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The absence of seroconversion after exposition to hepatitis C virus is not related to KIR-HLA genotype combinations (GEHEP-012 study)

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ABSTRACT

Background & aims: It has been reported that specific killer-cell immunoglobulin-like receptors (KIRs) and HLA genotype combinations, such as KIR2DS4/HLA-C1 with presence of KIRDL2 or KIRDL3, homozygous KIRDL3/HLA-C1 and KIR3DL1/≥2HLA-Bw4, are strongly associated with the lack of active infection and seroconversion after exposition to hepatitis C virus (HCV).
Objective: To determine whether these KIR-HLA combinations are relevant factors involved in that phenotype.
Patients and methods: In this retrospective case-control study, genotype data from a genome-wide association study previously performed on low susceptibility to HCV-infection carried out on 27 high-risk HCV-seronegative (HRSN) individuals and 743 chronically infected (CI) subjects were used. HLA alleles were imputed using R package HIBAG v1.2223 and KIR genotypes were imputed using the online resource KIR*IMP v1.2.0.
Results: It was possible to successfully impute at least one KIR-HLA genotype combination previously associated with the lack of infection and seroconversion after exposition to HCV in a total of 23 (85.2%) HRSN individuals and in 650 (87.5%) CI subjects. No KIR-HLA genotype combination analyzed was related to the HRSN condition.
Conclusions: Our results suggest that those KIR-HLA genotype combinations are not relevant factors involved in the lack of infection and seroconversion after exposition to HCV. More studies will be needed to completely understand this phenotype.

1. Introduction

It has been previously described that a small proportion of individuals remain seronegative after repeated exposure to hepatitis C virus (HCV), without detectable HCV-RNA (Hagan et al., 2008). These subjects, also termed high-risk HCV seronegative (HRSN) individuals or exposed uninfected subjects, could either show a low susceptibility to HCV infection or become infected but have an efficient viral clearance before seroconversion (Shawa et al., 2017a). In both cases, genetic factors could have a key role determining this phenotype.

On the one hand, and regarding the hypothesis of a low susceptibility to HCV infection, several studies have described the association of genetic variants linked to loci involved in lipid metabolism with that phenotype (Real et al., 2019a,b; Steba et al., 2019). On the other hand,

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supporting the hypothesis that an efficient viral clearance without seroconversion exists in HRSN individuals, several studies have highlighted that the specific combinations of genetically determined killer-cell immunoglobulin-like receptors (KIRs) and their HLA ligands are associated with this phenotype (Knapp et al., 2010; Thöns et al., 2017; Zúñiga et al., 2009). It was also suggested that these effects are relevant when the size of the inoculum is small, just as it occurs in intravenous drug users (IDUs) (Knapp et al., 2010). Interestingly, some of these KIR-HLA combinations were previously related to the spontaneous resolution of HCV infection (Khakoo et al., 2004; Knapp et al., 2010; Romero et al., 2008).

Firstly, Zuñiga et al. reported that the presence of KIR2DL2 and/or KIR2DL3 and HLA-C1 together with the activating receptor KIR2DS4 was overrepresented in HRSN individuals (Zúñiga et al., 2009). Later, Knapp et al. related the combination of KIR2DL3 and its ligand HLA-C1, both in homozygosis, to the possible resolution of HCV infection without seroconversion (Knapp et al., 2010). Nevertheless, this finding has not been replicated by others (Sugden et al., 2014a; Thöns et al., 2017).

Lastly, it was reported that the combination of KIR3DL1 and multiple HLA-Bw4 copies is associated with lack of seroconversion in HRSN subjects (Thöns et al., 2017). However, to our knowledge, no study has replicated this result nor that reported by Zúñiga et al., 2009).

Our aim was to determine whether the previously reported KIR-HLA genotype combinations are relevant factors related to the lack of seroconversion in HSRN individuals.

2. Material and methods

2.1. Study population

This retrospective case-control study included populations of HRSN individuals and HCV CI subjects previously described (Real et al., 2019a). Briefly, HRSN individuals were IDUs infected by HIV that had been sharing injection devices for longer than 3 months. The control group included chronically infected (CI), treated or not. Moreover, we also included spontaneous resolvers (SR) for analyzing the role of KIR-HLA genotype combinations in this phenotype. All of them were Spanish Caucasians individuals who attended the Infectious Disease Units at University Hospitals since 1999. Only those individuals in whom the imputation of at least one KIR-HLA analyzed combination was possible were included.

2.2. KIR and HLA genotypes imputation

Genotype data from a genome-wide association study on low susceptibility to HCV infection (Real et al., 2019a) was used for HLA and KIR genotype imputations.

KIR genes were imputed using the online resource KIR*IMP v1.2.0 (University of Melbourne, AUS) (http://imp.science.unimelb.edu.au/kir/) according to the manufacturer's instructions (Vukcevic et al., 2015). Only KIRs with ≥70% imputation accuracy were taken into account. KIR2DS4 exists as two major alleles, one of which encodes the functional one (KIR2DS4) and another allele that encodes a truncated version nonfunctional receptor (KIR2DS4-del). Only the functional allele was taken into account in this work.

HLA alleles were imputed using R package HIBAG v1.2223 (Zheng et al., 2014) on platform and ancestry specific reference panels available through HIBAG or trained in-house as previously described (Yu et al., 2021). As recommended by HIBAG developers, alleles with an imputation probability lower than 0.5 were considered undetermined.

HLA-A and HLA-B alleles considered as HLA-Bw4 were those reported by Thöns et al. (2017). Genomic alleles considered as HLA-C1 were those specified elsewhere (Gwozdowicz et al., 2019).

KIR gene frequencies in the Spanish population were those reported in Allele Frequency Net Database (http://www.allelefrequencies.net/default.asp) (Gonzalez-Galarza et al., 2020). Regarding KIR2DS4, no data about its frequency in Spain was available. HLA-Bw4 and HLA-C1 allelic frequencies in the Spanish population were those reported by de Arellano et al. (de Arellano et al., 2019) and Montes-Cano et al. (2005), respectively.

2.3. Statistical analysis

Categorical variables were expressed as frequencies (percentages). Comparisons of categorical variables were performed using the Pearson chi-square test or the Fisher test. Continuous variables were expressed as median (quartile 1 – quartile 2). Kruskal Wallis test was used for comparing age among groups. All these calculations were carried out using the SPSS software 26.0 (IBM Corporation, Somers, NY, USA). The p-value threshold for statistical significance was established at 0.05.

The estimations of power to detect KIR-HLA combinations associated with the HRSN condition were performed by the Episheet software (http://krothman.hostbyet2.com/episheet.xls).

2.4. Ethics

This study was in compliance with the Spanish legislation and it was performed according to the ethical guidelines of the Declaration of Helsinki. The study was approved by the Ethics Committee of the Hospital Universitario de Valme (internal reference number: 0422-N-16). Written consent was obtained from all individuals before sampling.

3. Results

3.1. Study population

It was possible to successfully impute at least one KIR-HLA combination previously associated with the lack of infection and seroconversion after exposure to HCV in a total of 23 (85.2%) out of 27 HRSN subjects, 650 (87.5%) out of 743 HCV CI individuals and 51 (87,9%) out of 58 SR. HRSN individuals were IDUs during a median (quartile 1-quartile 3) time of 12.1 (8.7–13.3) years. During that period, they reported to share injection devices during 6.6 (4.0–9.1) months, with a frequency of, at least, one injection per day. The main characteristics of all those individuals are depicted in Table 1.

3.2. KIR and HLA alleles frequencies

Among the study population, the presence of one or two copies of KIR3DL1 was successfully imputed in all individuals. KIR2DL3 was not accurately imputed in 7 (1.2%) CI subjects, 1 (4.3%) HRSN individual and 1 (1.9%) SR. The KIR2DS4 gene was not accurately imputed in 44 (6.7%) CI, 3 (13%) HRSN, and 5 (9.8%) SR subjects. HLA-C1 could not be imputed in 8 (1.2%) CI individuals and 1 (4.3%) HRSN subject. HLA-Bw4 alleles could not be determined in 8 (1.2%) CI individuals and in 1 (4.3%) SR.

The HLA-Bw4 and HLA-C1 allelic frequencies and the frequencies of the analyzed KIR genes among CI, HRSN and SR subjects, as well as those reported in the Spanish population, are depicted in Fig. 1.

Table 1
Main characteristics of the study groups.

Variables	CI (n = 650)	HRSN (n = 23)	SR *(n = 51)*	*p value*
Age, years[a]	51 (47–54)	48 (41–54)	49 (47–52)	0.068
Male sex, n (%)	539 (82.9)	20 (87.0)	43 (84.3)	0.859
HIV coinfection, n (%)	388 (59.7)	23 (100)	28 (54.9)	<0.001
IDUs, n (%)	539 (82.9)	23 (100)	45 (88.2)	0.062

CI, HCV Chronically infected subjects; HRSN, High-risk seronegative individuals; SR, Spontaneous resolvers; IDUs, intravenous drug users.
[a] Median (q1-q3).

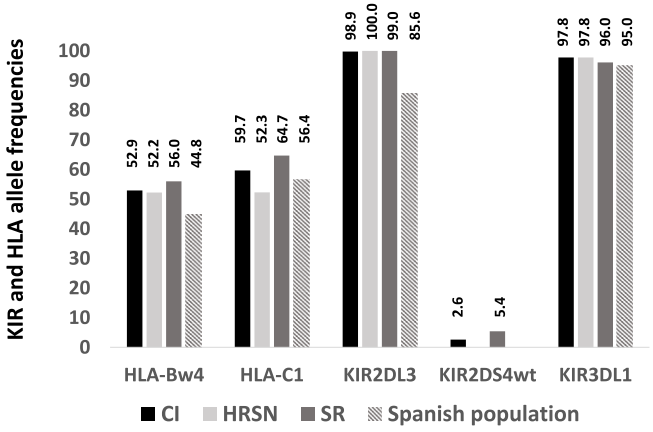

Fig. 1. KIR and HLA allele frequencies in HCV chronically infected (CI) individuals, HCV high-risk seronegative (HRSN) subjects, spontaneous resolvers (SR) and those reported in the Spanish population.

3.3. Analysis of KIR-HLA combinations previously associated with the lack of seroconversion in HRSN subjects

The frequencies of the KIR-HLA combinations in the studied CI and HRSN subjects are shown in Table 2. None of the analyzed combinations showed significant differences among CI and HRSN groups (Table 2).

When only IDUs infected by HIV were taken into account similar results were obtained (Table 3).

We explored if the presence of two of these KIR-HLA combinations could be associated with the HRSN phenotype. In a total of 595 (91.5%) out of 650 CI and 19 (82.6%) out of 23 HRSN subjects was possible to impute all KIR-HLA combinations analyzed herein. Among them, 78 (13.1%) CI and 1 (5.3%) HRSN individuals showed two favorable combinations ($p = 0.493$). When the analysis was restricted to IDUs infected by HIV, the results were similar (47 [14.9%] CI vs 1 [5.3%] HRSN, $p = 0.495$).

3.4. Analysis of KIR-HLA genotype combinations in spontaneous resolvers

We analyzed if those studied KIR-HLA combinations were associated with HCV spontaneous clearance. No significant differences were observed in the frequency of these combinations between CI subjects and SR (Supplementary Table 1). When the analysis was restricted to IDUs infected by HIV similar results were obtained (Supplementary Table 2).

4. Discussion

Our results indicate that the KIR-HLA combinations previously reported as associated with the lack of active infection and seroconversion in HRSN subjects are not relevant factors related to that condition. Similarly, none of these combinations is strongly related to HCV spontaneous clearance.

There are some evidences that the HRSN phenotype is related to enhanced NK cell activity in both kinds of HRSN subjects: IDUs (Golden-Mason et al., 2010; Mina et al., 2016; Sugden et al., 2014b; Thoens et al., 2014) and recipients of HCV-contaminated blood (Ow et al., 2018). Accordingly, it was observed that the KIR-HLA combinations associated with the HRSN condition are those that i) have a lower inhibitory effect on NK cells activation (KIR2DL3/HLA-C1 homozygous) (Khakoo et al., 2004; Knapp et al., 2010; Moesta et al., 2008), or ii) have a direct effect on NK cell activation through activating KIRs (KIR2DS4/HLA-C1) (Zúñiga et al., 2009), or influencing the IFN-gamma production by NK cells (KIR3DL1/≥2 HLA-Bw4) (Boudreau et al., 2016; Kim et al., 2008). However, the association of KIR2DL3/HLA-C1 was not confirmed by others (Sugden et al., 2014a; Thöns et al., 2017). We neither confirm that association nor the KIR2DS4/HLA-C1 or KIR3DL1/≥2HLA-Bw4 combinations with the HRSN phenotype. Furthermore, when we restricted the analyses to IDUs, similar negative results were also observed. Therefore, we also failed to confirm that the reported effects of these combinations are only evident when the inoculum is low, as previously suggested (Knapp et al., 2010). Nevertheless, and due to the low number of HRSN individuals included in our study, we can not rule out a low effect of these combinations on that condition. Interestingly, we neither observed the association of these combinations with HCV spontaneous clearance. Similarly, Montes-Cano et al. could not confirm any effect of KIR-HLA combinations among the HCV resolution in a sample from the same geographical area (Montes-Cano et al., 2005). This fact also reinforces our findings.

We have reported the first genome-wide association study on the low susceptibility to HCV infection (Real et al., 2019a). In that work, we found some suggestive associations with this phenotype, but none of them was linked to loci involved in the immune response. Moreover, we validated, at a nominal p-value level, the previously reported associations of rs5925 and rs688 polymorphisms, within the low-density lipoprotein receptor (LDLR) gene, with the lack of infection and seroconversion in HRSN subjects (Steba et al., 2019). In addition, we also published the association of LDLRAP1 with that condition (Real et al., 2019b). LDLRAP1 is an adaptor that interacts with the cytoplasmic tail of the low-density lipoprotein receptor (LDLR). Taken together, these findings suggest that the HRSN condition could be, at least in part, a consequence of genetic resistance to HCV infection related to lipid metabolism. In fact, it has been observed that the lipidomics profiling of HRSN individuals is different from that observed in HCV-infected individuals (Shawa et al., 2017b).

In spite of this, infection without seroconversion was previously observed in a few individuals and related to innate immune responses (Meyer et al., 2007; Post et al., 2004). However, and in accordance with our previous findings, the genetic resistance to HCV infection could be also a relevant factor involved in the HRSN phenotype. Therefore, the exposition to HCV without seroconversion could be a multifactorial condition related to both a low susceptibility to infection and an enhanced immune response that is not mainly determined by specific KIR-HLA genotype combinations.

This study has some limitations. First, due to the scarce number of HRSN individuals, the power of our study may be low. In accordance

Table 2
Association analysis of KIR/HLA genotype combinations with the absence of seroconversion in HRSN subjects.

KIR-HLA combinations	CI (n = 650)	HRSN (n = 23)	p-value
2DS4-(2DL2/3)/C1, n (%)	26 (4.3) [a]	0 (0) [b]	1.000
2DL3/C1 homozygous, (%)	225 (35.2) [c]	6 (27.3) [d]	0.446
3DL1/≥2Bw4, n (%)	195 (30.4) [e]	7 (30.4)	0.995

CI, HCV Chronically infected subjects; HRSN, High-risk seronegative individuals.
Combinations imputed in [a] 599, [b]19, [c] 640, [d] 22, [e] 642 individuals.

Table 3
Association of KIR/HLA genotype combinations with the absence of seroconversion in HRSN subjects. Analysis restricted to intravenous-drug users infected by HIV.

KIR-HLA combinations	CI (n = 344)	HRSN (n = 23)	p value[a]
2DS4-(2DL2/3)/C1, n (%)	16 (5) [a]	0 (0) [b]	1.000
2DL3/C1 homozygous, (%)	126 (37.5) [c]	6 (27.3) [d]	0.335
3DL1/≥2Bw4, n (%)	105 (30.9) [g]	7 (30.4)	0.964

CI, HCV Chronically infected subjects; HRSN, High-risk seronegative individuals.
Combinations imputed in [a] 319, [b] 19, [c] 336, [d] 22, [g] 340 individuals.

with the original articles, the odds ratio (OR) of the KIR2DS4/HLA-C1, KIR2DL3/HLA-C1 homozygous and KIR3DL1/≥ 2 HLA-Bw4 combinations were 2.65, 3.1 and 3.3 respectively (Knapp et al., 2010; Thöns et al., 2017; Zúñiga et al., 2009). It should be taken into account that with the sample size analyzed, the case-control ratio and the frequency of each combination in our population, our study had 31%, 81% and 81% power to detect each of those ORs, respectively. Therefore, only the analysis of the KIR2DS4/HLA-C1 combination had not enough power. However, the absence of this combination in all HRSN subjects suggests a very low effect on this phenotype. Second, KIR and HLA alleles have been imputed in our work instead of directly genotyped. Although errors could exist in the imputations, the tools used have demonstrated a high accuracy level (>90%) (Vukcevic et al., 2015; Zheng et al., 2014). Accordingly, the allelic or genetic frequencies imputed are similar to those observed in the Spanish population. Third, it is possible that some subjects considered as HRSN were not in contact with the HCV. However, all these subjects were IDUs who became infected by HIV through sharing needles during months. Since HIV is less transmissible and less frequent than HCV, aged IDUs infected by HIV show a high prevalence of HCV co-infection in Spain (Serrano-Villar et al., 2015). Therefore, these facts prove a high degree of parenteral exposure to HCV in those individuals.

In conclusion, we have not validated the association of specific KIR-HLA combinations with the absence of infection and seroconversion in HSRN individuals. This fact suggests that these combinations are not relevant factors involved in this phenotype. In accordance with our previous results, it is possible that resistance to becoming infected by HCV instead of an efficient viral clearance without seroconversion could be more frequent in HRSN individuals. Further studies will be needed to completely understand the existence of this phenotype.

CRediT authorship contribution statement

Carmen Martín-Sierra: Writing – review & editing, Writing – original draft, Investigation, Formal analysis. **María José Bravo:** Writing – review & editing, Formal analysis, Conceptualization. **María Eugenia Sáez:** Writing – review & editing, Software, Formal analysis. **Itziar De Rojas:** Writing – review & editing, Investigation. **Marta Santos:** Writing – review & editing, Investigation. **Jesica Martín-Carmona:** Writing – review & editing, Investigation. **Anaïs Corma-Gómez:** Writing – review & editing, Investigation, Conceptualization. **Alejandro González-Serna:** Writing – review & editing, Investigation. **José Luis Royo:** Writing – review & editing, Investigation. **Juan A. Pineda:** Writing – review & editing, Investigation, Funding acquisition, Data curation, Conceptualization. **Antonio Rivero:** Writing – review & editing, Investigation. **Antonio Rivero-Juárez:** Writing – review & editing, Investigation. **Juan Macías:** Writing – review & editing, Investigation, Funding acquisition, Data curation, Conceptualization. **Luis Miguel Real:** Writing – review & editing, Project administration, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Carmen Martín-Sierra, María José Bravo, María Eugenia Sáez, Itziar De Rojas, Marta Santos, Jesica Martín-Carmona, Anaïs Corma-Gómez, Alejandro González-Serna, José Luis Royo, Juan A Pineda, Antonio Rivero, Antonio Rivero-Juárez, Juan Macías, Luis Miguel Real.

Data availability

Data will be made available on request.

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5. Glossary

CI: chronically infected; HCV: hepatitis C virus; HLA: human leukocyte antigen; HRSN: high-risk HCV-seronegative; IDUs: intravenous drug users; KIR: killer-cell immunoglobulin-like receptors; *LDLR*: low-density lipoprotein receptor; SR: spontaneous resolvers.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.antiviral.2024.105795.

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