR genotyping and IFNL3 polymorphism combinations

We used logic regression to examine Boolean combinations (AND/OR/NOT) of binary covariates considered in this study, in order to derive a model for predicting chronic HCV treatment outcome. The final model obtained is summarised as follows. Combinations of KIR genotyping, IFNL3 and the intersections and unions of other factors can be expressed mathematically (figure 3).

The measure of the importance of a variable in the response to treatment is the frequency with which it appears independently in all models. From the summary, the contribution of each variable can be evaluated by the ratio of the number of times it was included in any of the models divided by the total number of models.

Logic regression revealed a strong association of the IFNL3 rs12979860 polymorphism in the prediction of response to combined treatment (PPV = 55.9%) (Table 4). It was improved when we incorporated different studies of KIR combinations in the logic model. The inclusion of the complete analysis of KIR genes and their combinations to the mathematical model yielded the best result (PPV = 75.3%). The ROC curve of different logic regression models was derived. In the best model, the area under the curve (AUC) was 0.729 (95% CI = 0.692 – 0.772) (Figure 4).

In conclusion, KIR2DL3/KIR2DL3-HLA-C1/C1 and the KIR centromeric haplotype motif (A/A genotype) are good predictors of SVR. On the other hand, KIR2DL2/KIR2DL2-HLA-C1/C2 and the KIR centromeric haplotype motif (B/B genotype) are associated with a high percentage of NSVR in combination with IFNL3 T/T. Our statistical analysis did not show any association between KIR2DS2 and treatment outcome.

### Discussion

Previous studies have described several biomarkers of treatment response in addition to IFNL3 polymorphisms [41,42]. Other study by our group has described the influence of KIR genes on the progression of chronic HCV infection and treatment outcome [29]. As well as KIR receptors, other genetic markers related to immune response have been reported [42]. One such marker is...
PDCD1, which allowed us to develop a model for predicting the response to conventional therapy. Combining the analysis of this marker with IFNL3 genotyping considerably improved the predictability of response to standard treatment established using other parameters such as viral load or viral genotype [30].

The current models predict the treatment response of each patient only approximately, so the search for new genetic markers is essential if we are to be able to establish the most appropriate therapy for each patient from the beginning of treatment [30]. As previously reported, factors such as the KIR-HLA genotype were relevant to this response. The KIR2DL3/KIR2DL3-HLA-C1C1 genotype was more frequent in the SVR group, while the KIR2DL2/KIR2DL2-HLA-C1C2 genotype was more frequent in NSVR patients. In relation with this, we found that the HLA-C*07 was associated with a good treatment response, especially in combination with KIR2DL3. This data is interesting because this HLA-C allele was previously associated with the HCV persistence [43] and, more recently, with high viral load [44].

Table 3. KIR genotypes and haplotype frequencies of the studied population, and combinations of Cen and Tel haplotypes with HLA-B and HLA-C.

| Genotype | NSVR (n = 498) | SVR (n = 313) | OR (95% CI) | p     |
|----------|----------------|---------------|-------------|-------|
| AA       | 117 (23.5)     | 88 (28.1)     | NS          |       |
| Bx²      | 381 (76.5)     | 225 (71.9)    |             |       |
| Centromeric haplotype segment | | | | |
| A/A      | 172 (34.5)     | 138 (44.1)    | 0.63 (0.47–0.84) | <0.005 |
| A/B      | 222 (44.6)     | 140 (44.7)    | -            | NS    |
| B/B      | 104 (20.9)     | 35 (11.2)     | 2.04 (1.35–3.03) | <0.001 |
| Telomeric haplotype segment | | | | |
| A/A      | 279 (56)       | 133 (55.3)    | NS          |       |
| A/B      | 176 (35.3)     | 115 (36.7)    |             |       |
| B/B      | 43 (8.6)       | 25 (8)        |             |       |
| Cen-HLA combinations | | | | |
| Cen-A/A-HLA-C1 | 121 (24.3) | 107 (34.2) | 0.62 (0.45–0.84) | <0.005 |
| Cen-A/B-HLA-C1 | 172 (34.5) | 110 (35.1) | -            | NS    |
| Cen-B/B-HLA-C1 | 92 (18.5) | 26 (8.3) | 2.5 (1.38–3.96) | <0.001 |

Note: ²B/B and A/B genotypes were included in this group. Tel-HLA-C, Cen-HLA-B and Cen-HLAB combinations were not significant.
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In order to determine specifically which allelic variant of these genes predominates in the two groups of patients, we carried out an oligotyping analysis of KIR2DL2 and KIR2DL3, as previously described [35,36]. In relation to KIR2DL3, we found that the KIR2DL3*001 allele was most common in SVR while KIR2DL2*001 was more frequent in NSVR. In addition, the relevance of KIR2DL3*001 to good treatment outcome is notably greater when analysed in combination with HLA-C1. These findings suggest these alleles are important in the response to standard treatment, and may be associated with a weak interaction with its ligand, as described for other alleles from KIR2DL2/3 [45].

Our results confirm the relevance of the KIR2DL3*001 alleles and the interaction with HLA-C in the treatment outcome, which could also be involved in the progression and chronicity of HCV infection. With regard to KIR3DL1-HLA-Bw4, Nozawa et al showed a significant association of this combination with SVR to Peg-IFN/RBV in Japanese population [46]. Nonetheless, we did not find statistical differences in our cohort, in line with previous reports in Caucasian populations. [29,47]. This data underscores the distinctive organisation of the human KIR locus that drives the generation of KIR gene-content diversity in different populations [48,49].

The human KIR locus contains highly conserved genes that are situated in the middle (KIR3DP1 and KIR2DL4) and at the ends (KIR3DL3 and KIR3DL2) of the cluster, creating a framework around two regions of variability in which highly homologous KIR genes are packed very close together in a head-to-tail configuration, separated by short and highly conserved intergenic regions. In different human populations, there is a variable balance between A and B groups of KIR haplotypes, which seems to be maintained by balancing selection for inhibitory and activating functions [50]. This selection is mediated, in part, by the interaction of inhibitory KIR with their HLA class I ligands. However, it should be taken into consideration that KIR-ligand interactions are different when considering activating receptors in patients with a good response. This association may have arisen because KIR2DL3 is present in the Cen A haplotype motif, while KIR2DL2 is part of Cen B haplotype motif. It has also been shown that KIR2DL2 and KIR2DL3 have different inhibitory capabilities arising from dissimilar interactions with their ligands [45]. KIR2DL1 has the strongest interaction with its ligands, the HLA-C2 alleles, while KIR2DL2 associates to HLA-C1 more intensely than KIR2DL3 [53]. Bearing these data in mind, we may surmise that the stronger interaction and therefore the stricter inhibition of NK cells would occur in individuals homozygous for KIR2DL2 who carry the HLA-C1/C2 genotype. Conversely, NK cells from patients who are homozygous for KIR2DL3 and for HLA-C1 would have a greater activation ability due to the lack of intense inhibition arising from the interaction between KIR2DL1 and the HLA-C2 ligand. However, it should be taken into consideration that KIR-activating receptors in the A haplotype are not reduced to KIR2DL4, because KIR2DL4 can sometimes act as an activating receptor [54,55].

Table 4. Prediction of treatment outcome in patients with chronic HCV infection using the proposed logic regression model.

|                | Sensitivity | Specificity | PPV³ | NPV⁴ | AUC² (95% CI) |
|----------------|-------------|-------------|------|------|---------------|
| IFNL3¹         | 51.7        | 74.3        | 55.9 | 71   | 0.658 (0.624–0.692) |
| KIR2DL2/3-HLA  | 16          | 95.8        | 70.4 | 64.5 | 0.596 (0.575–0.645) |
| IFNL3³ + KIR2DL2/3 | 25        | 93          | 69   | 66.3 | 0.684 (0.642–0.720) |
| IFNL3³ + KIR2DL2/3-HLA | 13.4  | 97          | 73.7 | 64   | 0.690 (0.651–0.733) |
| IFNL3¹ + Haplotypes study | 21.4 | 94.9        | 72   | 65.7 | 0.699 (0.662–0.735) |
| IFNL3¹ + KIR gene study² | 21.4 | 95.6        | 75.3 | 65.9 | 0.729 (0.692–0.772) |

Note:
¹IFNL3 polymorphism rs12979860.
²KIR2DL2/3, KIR2DL2/3-HLA and KIR haplotype study.
³Positive predictive value.
⁴Negative predictive value.
⁵Area under the curve.

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Figure 4. ROC curve of logic regression model using IFNL3 C/C and KIR/HLA gene determination (KIR2DL2/3, KIR2DL2/3-HLA and KIR haplotype study) (solid line). We also included the ROC curves of IFNL3 (dotted line) and KIR2DL2/3-HLA (dashed line) logic model determinations. It can be observed they do not overlap with the best logic regression model. Note: Area under the curve (AUC) of the best model: 0.729 (95% CI, 0.692–0.772).

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Suppiah et al [56], observed a significant association between HLA groups C1 and C2 with treatment outcome and established a logistic model to predict a failure to clear virus on therapy with PegIFN/RBV. We did not find any association with HLA-C groups and treatment except HLA-C*07 allele. Nonetheless, we observed a clear association between KIR2DL2/3 and therapy response. Also, we studied all KIR genes to establish the different haplotypes of these receptors, and found a statistical association with SVR. These different results may be due to patient selection and different study population. To assess the significance of these results to treatment outcome, we developed a logic regression model [37], different from Suppiah. This showed that genotyping IFNL3 alone had a specificity of 74.3% and a positive predictive value (PPV) of 55.9%. However, this result was improved by the determination of KIR genes, increasing the specificity to 95.6% and the PPV to 75.3%. Although the sensitivity decreased from 51.7% to 21.4%, these results are further evidence of the important role that KIR receptors play in the response to combined therapy in HCV-infected patients.

In conclusion, based on our results we propose a novel relationship between KIR/HLA genotypes and KIR haplotypes (A/B) in the chronicity of HCV infection. These could be used as tools in combination with other parameters, such as the determination of IFNL3 rs12979860, to predict treatment outcome and in order to establish the best cost-effective treatment between the classical regimen and the new arrival therapies. Further research is required to determine the molecular mechanisms involved in this process.

Supporting Information

Table S1

A) HLA-C alleles distribution in patients with HCV chronic infection. B) KIR2DL2 and KIR2DL3 association with HLA-C*07 allele.

(DOC)

Author Contributions

Conceived and designed the experiments: JVC ALV CLL. Performed the experiments: JVC ALV JMB. Analyzed the data: JVC CLL PMC. Contributed reagents/materials/analysis tools: JP RLR PSC JdlV LR RPL RPA. Wrote the paper: JVC ALV RPA CLL.

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