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Association of IFN λ 4 rs12979860 Polymorphism with the Acquisition of HCV and HIV Infections among People Who Inject Drugs

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Abstract

We investigated the presence of a single nucleotide polymorphism designated rs12979860 in the interferon-lambda-4 (IFN λ 4) gene among 345 people who inject drugs (PWID) and 495 blood donors to evaluate associations between the rs12979860 genotypes and HIV/HCV.

The rs12979860 TT genotype was overrepresented among HIV+ PWID than HIV– PWID and blood donors (16% vs. 8% and 10%, $p=0.03$, respectively). PWID with TT genotype had approximately twice the probability of being HIV+ (OR=2.19, 95% CI=1.11–4.33) than PWID without TT. Every additional year of intravenous drug use (IVDU) decreased the odds ratio 1.16 times (OR=0.86, 95% CI=0.75–0.98).

This suggests that rs12979860 TT increases susceptibility to HIV and this impact decreases with increasing duration of IVDU.

Keywords

IL28B; IDU; HIV/HCV co-infection; lambda interferon

Introduction

Hepatitis C virus (HCV) and human immune deficiency virus (HIV) are widely spread around the world, and with their high rate of co-infection, these viruses continue to cause increased mortality, especially among people who inject drugs (PWID). In addition to sociodemographic factors, host genetics might influence susceptibility to these viruses (1–4). As interferon-lambdas (IFN λ s), and interferons in general, are essential in innate immune responses polymorphisms located in these genes are of interest.

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The IFN λ s induce a variety of biological activities similar to type I interferons, including antiviral activity (5). IFN λ s activate the Jak-STAT pathway via the heterodimeric receptor including the chains of IFN λ R1 and IL-10R2 and upregulate interferon stimulated genes (6, 7). The expression of the IFN λ R1 is limited to hepatocytes, cells of myeloid lineage, and epithelial cells of the lung, intestine, and skin (6, 8). Indeed, IFN λ s are essential to fighting viral respiratory infections (8) and IFN λ s have been shown to successfully inhibit HCV and HIV replication in vitro (6).

In HCV infection, genome-wide association studies have shown that the single-nucleotide polymorphism (SNP) rs12979860 (also referred to as interleukin-28B rs12979860 polymorphism), located in the interferon-lambda-4 (IFN λ 4) gene, influences the outcome of interferon-based therapy and spontaneous HCV clearance (9, 10). Persons with the IFN λ 4 rs12979860 C allele have higher probability of achieving sustained virologic response compared to persons without this allele when receiving interferon based therapy (9). The same allele is also responsible for a higher rate of spontaneous viral elimination of HCV (11, 12).

Few studies have evaluated the effect of IFN λ 4 rs12979860 polymorphism on HIV disease progression by comparing typical progressors to long-term non-progressors (LTNPs) or controllers and assessing HIV acquisition among people who inject drugs (PWID) (11, 13, 14). Machmach et al. found an overrepresentation of the rs12979860 CC genotype among HIV controllers compared to non-controllers, suggesting a protective role of CC genotype on HIV disease progression (15). In contrast, others found no association between IFN λ 4 rs12979860 genotypes and HIV acquisition or disease progression (11, 13, 14). However, associations between IFN λ 4 rs12979860 genotypes and HCV acquisition are widely unknown. We hypothesised that the IFN λ 4 rs12979860 polymorphism affects the acquisition of HCV and HIV infection.

Materials and methods

A cross-sectional study was conducted among Caucasian PWID and blood donors. The study group included 345 PWID who were recruited using the respondent-driven sampling method from a syringe exchange program in Tallinn, Estonia in 2011 (16). Plasma and peripheral blood mononuclear cells were separated from whole blood and stored for all individuals. All sociodemographic data and information about receiving antiretroviral treatment (ART) was collected through self-reported questionnaire. Informed written consent to genetic testing and participation in the study was obtained from all the PWID. The control group included 495 blood donors whose leftover blood samples were collected from blood donation centres in Tallinn and Ida-Viru County in 2010. All blood donors had agreed to the use of their leftover blood for research purposes. The study was evaluated and approved by the Ethics Committee of the University of Tartu.

HIV, HCV, and hepatitis B (HBV) serostatuses were determined as described previously (1). To detect IFN λ 4 rs12979860, DNA was extracted using a Pure-Link[®] Pro 96 Genomic DNA Purification Kit (Invitrogen, Carlsbad, CA, USA), and the genotyping was done using allelic discrimination analysis with the following primers and probes: 5'-

TGCCTGTGCTGTACTGAA-3' (sense), 5'-GAGCGCGGAGTGCAATTC-3' (antisense), 5'-TCCCCGAAGGCGTGA-3' (VIC-probe), 5'-AAGGCGCGAACCA-3' (FAM-probe) (17), and the ABI7900HT Sequence Detection System (Applied Biosystems).

Statistical analyses were performed using the program R 2.13.1 (www.r-project.org) and Stata 14 (www.stata.com). Differences in the distributions of IFN λ 4 genotypes between the groups were examined using a Fisher exact test. Logistic regression analysis was used, and odds ratios with corresponding 95% confidence intervals (CI) were calculated to further assess the impact of IFN λ 4 rs12979860 TT genotype on HIV positivity. To determine possible confounders to be included in multivariable logistic regression analysis, the software DAGitty version 2.3 was used (18). To explore statistical interaction, interaction term was included in the model, and marginal effects at representative values were calculated using predictions from this model. *P*-values below 0.05 were considered to be significant.

Results

The majority of the PWID included in the study were male (79%), the median age 30 years (interquartile range [IQR] 25–34 years), and the median duration of intravenous drug use 11 years (IQR=7–14 years) (1, 2) (Table 1). Half of the PWID were HIV seropositive (HIV+), 89% HCV seropositive (HCV+), 77% HBV seropositive (HBV+), 49% HIV/HCV dual and 40% HIV/HCV/HBV triple seropositive. The prevalence of HCV and HBV seropositivity was higher among HIV+ PWID compared to HIV– PWID (98% vs. 79% for HCV, 82% vs. 53% for HBV; *p*<0.05). In addition, HIV+ PWID had used intravenous drugs for longer periods of time compared to HIV– PWID (12 years vs. 10 years; *p*<0.05). In total, 91 PWID admitted of taking ART in the past and 77% of them (70/91) admitted of being on ART at the moment of the recruitment. Demographic data for blood donors were unavailable.

In all groups IFN λ 4 rs12979860 was in Hardy-Weinberg equilibrium with a TT genotype frequency ranging from 5% to 16% (Supplementary Information, Table S1). First, we analysed the distribution of rs12979860 genotypes among HCV– and HCV+ PWID regardless of HIV serostatus and found no differences between the HCV+ and HCV– PWID groups. Second, we analysed the distribution of rs12979860 genotypes among HIV– and HIV+ PWID regardless of HCV serostatus. We found the IFN λ 4 rs12979860 TT genotype to be slightly over-represented among HIV+ PWID compared to HIV– PWID and blood donors (16% vs. 8% *p*=0.03 and 16% vs. 10% *p*=0.024, respectively). In univariate analysis, the presence of TT genotype increased the odds of being HIV positive approximately two times (Table 2, Analysis A) compared to PWID with non-TT genotypes. To understand whether the association between the acquisition of HIV and IFN λ 4 rs12979860 TT genotype changes due to the duration of IVDU, we included the interaction between TT genotype and duration of IVDU in the model (Table 2, Analysis B). The interaction between TT genotype and the duration of IVDU was statistically significant, indicating that having TT genotype increased the probability of being HIV+ to a greater extent for people who had injected drugs for shorter periods of time. The odds ratio was largest when the duration of IVDU was less than one year (OR=13.16, 95% CI=2.16–80.21, *p*=0.005). Every additional year of injecting drugs decreased the odds ratio 1.16 times (OR=0.86, 95% CI=0.75–0.98,

$p=0.024$). According to our model, the IFN λ 4 rs12979860 TT genotype affects the acquisition of HIV among people who have been using intravenous drugs for 12 years or less (Figure 1). The effect disappears among PWID with the duration of IVDU over 12 years.

Based on HCV and HIV serostatuses we divided the study population into four subgroups (HIV-HCV–, HIV-HCV+, HIV+HCV–, and HIV+HCV+) and evaluated the distribution of IFN λ 4 rs12979860 TT genotype between these groups. There was a non-significant trend of TT genotype frequency increase between study groups as follows: 3% among HIV-HCV– (N=35), 9% among HIV-HCV+ (N=137), 14% among HIV+HCV– (N=169), and 33% among HIV+HCV+ (N=3) (Table 3). Since we found no significant association between the IFN λ 4 rs12979860 polymorphism and acquisition of HCV, this trend might be due to the association between IFN λ 4 rs12979860 polymorphism and acquisition of HIV.

Discussion

To the best of our knowledge, this is the first study evaluating the effect of IFN λ 4 rs12979860 polymorphism on HCV acquisition in a population at risk of acquiring blood-borne infections. Based on our hypothesis, we did not find an effect of IFN λ 4 rs12979860 polymorphism on HCV susceptibility. However, we found the IFN λ 4 rs12979860 TT genotype to increase the probability of being HIV positive in our study population. In addition, we found that the scale of the influence of IFN λ 4 rs12979860 TT genotype on the acquisition of HIV decreased with the increase of the duration of IVDU.

Previous studies investigating the role of IFN λ 4 rs12979860 on HCV infection have focused on treatment outcomes and spontaneous viral clearance and demonstrated the beneficial effect of CC genotype on treatment response and HCV clearance (9, 10, 12). The few studies evaluating the effect of rs12979860 on HCV acquisition are controversial (3, 4, 19). An Iranian study comparing chronically HCV infected and healthy controls found no association between rs12979860 genotypes and HCV susceptibility (19). In contrast, other studies have found that the rs12979860 T allele and non-CC genotypes increase the odds of being HCV+ and that the CC genotype is protective against HCV infection (3, 4). We showed that the distribution of IFN λ 4 rs12979860 genotypes did not differ between our Caucasian HCV+ and HCV– PWID and blood donors. However, as majority of the studied PWID were HCV seropositive we acknowledge that the statistical power might have been insufficient for the detection of associations between rs12979860 genotypes and the acquisition of HCV. Unfortunately, HCV seropositivity is generally rather high among populations at risk for blood-borne infections such as PWID.

In terms of HIV infection, the few reports investigating the effects of IFN λ 4 rs12979860 on HIV susceptibility did not detect associations between rs12979860 genotypes and HIV acquisition (13, 14). Rallon et al. had a rather small sample size which might have influence the detection of any association (13). Though Martin *et al.* included a very good size study population it was a heterogeneous cohort which is not ideal for genetic studies (14). In contrast, others have found the rs12979860 CC genotype to have a beneficial effect on HIV disease progression (15, 20). While Machmach et al. found an over-representation of the

rs12979860 CC genotype among HIV controllers compared to non-controllers (15), Srinidhi et al. showed the CC genotype to be associated with increased CD4 T cell count following ART initiation (20).

The present study focused on HIV susceptibility and found the rs12979860 TT genotype to increase the odds of being HIV+ compared to non-TT genotypes. This association could reflect the influence of a functional SNP located in the same gene. The IFN λ 4 rs12979860 polymorphism is in strong linkage disequilibrium with a dinucleotide polymorphism rs368234815 (TT or G), and the unfavourable rs12979860 T allele correlates well with the rs368234815 G allele in Europeans ($r^2=0.92$) (21). The rs368234815 G variant creates a frame shift which results in the production of the IFN λ 4 protein (21). The rs368234815 TT/TT genotype, which marks the rs12979860 CC genotype, has been shown to be more frequent among highly exposed seronegative individuals compared to HIV+ subjects (22, 23). Accordingly, we found the IFN λ 4 rs12979860 TT genotype to be overrepresented among HIV+ PWID compared with HIV- PWID and blood donors. In addition, we observed the influence of TT genotype change overtime depending on the duration of IVDU. The effect of TT genotype was highest among PWID with duration of IVDU of less than one year, and the effect lasted until the duration of IVDU became 12 years. Among PWID with longer durations of IVDU, the influence of IFN λ 4 rs12979860 TT genotype on the acquisition of HIV was not significant. As previous studies have shown, intravenous drug use, especially sharing injecting equipment, is a major risk factor for acquiring HIV infection (24, 25). Thus, within a high risk population like PWID the overall HIV prevalence increases and the incidence decreases with increasing duration of IVDU. This all suggests that although the IFN λ 4 rs12979860 polymorphism affects susceptibility to HIV, the duration of IVDU has a greater impact on HIV acquisition than the IFN λ 4 rs12979860 TT genotype.

This study has some limitations. First, the data on HCV viral load were not available, and thus we were unable to distinguish between patients with active and past HCV infections or whether the rs12979860 polymorphism affects spontaneous viral clearance in our population. Second, we had rather small groups of HIV-HCV- and HIV+HCV- PWID which might have resulted in lack of statistical power to detect any associations regarding studied polymorphism and HIV and HCV serostatus based groups. As PWID are very hard-to-reach population it is difficult to recruit participants based on infection statuses. Third, as the HIV treatment information was collected through self-reported questionnaire we had no information with regards to the length or the combination of the treatment or the HIV viral load which prevented us to determine any associations between this polymorphism and HIV disease progression and/or treatment. Nevertheless, we believe the importance of our results is not diminished by these limitations.

In conclusion, the study results support our hypothesis regarding the influence of the IFN λ 4 rs12979860 polymorphism affecting the susceptibility to HIV infection but not regarding the acquisition of HCV infection in a Caucasian PWID population.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Prognosis of the logistic regression model used in interaction analysis. The effect of IFN λ 4 rs12979860 TT genotype on the acquisition of HIV is decreasing with each year of intravenous drug use and with the duration of IVDU over 12 years the effect is lost. Note. The durations of IVDU are included in full years. If the duration of IVDU was less than one year, it was referred to as 0.

Table 1.

Characteristics of People Who Inject Drugs (PWID)

Variables	PWID (n = 345)
Male, n (%)	272 (79%)
Median age,(IQR)	30 (25-34)
Median duration of IDU, (IQR)	11 (7-14)
HIV+, n (%)	172 (50%)
HCV+, n (%)	306 (89%)
HBV+, n (%)	232 (67%)
HBV vaccinated, n (%)	43 (12%)
HIV+HCV+, n (%)	306 (89%)

Note. Hepatitis B positive (HBV+) group included persons who were positive for HBsAg and/or anti-HBc. HBV vaccinated group included persons who were positive for only anti-HBs. Age and the duration of IDU are measured in full years. HIV – human immunodeficiency virus; HCV – hepatitis C virus.

Table 2.

Associations by Logistic Regression Analysis of IFN λ 4 rs12979860 with HIV Infection and Duration of Intravenous Drug Use (IVDU) Among People Who Inject Drugs (PWID)

A) Univariate analysis		Outcome: HIV+	
	OR (95% CI)	P	
IFNλ-4			
Non-TT genotype ^a	1.0		
TT genotype	2.19 (1.11-4.33)	0.002	
B) Interaction analysis		Outcome: HIV+	
	OR (95% CI)	P	
IFNλ-4			
Non-TT genotype ^a	1.0		
TT genotype	13.16 (2.16-80.21)	0.005	
Duration of IVDU [#]	1.08 (1.03-1.13)	0.001	
IFNλ-4-TT*IVDU	0.86 (0.75-0.98)	0.024	

Note.

^aReference group.

[#]Categorized as a continuous variable, data reflects an increase in OR with each additional year of duration of IDU.

Table 3.

Distribution of TT and non-TT Genotypes among HIV and HCV Serostatus Based Subgroups

	TT	non-TT	Total
HIV-HCV-, n (%)	1 (2.9)	34 (97.1)	35
HIV-HCV+, n (%)	12 (8.8)	125 (91.2)	137
HIV+HCV+, n (%)	23 (13.6)	146 (86.4)	169
HIV+HCV-, n (%)	1 (33.3)	2 (66.7)	3

Note. Comparison of these groups revealed no significant differences (all p-values > 0.05)