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BRIEF REPORT

A Knockout *IFNL4* Variant Is Associated With Protection From Sexually Transmitted HIV-1 Infection

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An interferon λ4 gene (*IFNL4*) knockout allele (rs368234815; TT) is associated with spontaneous and IFN-α–dependent cure of hepatitis C virus infection. The role of this polymorphism in the susceptibility to human immunodeficiency virus type 1 (HIV-1) infection is controversial. This study aimed to assess the association of this knockout *IFNL4* variant and sexually transmitted HIV-1 infection. A total of 228 HIV-1–positive individuals and 136 HIV-exposed seronegative individuals were investigated for their association with *IFNL4* rs368234815 genotypes. The *IFNL4* ΔG functional allele is associated with increased susceptibility to HIV-1 infection through the sexual route (odds ratio [OR], 2.1; 95% confidence interval [CI], 1.2–3.6; $P = .004$). A meta-analysis including a population of injection drug users suggests a codominant mode of inheritance of this risk factor (OR, 2.0; 95% CI, 1.3–3.2; $P = .001$).

Keywords. HIV-1; high exposed seronegatives; IFNL4; USP18; IFNA.

Human immunodeficiency virus type 1 (HIV-1)–exposed seronegative (HESN) individuals remain HIV-1 uninfected after frequent risk behaviors, including injection drug use with needle sharing or sexual intercourse with infected partners. The study of genetic or immunological factors associated with the HESN phenotype is a key model for the discovery of alternative antiviral targets and the development of novel vaccine strategies. The study of this group is conditioned by confounding factors such as risk scores, infection route (parenteral vs sexual), or sex-dependent innate immune responses. Using hypothesis-driven candidate gene strategies, several loci have been identified as being associated with HESN status [1]. Most of these associations were not confirmed in independent populations [2]. Moreover, several large genome-wide association studies did not find any association between these loci and innate resistance against HIV-1 infection [2], and *CCR5* Δ32 appears to be the only confirmed genetic polymorphism that confers resistance to HIV-1 infection.

The human interferon λ gene (*IFNL*) cluster includes 4 paralog genes, *IFNL1–IFNL4* (type III IFNs). The antiviral activities of IFNLs are similar to that of INFA (type I IFN). However, the expression of the IFNL receptor is more restricted than that of the INFA receptor. The main tissues sensitive to the action of IFNLs are the liver and epithelial and myeloid cells [3].

Several genome-wide association studies discovered that several single-nucleotide polymorphisms (SNPs) upstream of *IFNL3* (formerly known as *IL28B*) are strong susceptibility loci for spontaneous and treatment-dependent cure of HCV infection. Subsequently, the functional polymorphism (rs368234815) was identified in a previously unknown gene upstream to *IFNL3*, termed *IFNL4* [4]. This polymorphism includes a SNP plus an insertion that produces a frameshift in the coding region (ΔG/TT), generating a natural knockout gene and a total absence of IFNL4 (owing to the presence of the TT allele). This genetic association is paradoxical because the protective allele does not produce INFL4, a potent antiviral protein [4].

Several studies have explored the potential role of *IFNL4-IFNL3* polymorphisms in the course of HIV-1 infection. Machmach et al [5] found that the *IFNL4* ΔG variant is associated with unfavorable clinical and immunological statuses independently of HIV viremia. Dominguez-Molina et al [6] found that *IFNL4* variants are associated with the long-term nonprogressor controller phenotype. Additionally, Martin et al [7] and Rallón et al [8] did not find any association between the genotype of *IFNL4* and susceptibility to HIV-1 infection in HESN individuals, but these studies did not test the functional polymorphism in the coding region of *IFNL4*. Conversely, Real et al [9] found a strong association between the knockout allele of *IFNL4* and innate resistance to HIV-1 infection in injection drug users. Based on the previous observations, we tested the association between the functional *IFNL4* polymorphism and sexually transmitted HIV-1 infection in well-characterized populations of HESN individuals, healthy donors, and HIV-1–infected individuals from Spain and Italy.

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METHODS

Study Population

We analyzed 364 individuals, from southern Spain (Cordoba and Jaen), central Spain (Madrid), and Italy (Florence) with sexual exposure to HIV-1. Among them, 228 HIV-1–seropositive individuals and 136 HESN individuals (discordant couples for whom sexual intercourse was routinely unprotected, as well as men who have sex with men). Additionally, 211 anonymous healthy blood donors from Hospital Ciudad de Jaen (Spain) were genotyped. Data from a previous study of 213 seropositive Europeans who were exposed to HIV-1 through injection drug use and shared needles for >3 months and 188 HESN individuals were included in our study [9]. The inclusion criteria and main characteristics of these patients have been previously described in detail elsewhere [8, 10].

Genotyping

DNA was extracted from whole-blood specimens, using the Blood QuickPure kit (Macherey-Nagel, Duren, Germany). *CCR5* Δ32 and *IFNL4* rs368234815 polymorphisms were genotyped by high-resolution melting and Taqman real-time polymerase chain reaction analysis (PCR), respectively, as previously described [9], on the Eco Real-Time PCR system (Illumina, San Diego, CA).

Statistical Analysis

Hardy-Weinberg equilibrium was estimated using the online tool of the University of Munich (available at: https://ihg.gsf.de/cgi-bin/hw/hwa1.pl). Logistic regression analyses adjusted for age and sex were performed using PLINK and SPSS, version 19, software. Results from the 3 cohorts (ie, individuals from southern and central Spain and from Italy) were combined with results from the cohort of injection drug users, using a random effects meta-analysis. This meta-analysis was estimated with Ken Rothman's Episheet (available at: http://www.krothman.org/episheet.xls). We then calculated the pooled odds ratios (ORs) with 95% confidence intervals (CIs) for each genetic model. The ORs of the individual populations (examining the association between transmission route and the *IFNL4* rs368234815 variant), together with ORs from meta-analyses, are presented using a forest plot. Forest plots were made using StatsToDo (available at: https://www.statstodo.com/ForestPlot_Pgm.php).

Ethics

This study was designed and performed according to the principles of the Helsinki Declaration and was approved by the institutional review boards at the participating hospitals and the Universidad de Jaen. The participating centers and hospitals were Complejo Hospitalario de Jaén (Jaén), Hospital Universitario Reina Sofía (Cordoba), Centro Sanitario Sandoval (Madrid), and S. Maria Annunziata Hospital (Florence). All patients and healthy blood donors provided written informed consent to participate in this study.

RESULTS

Sociodemographic and clinical variables of the subjects were previously described [8, 10]. Two individuals in the HESN group were homozygous for *CCR5* Δ32 and were excluded from subsequent analysis. The genotyping call rate was 98.7%. A slight deviation from Hardy-Weinberg equilibrium ($P = .04$) was observed for *IFNL4* rs368234815 in HESN individuals from Spain.

We observed a significant difference in the genotypic distribution of the *IFNL4* rs368234815 polymorphism in HIV-1–positive patients as compared to HESN subjects (Table 1). Thus, the frequency of harboring genotypes including the functional allele (ΔG/ΔG + ΔG/TT) was significantly higher in Spanish HIV-1–positive subjects as compared to HESN individuals (64% vs 39%). The OR for a dominant model with the ΔG/ΔG + ΔG/TT genotypes being a susceptibility factor against HIV-1 infection was 2.6 (95% CI, 1.3–5.3; $P = .005$, by logistic regression after adjustment for age and sex; Table 1). The presence of only 1 copy of the ΔG allele (vs TT/TT) was also associated with susceptibility to HIV-1 infection in this population (OR, 2.6 [95% CI, 1.2–5.5]; $P = .008$). A similar pattern was found when comparing the Spanish healthy donors to HIV-1–positive patients (64% vs 49.5%; OR, 1.8 [95% CI, 1.2–2.8]; $P = .003$; Table 1). The same trend for HIV-positive subjects as compared to HESN subjects was observed in the Italian population (66% vs 53%), but the differences did not reach statistical significance (OR, 1.5 [95% CI, .6–3.6]; $P = .30$; Table 1). Heterozygous individuals with sexual exposure to HIV-1 were more susceptible to infection than those with parenteral exposure [9] ($P = .0003$ vs $P = .4$; Supplementary Table 1). Results for the populations at risk for sexual acquisition were combined through a random-effects meta-analysis and yielded a significant difference for the dominant model (ΔG allele) comprising the genotypes ΔG/ΔG + ΔG/TT (OR, 2.1 [95% CI, 1.2–3.6]; $P = .004$; $P = .33$, by the heterogeneity test; Figure 1). Furthermore, the results of the populations at risk for sexual acquisition, as well as the previously described population of male injection drug users [8], were combined through a meta-analysis and yielded a significant difference for the dominant model including the genotypes ΔG/ΔG + ΔG/TT (OR, 1.5 [95% CI, 1.1–2.2]; $P = .007$; $P = .22$, by the heterogeneity test; Figure 1).

DISCUSSION

The role that *IFNL4* plays in innate resistance to HIV-1 infection is controversial. A large study reported no association of *IFNL4* rs12979860 with resistance to HIV-1 infection; it included 1221 HIV-1–positive patients and 291 HESN individuals from several cohorts in the United States (ie, men who have sex with

Table 1. Allele and Genotype Distributions Among Human Immunodeficiency Virus Type 1 (HIV-1)–Infected Subjects, HIV-1–Exposed Seronegative (HESN) Subjects, and Healthy Donors

	Allele	Genotype		
Location, Group	ΔG	ΔG/ΔG	ΔG/TT	TT/TT
Spain				
Overall, no. (%)[a]				
HIV-1 infected	135 (39.2)	25 (14.5)	85 (49.4)	62 (36.0)
HESN[b]	38 (24.7)	8 (10.4)	22 (28.6)	47 (61.0)
Healthy	123 (29.1)	20 (9.5)	83 (39.3)	108 (51.2)
HIV-1 vs HESN	ΔG vs TT	ΔG/ΔG vs TT/TT	ΔG/TT vs TT/TT	ΔG/ΔG + ΔG/TT vs TT/TT
OR (95% CI)	2.0 (1.3–3.0)	1.7 (.9–3.2)	2.7 (1.3–5.6)	2.7 (1.3–5.3)
P[c]	.001	.079	.008	.005
HIV-1 infected vs healthy	ΔG vs TT	ΔG/ΔG vs TT/TT	ΔG/TT vs TT/TT	ΔG/ΔG + ΔG/TT vs TT/TT
OR (95% CI)	1.6 (1.1–2.1)	2.2 (1.1–4.2)	1.8 (1.1–2.7)	1.8 (1.2–2.8)
P[c]	.003	.002	.008	.003
	Allele		Genotype	
Italy	ΔG	ΔG/ΔG	ΔG/TT	TT/TT
Overall, no. (%)[a]				
HIV-1 infected	42 (37.5)	5 (8.9)	32 (57.1)	19 (33.9)
HESN	34 (28.8)	3 (5.1)	28 (47.5)	28 (47.5)
HIV-1 infected vs HESN	ΔG vs TT	ΔG/ΔG vs TT/TT	ΔG/TT vs TT/TT	ΔG/ΔG + ΔG/TT vs TT/TT
OR (95% CI)	1.4 (.8–2.9)	2.1 (.7–6.7)	1.4 (.6–3.4)	1.5 (.6–3.7)
P[c]	.16	.177	.425	.305

Abbreviations: CI, confidence interval; OR, odds ratio.

[a]Data are no. (%) of subjects with the specified allele or genotype.

[b]Two individuals were homozygous for *CCR5* Δ32 and were excluded from this analysis.

[c]Tests for association were performed using the Fisher exact test.

men, injection drug users, and hemophiliacs) [7]. However, this study did not genotype the functional polymorphism in *IFNL4*, and the confounding factors of the population's admixture were not evaluated. The allelic frequencies of *IFNL4* polymorphisms are dependent of the population´s origin, with the ΔG allele significantly more frequent in individuals of African ancestry (78%), compared with those of European (32%) and Asian (10%) ancestry. Nonetheless, we have previously communicated the association of the functional allele of *IFNL4* with an increased risk of HIV-1 infection in a well-characterized

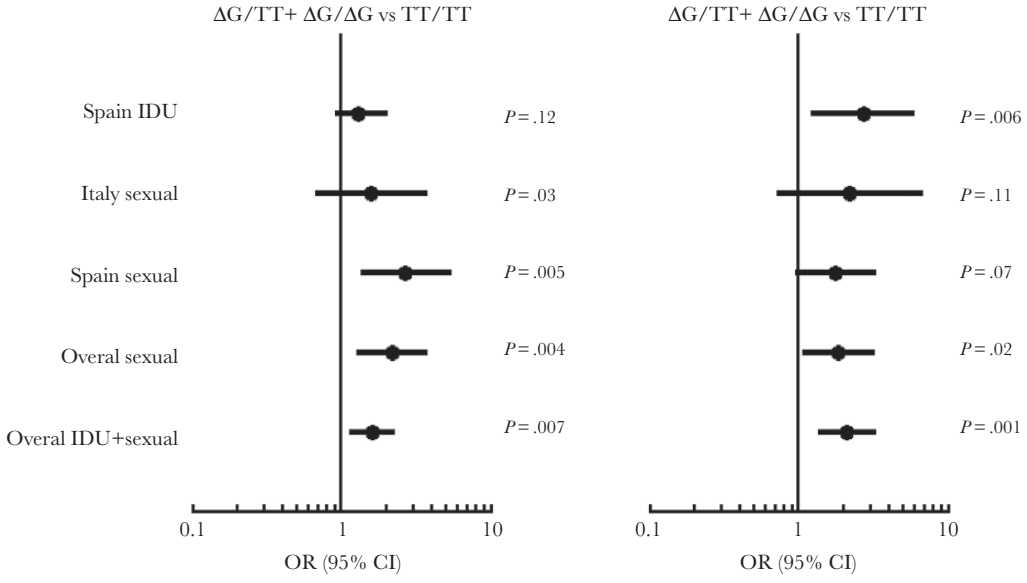

Figure 1. Meta-analysis of the sexual and injection drug users (IDU) populations: OR and 95% Confidence interval (95% CI) and Logistic regression or random-effect meta-analysis (overall sexual and overall IDU+sexual cohorts) *P* values.

population of injection drug users in Spain [9], including 213 HIV-1–positive individuals and 188 HESN individuals. Owing to these discrepancies, in this report we evaluated whether the functional polymorphism in *IFNL4* was associated with sexually transmitted HIV-1 infection in 2 European populations. The main finding of this study is that, in an independent population at risk of sexual acquisition of HIV-1, we replicated our results from a previous study of injection drug users. Because the ΔG allele was significantly associated with a higher risk of being HIV-1 infected, we can deduce that the presence of IFNL4 is a risk factor for HIV-1 infection independently of the infection route.

The role of IFNL4 in the innate immune response is a matter of considerable interest. *IFNL4* messenger RNA (mRNA) expression after induction with polyinosinic:polycytidylic acid (an agonist of double-stranded RNA) or experimental infection with hepatitis C virus or Sendai virus is very fast and transient, compared with expression of *IFNL3* or *IFNA* mRNA [4, 11]. IFNL4 appeared earlier than other IFNLs in inducing antiviral genes, as well as genes that negatively regulate the IFN response, such as *USP18* and *SOCS1* [11]. Transient treatment of hepatocytes with IFNL4 but not IFNL3 caused durable and sustained induction of SOCS1 and refractoriness to further stimulation with IFNL3 [11]. Levels of IFNs inhibiting ISG15 and USP18 are increased in IFNL4-expressing or treated cells and mediate IFNA insensitivity in an IFNL4-dependent way [12]. Additionally, *IFNL4* genotype correlates with *ISG15* or *USP18* mRNA expression, with genotypes containing the ΔG allele displaying higher levels of ISG15 mRNA as compared to those containing the TT allele [13]. Also, IFNL4 inhibits the JAK-STAT signaling pathway by inducing USP18-mediated IFNAR2 desensitization [12] and by reducing *IFNAR1* mRNA levels; this is also dependent on *IFNL4* genotype, with the ΔG allele associated with a significantly lower level of *IFNAR1* mRNA [14]. The USP18-ISG15 tandem appears to be a main effector in the negative feedback control of IFN signaling; *USP18* mutations leading to loss of function underlie interferonopathies leading to severely enhanced IFN-induced inflammation [15]. Chronic IFN activation is highly toxic [15], and it is probable that IFNL4, through USP18-ISG15 upregulation, contributes to repress the cell´s responsiveness to prolonged IFN stimulation [11]. We predict that the *IFNL4* ΔG allele could be a protective genetic factor in pathologies related to enhanced IFN activation, such as type I diabetes; and in interferonopathies, such as Aicardi-Goutieres syndrome or systemic lupus erythematosus, where mutations in nucleic acid metabolizing enzymes lead to accumulation of nucleic acids and pathogenic IFN production [15].

Furthermore, individuals harboring the functional *IFNL4* allele display poorer clinical responses to IFNA treatment in chronic hepatitis C virus infection. This fact strongly suggests that INFL4 acts as a potent antagonist or desensitizing factor of IFNA in vivo [4].

Regarding the assessment of an association between HIV-1 transmission routes and *IFNL4* rs368234815, we found that the ΔG allele appears to follow a recessive model of inheritance in the case of parenteral transmission [9] but a dominant model in the case of sexual transmission. These discrepancies could be due to the small size of the cohorts included in this study. However, the meta-analysis suggests a codominant mode of inheritance, which best fits a model in which a higher HIV-1 infection risk is correlated to increasing cumulative levels of IFNL4. These differences in the pattern of susceptibility to HIV-1 infection could be related to the expression of the IFNL4 receptor. In the sexual mucosa, lower levels of IFNL4 could create a favorable environment for viral escape, in contrast to the parenteral route, in which higher levels of IFNL4 could be necessary for such an effect. It has not escaped our notice that the results presented here immediately suggest that IFN-dependent signaling is a core defense against HIV-1 infection.

Nevertheless, the main limitation of our research is the small number of populations included in the meta-analysis and the possible bias associated with combining the populations from Spain and Italy. The ΔG/ΔG genotype has a low frequency (between 5% and 14 %), and small random deviations could have an influence on the statistical association. Further research is needed to resolve this complex genotype-phenotype relationship and the apparent paradox of IFNL4 and its role in innate immunity against viral infections.

In conclusion, the present study provides genetic evidence that the *IFNL4* TT/TT genotype is associated with innate resistance to HIV-1 infection, regardless of transmission route (sexual vs parenteral), which supports the importance of IFN type I and type III crosstalk in the pathogenesis of HIV infection.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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editors consider relevant to the content of the manuscript have been disclosed.

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